MODIFICATION OF FLORAL MORPHOGENESIS IN THE TOMATO (LYCOPERSICUM ESCULENTUM) WITH N-m-TOLYLPHTHALAMIC ACID

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By

JANE YUPEI SHEN

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
State University of Agriculture and Applied Science
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Approved S. H. Wittwer

Certain aspects of floral morphogenesis in the tomato plant as influenced by treatment with low temperature (55° F) and N-m-tolylphthalamic acid were pursued. Apical meristems were collected daily for histological examination from the time of seedling emergence until 15 days after the 2-3 leaf stage. At cotyledon expansion only the two plumule leaves had been initiated. Vegetative-reproductive transition came two days earlier (8th day after germination) in the cold-treated (55° F) plants than in the non-treated (72° F). Cold-treated plants also had fewer leaves (nodes) subtending the reproductive primordium than the non-treated. In the N-m-tolylphthalamic acid treated seedlings, the sympodial bud subjacent to the inflorescence was retarded in development, consequently giving the inflorescence a longer period of apical dominance, and resulting in polychotomous branching of the inflorescence. Treatment with N-m-tolylphthalamic acid prolonged juvenility of the sympodial bud, while at the same time projected a direct induction of greater flower numbers in the inflorescence.

A comparison of several methods of application over a wide range of concentrations suggested that the primary site of action of N-m-tolylphthalamic acid was the terminal meristem. Measureable increases in flower numbers were obtained with as little as 0. 3 microgram applied directly to the apical meristem. The optimum time for treatment to obtain polychotomy

and greater numbers of flowers in the first inflorescence was at the 2-3 leaf stage of seedling development when the transition from vegetative to reproductive development occurred in the apical meristem.

The translocation pattern of C-14 N-m-tolylphthalamic acid in the tomato plant revealed that more than 50 percent of the foliar absorbed chemical was not recovered 24 hours after treatment. This observation and the temporary effect of the chemical on flower formation suggest that N-m-tolylphthalamic acid is extremely labile in the biological system of the intact tomato plant. However, the recovered chemical was in the form of intact N-m-tolylphthalamic acid as substantiated through paper chromatography of acetone extracts of treated leaves, which gave Rf values typical of N-m-tolylphthalamic acid. Autoradiograms of intact plants following treatment of one of the plumule leaves with C-14 labeled N-m-tolylphthalamic acid indicated a high concentration of radio-activity in the apical meristem.

Possible breakdown products of N-m-tolylphthalamic acid were not active in inducing polychotomous inflorescences in the tomato. An intact molecule of the chemical was required. Avena straight-growth bioassays and parthenocarpic development of tomato ovaries suggested that N-m-tolylphthalamic acid possesses "auxin-like" activity.

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TO

MY PARENTS

Dr. and Mrs. Shen Yi

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INTRODUCTION

N-m-tolylphthalamic acid has been applied to seedling greenhouse tomato plants to increase the flower number in the first inflorescences. This has led to a two-fold higher early yield of fruit (109, 110), and has become a standard practice employed by many Michigan, Indiana and Ohio greenhouse tomato growers.

DeZeeuw (24) has suggested that the young leaves of the sympodial bud produce an inhibitor which retards the development of the adjacent inflorescence, and that N-m-tolylphthalamic acid acts not as an auxin, but accelerates maturation of these young leaves, and thereby alleviates the inhibiting effect. He has found that in the absence of the sympodial bud N-m-tolylphthalamic acid inhibits flower formation, while a typical auxin, naphthaleneacetic acid, causes increased flower numbers. An alternate interpretation which DeZeeuw (24) suggests is that the young developing leaves of the sympodial bud compete with the flower primordia for an available auxin supply, and in this way prevent continued flower formation. In opposition to DeZeeuw's first hypothesis, Teubner and Wittwer (110) have found that increased flower numbers can be obtained with indoleacetic acid even in the presence of the sympodial bud.

Rahn (93) and Walhood et al. (121) found N-m-tolylphthalamic acid prevented abscission of lima bean pods and cotton squares (buds) during hot, dry conditions. Hoffman et al. (48) obtained parthenocarpic fruit-set of tomato with several N-aryl-phthalamic acids including the N-m-tolyl derivative. While these characteristics (stop-drop, and fruit-set) do not provide the classical proof required of auxins, they do indicate "auxin-like" activity.

Since only a few species clearly respond to auxin with earlier flower initiation or increased flower numbers, studies were made on morphological and physiological changes associated with the response of tomato plants to N-m-tolylphthalamic acid. These studies are of significance not only with respect to fundamental aspects of tomato flowering, but should be of considerable practical value in the chemical control of fruiting in greenhouse and field tomatoes.

LITERATURE REVIEW

GENERAL ASPECTS OF MORPHOLOGY AND ANATOMY

The pattern of growth of the tomato plant (Lycopersicum esculentum) is determined, to a large extent, by its flowering habit. Alpatev and Polumordvinova (2) examined the growing points of several varieties of tomato and found the first inflorescence was initiated after seed germination. This floral initiation takes place through a change in the terminal meristem after 8 to 14 leaves have been formed. The number of leaves initiated prior to the inflorescence is related not only to variety, but is also dependent on environmental conditions (18, 23, 65, 70, 78, 95, 123, 126, 134, 135, 136, 137). Discussion of these factors will be given in later sections.

The tomato plant is a sympodium (124). Gray (37) describes a sympodium as a stem having a simple axis, terminated by a flower or inflorescence, in which the bud in the axil of the leaf subtending the inflorescence develops into a new axis or branch. The vigorous growth of this sympodial bud forces the adjacent inflorescence into a lateral position and thereby assumes a dominant role. This axis (developed from a sympodial bud) bears its leaves and terminates, like its predecessor, with an inflorescence and this pattern of growth may be repeated indefinitely. Tomato plants exhibiting this habit of growth are designated as an indeterminate type. A second distinct growth

type can also result from the sympodial pattern of development. In this case growth of the sympodial bud is suppressed and the main axis terminates after the appearance of one to two inflorescences. Subsequent vegetative growth, however, may occur from other lateral buds which, in turn, produce one to two inflorescences before termination, and the process repeats indefinitely. Tomato plants having this pattern of development are referred to as determinate.

While the transition of the tomato terminal meristem from vegetative to reproductive has not been studied in detail, it appears that the development is similar to plants in general, as discussed by Esau (27). The "pointed" vegetative apex begins to flatten through increased meristematic activity in the peripheral cells. Shortly thereafter, differentiation of flower parts begins in the first flower (27, 60, 88, 89, 90, 91, 94). In development of the individual flowers, a small protuberance of meristematic tissue develops at the base of the preceding flower. This meristematic tissue develops into a flower bud and forces the preceding flower bud to one side and itself assumes the terminal position. The succeeding flower buds develop likewise until 7 to 8 buds are formed (20). Ordinarily, it is unusual to have two flowers opening at the same time; and because of this progressive development, a single inflorescence or flower cluster may simultaneously exhibit small fruits, open flowers, and developing buds (44).

Cooper (20) and Smith (101) describe the tomato inflorescence as a racemose cyme. In contrast, Bouquet (10) points out that the inflorescences occur in mixed populations of simple racemes, as well as racemose cymes having dichotomous and polychotomous branching. While one type may predominate, all three types have been found on the same plant. The first formed inflorescences are frequently simple racemes and the polychotomous type is characteristic of later inflorescences.

The simple raceme is due to a single gene, which is completely dominant (22). Occurrence of this dominant character was found by Bouquet (10) in all common varieties studied with the exception of Earliana. This dominance may be altered by light, temperature, nutrition (17, 70, 111, 118, 127, 134, 135, 137) and chemicals, such as triiodobenzoic acid and N-m-tolylphthalamic acid (34, 35, 36, 109, 110, 139, 140, 141). Detailed discussion of these will follow.

Tomato flowers are perfect, hypogynous and regular; and except in cultivated varieties, the number of floral parts in each whorl is variable (4, 10, 20, 51, 87). An individual flower consists of: a short calyx tube terminated by 5 to 6 lobes; a corolla also having a short tube which expands into five or more lobes; stamens having short filaments, with anthers joined laterally to form a hollow cone around the pistil; and a pistil consisting of two to several carpels with stigma and style extending through the encircling

androecium (stamens) (44). The sequence of floral whorl differentiation follows a centripetal pattern. The calyx (sepals) is the first to differentiate, followed by the corolla (petals), stamens and finally pistils, respectively. Primordia of each whorl alternate in position with the adjacent whorl (20). As with the development of the inflorescence, the structure of the floral parts is also modified by environmental conditions and the nutritional status of the plants (13, 49, 50, 51, 100, 102, 123).

Tomato flowers were found to be hexamerous by some investigators (20, 51) and pentamerous by others (4, 38). A recent paper by Palmer (87) dealing with varieties Ailsa Craig and Harbinger indicates the number of sepals and petals can vary from 5 to 8. The number of anthers varies directly with the number of sepals (87). The first flower on the truss often has seven sepals and petals and multiple locules. This condition in its extreme form is referred to as fasciation. Fasciation of floral parts seems to occur in the tomato inflorescences quite frequently. Some investigators (18, 103) have discussed it as an abnormality. The flowers were described as being very large with numerous sepals, petals and stamens; the styles were fasciated in the form of a hollow cylinder in which more whorls of petals and sepals arose (18).

White (129) suggested that "The basic cause of fasciation is a disturbed metabolism, involving excessive nutrients which mobilize energy that must be

utilized. This energy once accumulated, must go into growth, and it becomes 'widely' expanded into extravagant, abnormal and unpredictable tissue production, generally to the detriment of the plant. Various agencies, innate and external, bring this about."

Fasciation of tomato flowers is due to a recessive gene (138). Crosses between homozygous fasciated and nonfasciated lines, yielded, in the F_1 generation, non-fasciated flowers. However, the average F_1 locule number always exceeded the average number of the non-fasciated parents. In 36 varieties studied, Zielinski (138) found a definite trend toward lower locule number in later formed inflorescences.

LIGHT

While the photoperiodic control of floral induction in long and short day plants has been extensively studied, little is known of the role of light on flowering of a large group of plants broadly classified as day neutral. Allard (1) studying the flowering behavior of several day neutral plants found partial to complete inhibition of flowering when the photoperiod was too short or too long (shorter than 12 and longer than 16 hours). Thus, it is apparent that these plants could not be considered day-neutral, since they require an intermediate day-length. The tomato, Lycopersicum esculentum, which shows no inductive response to long or short photoperiods, has been classified as one

of this less explored group. Nevertheless, the number of primordia initiated and the rate of floral differentiation within a tomato inflorescence depend a great deal on photoperiod and photointensity (95). Tomato plants produce small and weak inflorescences in the fall and winter months which ultimately lead to lower yields (46, 132). Short days and low light intensity are the suggested causes (95). Reinders-Gouwentak (95) found a delay of tomato flower initiation (in terms of leaf number and number of days) and retardation of primordial initiation within an inflorescence when a base photoperiod of 9 hours (high tension Mercury lamp HO 2000) was extended with a period of low light intensity. On the other hand, if the base photoperiod of the same intensity was 7 hours, a short duration of low light intensity (4 1/2 hours or less) gave earlier initiation, and more primordia were evident within the inflorescence during early development than without the added low light period. Since the total flower number was not changed by photoperiod, it would appear that the rate of flower initiation was affected. These data indicate that the tomato is a plant requiring an intermediate day-length for optimum flowering.

Morphologically, photoperiod also affects the rate of vegetative growth in tomato (95). More leaves were formed before initiation of the first inflorescence with a base photoperiod of 7 1/2 hours than with one of 12 hours. However, if 2 hours of incandescent or red light were used to supplement these base photoperiods (7 or 12 hours), the results were reversed. It would

appear that when a photoperiod is insufficient to meet the flowering requirement of a plant, extension of the photoperiod enhances reproduction. On the other hand, if the photoperiod has met this requirement, an extension of the photoperiod may inhibit flowering (95).

Learner and Wittwer (65) were able to reduce the time to first anthesis and harvesting of ripe tomato fruit 2 to 3 days in winter months when the normal day length was lengthened to 16 hours with a low light intensity of 300 f. c. However, Wittwer and Teubner (134) found a delay (in terms of node number) in initiation of the first flower cluster under similar conditions. The extent of flower initiation and polychotomy within an inflorescence of the tomato plants was increased by high light intensity and extra illumination (70, 118). With increasing light intensity, the number of leaves subtending the inflorescence decreased (23, 64, 118).

Under short days and low light intensity, the over-all vegetative growth of tomato plants is retarded (83, 95, 118). Chemical analyses show tomato plants receiving a short photoperiod of low intensity have a lower carbohydrate content than plants with either a long photoperiod or higher light intensity (3, 92, 99, 126). Went (125) suggests "The light process with an optimum temperature of 26°C which controls growth during the day is probably photosynthesis."

In addition to affecting morphology of the tomato inflorescence, photo-

period and photointensity also modify the development of individual flowers. Under short day and low light intensity, the flower develops an extremely long pistil in relation to the stamens and this interferes with normal self-pollination (13, 51, 101). This may well be a contributing factor affecting flower abscission in tomato. Johnson, Hall and Livermann (54) showed that far-red radiation accelerates abscission of first formed flowers and reduces the number of late formed flowers, and sometimes produces unfertilized fruits.

TEMPERATURE

Roberts (96) after observing the performance of numerous plants suggested that "The temperature during the night rather than the day largely determines the type of response which the plants make to temperature." Phaenopsis plants failed to develop in constant 26.5°C temperature, but grew normally when the night temperature was lowered to 20°C (122). Cosmos grown at day and night temperature of 26.5° and 19°C, respectively, weighed twice as much as those kept constant at either of those two temperatures (7). Flowering of Dendrobium crumentum did not occur until the plants were exposed to a cold temperature (21, 58).

Went (124) found that a diurnal fluctuation of temperature, "thermoperiodicity", was of paramount importance in the development of the tomato plants. With the variety San Jose Canner, there was a gradual shift of optimal

night temperature for growth from 30°C in the seedling stage to 18°C in the early fruiting stage (126). There was definite relationship between temperature and light. The optimum night temperature increased with increasing light intensity (126). Hoffman (47) working with Globe, Strain A, tomatoes under Ohio greenhouse conditions suggested that the night temperature be kept around 15 to 16°C, and raised to 21 to 24°C during sunny days.

Attempts to shorten the ripe-to-flower period by vernalizing germinating tomato seeds have yielded inconsistent results. Burr and Turner (14) found reduction in germination proportional to length of cold treatment. The vernalized seeds lagged behind the control in growth, fruiting, time of ripening and yield of fruit. In a subsequent experiment (116), however, they were able to obtain earlier flowering and ripening from vernalized seeds provided the seedlings were given 12 days of continuous light. Stier (104) came to essentially the same conclusion that continuous light accelerated flowering when seeds were exposed to a short cold period (15 days). This accelerating effect was nullified by prolonged low temperature (32°F) treatment. Stier concluded that vernalization was not important for early flowering, fruit ripening and did not affect total yield.

Goodall and Bolas (33) found an increase in flower number in the first few flower clusters from vernalized seeds, but not earlier ripening of fruit. Junges (55) and Sutov and Beljaev (107) obtained higher yield from vernalized

seeds and found continuous chilling (3° C) for 10 to 15 days was particularly favorable for earlier yield. However, when Calvert (17) later repeated Junges' experiment, he could not reproduce these results. Similarly, Wittwer and Teubner (137) were unable to observe any effects of seed vernalization on flowering.

While tomato seeds are not consistently responsive to vernalization, diurnal temperature fluctuation during seedling and plant growth does have striking effects. Went (124, 126, 127) observed that tomato plants grown at 78° and 55° F day and night temperatures, produced inflorescences that were more branched and had greater flower numbers than plants at lower day temperature accompanied by higher night temperature. Verkerk (118) suggested this temperature fluctuation favored earliness and increased yield. However, a differential of 10° F or more between night and day temperature may retard growth and reduce yields (118).

Lewis (70) has shown that the temperature sensitive period of tomato plants with respect to increased flowering is between cotyledon expansion and initiation of first flower primordia. Cold treatment of 13° C during this period will induce a larger first inflorescence, an effect which may carry over to the fifth inflorescence.

A series of papers from the John Innes Horticultural Institute (61, 62, 63, 64) report a decrease in nodes to first inflorescence accompanied by in-

creased flower number in tomato palnts exposed to low temperature (55°F) at the seedling stage.

Wittwer and Teubner (134, 135, 136, 137) and Kurki and Wittwer (57) confirmed that the number of nodes subtending the first inflorescence was less and the number of flowers in the first cluster greater when tomato seedlings were exposed for 2 to 3 weeks to a temperature of 50 to 55°F, as compared to plants grown at 65 to 70°F. They further concluded that day exposure to cold treatment is as effective as night exposure. It is the accumulated cold treatment that determines the node (number of nodes or leaves subtending the first inflorescence) and flower number, not the pattern of cold exposure. This is in agreement with conclusions of Went (126) and Calvert (17).

Kramer and Went (56) found that the auxin content of tomato stem tips increased with age and when plants were exposed to low (46°F) as compared with high temperature (72°F). They concluded on the basis of the diffusion coefficient and stability in acid and alkali that auxin obtained from tomato stem tips is mainly 3-indoleacetic acid with perhaps in admixture of a small amount of an auxin of higher molecular weight. Other workers, using paper chromatographic techniques, have been unable to detect 3-indoleacetic acid in tomato roots (11), stems (32), leaves (119) or fruits (85). Unidentified non-indole auxins of tomato roots and fruits (11, 85), however, were active (6) in stimulating Avena straight growth.

The increase of flower number in an inflorescence is directly related to early yield of tomato fruit (134). Although a delay in growth and flowering is observed in cold exposed seedlings (57), accelerated growth of these plants, when the night temperature is raised, often overcomes this initial delay.

Calvert (16) in a recent report stated that tomato plants exposed to cool soil temperature, 57 to 61°F, gave increased early yields. Teubner and Wittwer (111) have shown that while top temperature controls the time of initiation of the first inflorescence, it is the root temperature that influences flower number.

Temperature also has an effect on the development of floral parts of the tomato inflorescence. Went (104) found at a low night temperature (54 to 59°F) tomato flowers showed phyllody of the calyx and had a greater tendency to fasciate. High temperature accompanied by low humidity caused styles to develop abnormally (102). The styles elongated several days before anther dehiscence and consequently no self-pollination could occur. Germination of pollen was best at 75 to 85°F, and 70°F was most favorable for pollen tube growth (102). According to Smith (100, 102) the stigmatic area tends to dry out faster under higher temperatures, and low humidity and is non-receptive to pollen germination. Flowers developing under such conditions usually abscissed.

CHEMICALS

Cajlachjan (15) was the first to suggest that a chemical substance ("hormone") was responsible for photoperiodic induction. It was later demonstrated by Withrow and Withrow (130) that the flowering stimulus could be obtained when a photo-induced leaf was grafted to a non-induced plant. Isolation of this stimulus, "florigen", has not been accomplished (59).

Numerous experiments have demonstrated that application of auxin can alter the flowering pattern in plants. Leopold (67) in a recent review suggested that auxin is capable of modifying flowering in four ways: (a) Earliness in flowering from seed treatment; (b) promotion of flowering; (c) delay in flowering; and (d) altering flowering morphology.

As with vernalization treatment, auxin treatment of tomato seeds has not proven of definite value since the results obtained are inconsistent (5, 105, 108, 114). One of the earliest reports of promotion of flowering was that of Cholodny (19). Thimann and Lane (114) and Stier and DuBuy (105) reported hastening of anthesis and an increase in the flower number when tomato seeds were treated with naphthaleneacetic acid and indoleacetic acid (100 ppm). Tang and Loo (108) found hastening of flowering in indoleacetic acid (1-100 ppm) treated tomato, rice and mustard seeds. They also observed accelerated vegetative development of these plants. Barton (5) found

no beneficial effect in treatment of tomato and a number of other seeds with auxins (naphthaleneacetic acid and indolebutyric acid, 1-300 ppm).

Teubner and Wittwer (110) increased the number of tomato flower buds in the first inflorescence by treating the young seedlings with indoleacetic acid. Similar results were also obtained with other compounds which are not yet classified as typical auxins. Triiodobenzoic acid, which has been variously considered as an auxin (79), an anti-auxin (3) and an auxin synergist (115), increased the number of flower primordia in tomato and bean plants (30, 34, 35, 36, 139, 140). Osborne and Wain (86) found α (2-naphthoxy) phenylacetic acid produced similar responses as those tomato plants treated with 2, 3, 5-triiodobenzoic acid. However, triiodobenzoic acid was quantitatively more active than α(2-naphthoxy) phenylacetic acid in respect to flower numbers in the inflorescence (86). N-m-tolylphthalamic acid and other N-aryl-phthalamic acids increased flower number in the tomato (109, 110, 111, 113). The effect of N-m-tolylphthalamic acid on flower number is particularly noticeable in higher early yields of greenhouse tomato fruit when the environmental conditions are unfavorable for flowering (109, 110). Bukovac, Wittwer and Teubner (12) found an increase in node number to, and fewer flowers in the first inflorescence of the tomato plants treated with gibberellin. However, growth of the treated plants was greatly accelerated and flowering of the first inflorescence occurred earlier than with the non-treated plants (12).

The time and concentration of auxin or chemical application are apparently most critical in the type of response a plant may show. Hemphill (45) has pointed out: "To obtain the desired results from hormone treatment, not only must the proper concentration of a specific chemical be used, but the application must be accurately timed. This can not be over-emphasized. A given concentration may retard growth at one stage of development, while later it may accelerate it. There may even be a stimulating effect on one part of the plant and an inhibiting effect on another." The optimum time for N-m-tolylphthalamic acid application to affect the first inflorescence is nine days after cotyledon expansion of tomato seedlings and 18 days after cotyledon expansion for the second inflorescence (109, 110). The optimum concentration for maximum flower numbers without complete inhibition of the sympodial bud is 100 to 200 ppm (110).

EXPERIMENTAL

I. GENERAL METHODS

Plant Growing:

For uniform tomato plants, homozygous varieties of Ohio WR7 and Michigan-Ohio (F_1 Hybrid), mainly the latter, were used throughout the investigation. Seeds were germinated at 75 to 80° F in sterilized wooden flats containing vermiculite. After germination, the seedlings were transplanted to sterilized 4-inch clay pots of soil (1:1 muck and loam). The plants were maintained thereafter at a night temperature of 58 to 65° F. The seedlings were fertilized at transplanting with a solution of diammonium and monopotassium phosphate mixture (10:52:17) at a concentration of 1 ounce per gallon, and at a rate of one-quarter pint per pot. Subsequent fertilization procedures were the same as outlined by Wittwer and Teubner (136).

Chemical Treatment:

N-m-tolylphthalamic acid or Duraset-20W[1] was furnished by the United States Rubber Company, Naugatuck Chemical Division, of Naugatuck, Connecticut. N-m-tolylphthalamic acid solutions were applied at the 2-3 leaf stage (Figure 1c) of seedling development, at which time flower number

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[1] Duraset-20W is a trade name for N-m-tolylphthalamic acid. It is a wettable powder containing 20 percent N-m-tolylphthalamic acid. This formulation has wetting and suspending properties.

in the first inflorescence could be most significantly affected. The seedlings were sprayed to the drip point, unless otherwise specified, with a "Sure-Shot" self-contained compressed air sprayer[1] at 100-pound pressure. Thirty to forty days after transplanting the plants were transplanted to sterilized 6-inch pots of the same soil mixture.

Histological Preparations:

In histological studies the freshly collected meristems were killed and fixed in 70 percent FAA[2] solution. The tissue was then dehydrated using the tertiary butyl alcohol method described by Johansen (53). The material was then infiltrated with several changes of Matheson Histowax and embedded in Fisher tissuemat. The embedded material was cut longitudinally into sections of 10 to 12 microns thick with a model 820 American Optical Company rotary microtome. The sections were mounted in series on standard non-corrosive half-white glass slides with Haupt's adhesive smear and drops of 3 to 4 percent formalin solution (53). The tissuemat was removed from the sections with xylol, and the sections stained with safranin and fast green (53). The slides were mounted with piccolyte[3] and covered with 50 x 24 millimeter Fisher No. 1 micro-corning glass covers. They were dried in a 40°C oven

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[1]Milwaukee Sprayer Manufacturing Company, Milwaukee, Wisconsin.

[2]FAA - Formalin (37 to 40 percent formaldehyde): Glacial acetic acid: Ethyl alcohol (70 percent), 5: 5: 90 milliliters.

[3]Turox piccolyte dry resin dissolved in xylol having the same refractive index as balsam or clarite.

for a week before cleaning and microscopic examination. All microscopic examinations were made with a Bausch and Lomb Optical Company LB8982 microscope.

Photomicrographs were taken with an Exacta VX camera using a microscope adapter with a light source from a 31-33-26 Bausch and Lomb microscope lamp with a ground glass filter (39375) and a blue glass filter (39370) placed between the light source and the substage mirror of the microscope. Kodak Plus X film was used with an exposure B and time of 1/50 second for taking black and white photomicrographs.

II. HISTOLOGICAL AND MORPHOLOGICAL CHANGES IN THE DEVELOPING TOMATO INFLORESCENCE FROM VARIOUS TREATMENTS

A. Cold Treatment

As mentioned in the literature review, low temperature (50 to 55°F) exposure of seedlings for 2 to 3 weeks after cotyledon expansion, reduces the number of nodes (leaves) subtending, induces branching, and increases the flower number in the first tomato inflorescence (17, 62, 63, 70, 124, 126, 134, 135).

Seeds of Michigan-Ohio F_1 Hybrid were germinated and the seedlings transplanted to 4-inch pots (Figure 1a). The cold treatment was imposed after transplanting and the cotyledons had expanded. The seedlings were then moved daily for 10 days into a darkened room (55±1°F) at 5 p.m. and out at 8 a.m. This provided a daily cold exposure of 15 hours and 9 hours of photoperiod. During the day the plants were held together with control plants in a cold-frame. The controls were covered with an opaque plastic at the time the cold treated plants were placed in the cold (55°F) room. The mean night temperature under the plastic cover was 72°F. Apical meristems were harvested in six replicates at cotyledon expansion and on alternate days thereafter. The meristems were collected at 8 a.m. from the control and the cold treated plants after they were removed from the cold room. A total

a b c

Figure 1. Stages of development of Michigan-Ohio F_1 Hybrid tomato seedlings.
a. Cotyledon expansion.
b. After 10 days in 55° F night temperature.
c. After 10 days in 72° F night temperature (2-3 leaf stage).

of six collections were processed for histological examination, according to methods outlined in the general methods section.

Figure 1a, 1b and 1c show stages of development of Michigan-Ohio Hybrid seedlings at cotyledon expansion, 10 days after cold (55°F) exposure and control plant (2 to 3 leaf stage) grown in 72°F for 10 days.

Histological examination of apical meristems of tomato seedlings at cotyledon expansion revealed that only the two plumule leaves had been initiated at this stage (Figure 2) in control plants. Two more leaves were initiated during the first two days after cotyledon expansion, and an additional leaf was initiated every two days thereafter (Table I).

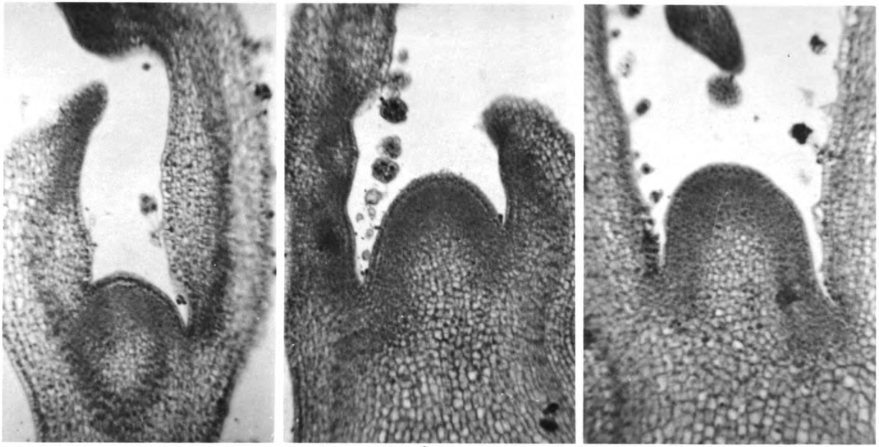
Plants receiving the cold treatment showed a similar pattern of development for the first two days, and then a slower rate of leaf initiation. Eight days after imposing the cold treatment, leaf initiation ceased, the apical meristem remained undifferentiated, and a transition from a vegetative to a reproductive apex began (Figure 2b, c). A total of six leaves were initiated in the cold treated plants prior to formation of the inflorescence. This transition occurred two days later in the control meristems, after approximately eight leaf initials had developed.

TABLE I

Rate of Leaf Initiation on Control and Cold Exposed Tomato Seedlings

Days from Cotyledon Expansion	Leaf Number	
	Control	Cold Exposed
0 (control)	2.0	2.0
2	4.0	4.0
4	5.0	4.5
6	6.0	5.9
8	6.8	6.0*
10 (2-3 leaf stage)	7.7*	6.3*

*Apical meristem changing from vegetative to reproductive.



a b c

Figure 2. Tomato apical meristems.

a. At cotyledon expansion (200X)
b. and c. Control and cold treated (55°F) meristems 8 days after cotyledon expansion, showing a vegetative apex and a reproductive apex, respectively (200X).

B. N-m-Tolylphthalamic Acid Treatment

Teubner and Wittwer (109, 110) reported that application of N-m-tolylphthalamic acid to tomato seedlings at the 2- 3 leaf stage, or approximately 9 days after cotyledon expansion, will increase the number of flower buds in the first inflorescence.

A preliminary study of various N-m-tolylphthalamic acid concentrations on tomato flowering was conducted using the variety Ohio WR7. The seedlings were sprayed at 2-3 leaf stage using Duraset-20W at 0, 50, 200 and 500 ppm concentrations. Each treatment had six single plant replicates. Measurements were taken on the following:

1. Number of foliar injuries
2. Formative effects:
   (a) Vegetative termination
   (b) Fasciated flowers
3. Number of nodes to first inflorescence
4. Height to first inflorescence
5. Number of buds in first inflorescence
6. Number of branches in first inflorescence

Results are presented in Table II. The number of nodes subtending the first inflorescence was significantly decreased by the 500 ppm treatment. This

TABLE II

The Effect of N-m-Tolylphthalamic Acid on Development of the First Inflorescence of the Ohio WR7 Tomato Variety

Concentration (ppm)	Node[1] (No.)	Height (Ins.)	Flower (No.)	Branch (No.)
0 (control)	9.2	10.3	5.3	1.0
50	9.5	9.5	8.8	2.5
200	9.0	9.2*	14.6**	4.0
500	7.5	8.7*	16.6**	4.4

*, ** Significantly different from control at 5 and 1% level, respectively.

[1] Nodes subtending the first inflorescence.

had not been noted in other experiments and may be an anomaly. Treated plants (200 and 500 ppm) were significantly shorter, and flower and branch numbers in the inflorescences significantly greater. Figures 3 and 4 show a typical simple raceme, as well as di-, tri-, and polychotomous inflorescences from control and treated plants, respectively.

Of the plants treated with 200 ppm, 17 percent exhibited vegetative termination, and 50 percent were terminated by the 500 ppm concentration. This termination resulted from suppressed development of the sympodial bud below the first inflorescence. Figure 5 illustrates a plant vegetatively terminated by treatment of 500 ppm. There was no observable foliage injury in any treatment; however, all treated plants had an average of two fasciated flowers (individual floral whorl failed to diverge). The degree of fasciation increased with increasing concentration of N-m-tolylphthalamic acid.

Histological studies were made of meristems from Michigan-Ohio Hybrid seedlings treated with 200 ppm N-m-tolylphthalamic acid at the 2-3 leaf stage. Meristems of plants were collected one day after treatment and every other day thereafter until 11 days after treatment, making a total of six collections, for histological examination. The histological procedures and photomicrography were as previously described under general methods. Drawings of the tomato apical meristems of the various stages of development

Figure 3. Control and N-m-tolylphthalamic acid sprayed tomato inflorescences showing from left to right and top to bottom a simple raceme (control), dichotomous, trichotomous and polychotomous branching.

Figure 4. An intact polychotomous tomato fruit cluster resulting from N-m-tolylphthalamic acid treatment.

Figure 5. A tomato plant, terminated at the first inflorescence due to N-m-tolylphthalamic acid treatment.

were reconstructed from serial microsections using a Victor V-200 model magnascope.

Examinations of advanced stages of inflorescence development 15 days after treatment were carried out by dissecting the meristems under a low power binocular microscope. Glycerine was applied to the tissue to prevent drying during dissection and examination. Free-hand sketches were made of these meristems.

Although efforts were made to select uniform plants, microscopic examinations indicated wide variations (Figure 6a, b, c, d). The meristems varied from one undifferentiated flower primordium to 2-3 primordia plus a sympodial bud at time of treatment (2-3 leaf stage).

Treated and control meristems showed no differences in development the day after treatment with N-m-tolylphthalamic acid and none had advanced beyond the stage represented by Figure 6c, d. A slight difference was apparent three days after treatment when the sympodial bud of the control was slightly larger than the treated plant (Figure 6e, f). Five days after treatment a difference in flower bud development was obvious (Figure 6g, h). In the non-treated meristem, sepals and petals of the first formed flower buds had differentiated, and more new buds had initiated. While the flower buds in the treated meristems increased in size, they remained undifferentiated, and no new buds were being initiated.

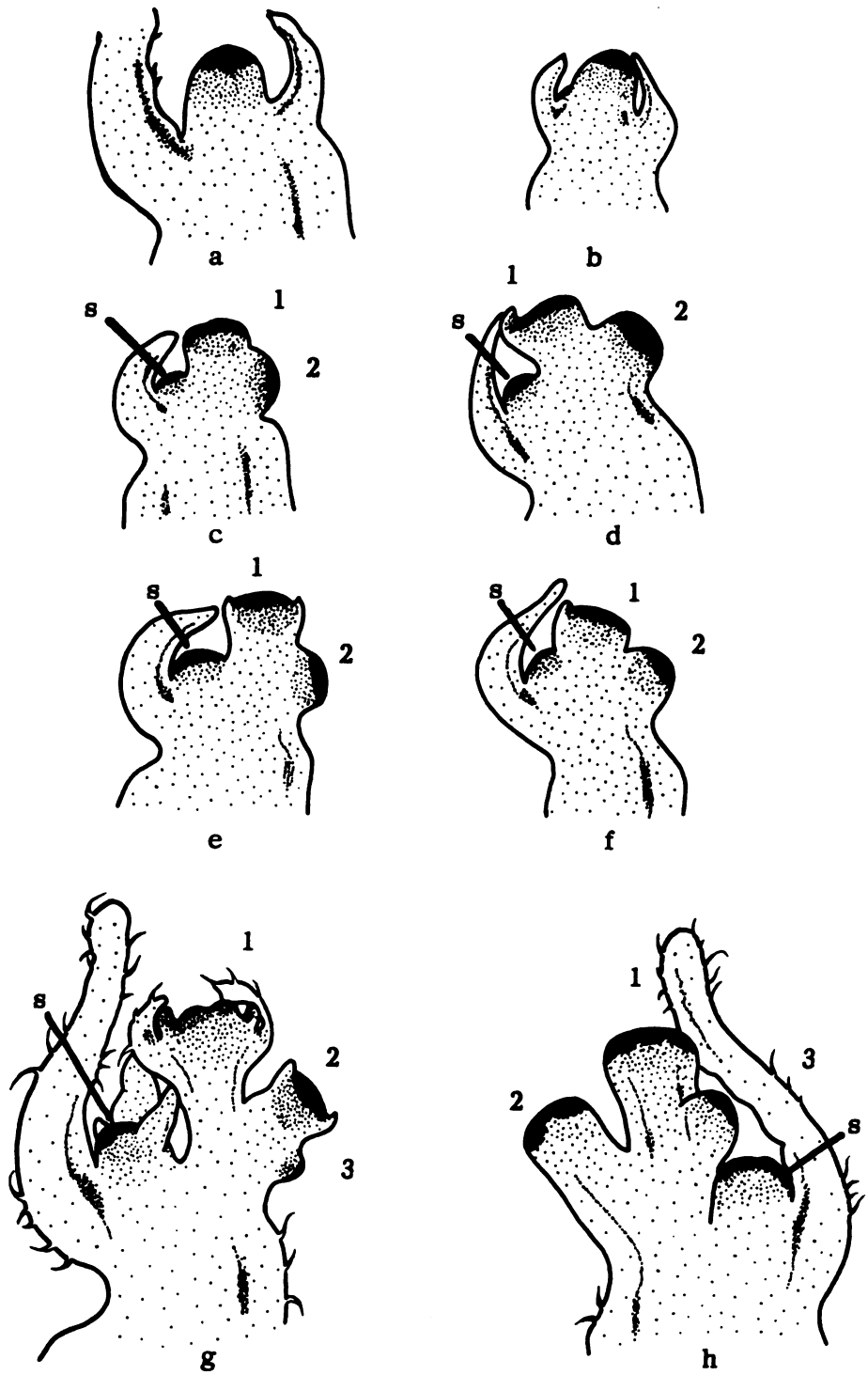


Figure 6. Differentiation of control and N-m-tolylphthalamic acid treated tomato meristems.
a, b, c, d. Variation of development at time of treatment (65, 55, 51, 55X).
e, f. Three days after treatment, non-treated and treated meristems, respectively (55X).
g, h. Five days after treatment, non-treated and treated meristems, respectively (42, 54X).
Key: 1, 2, 3 - sequence of floral primordia formation; s - sympodial bud.

In meristems of non-treated plants there was a day lapse between initiation of a flower bud and differentiation of floral parts. This period was extended to six days in the N-m-tolylphthalamic acid treated meristems.

Seven days after treatment, the undifferentiated flower primordia of the treated meristem began to subdivide into several smaller primordia (Figure 7, b). Flower primordia initiation in the first inflorescence was complete in the control meristems of this age and little or no further flower initiation in the inflorescence took place (Figure 7a, c; and Figure 8a).

Multiple primordia formation became more apparent in the treated meristems by the 9th day (Figure 7, d). The individual primordia developed into simple racemes (Figure 7, e and Figure 8, b), and the matured flower cluster was a polychotomous inflorescence (Figures 3, 4).

Development of the sympodial bud on treated plants was retarded. Seven days after treatment the sympodial bud of the control plants had developed three young leaves, a second inflorescence containing two primordia, and a second sympodial bud (Figure 7, a). The sympodial bud of the treated meristems produced three leaves and remained undifferentiated nine days after treatment (Figure 7, d), while the control had floral whorl differentiation in the second inflorescence.

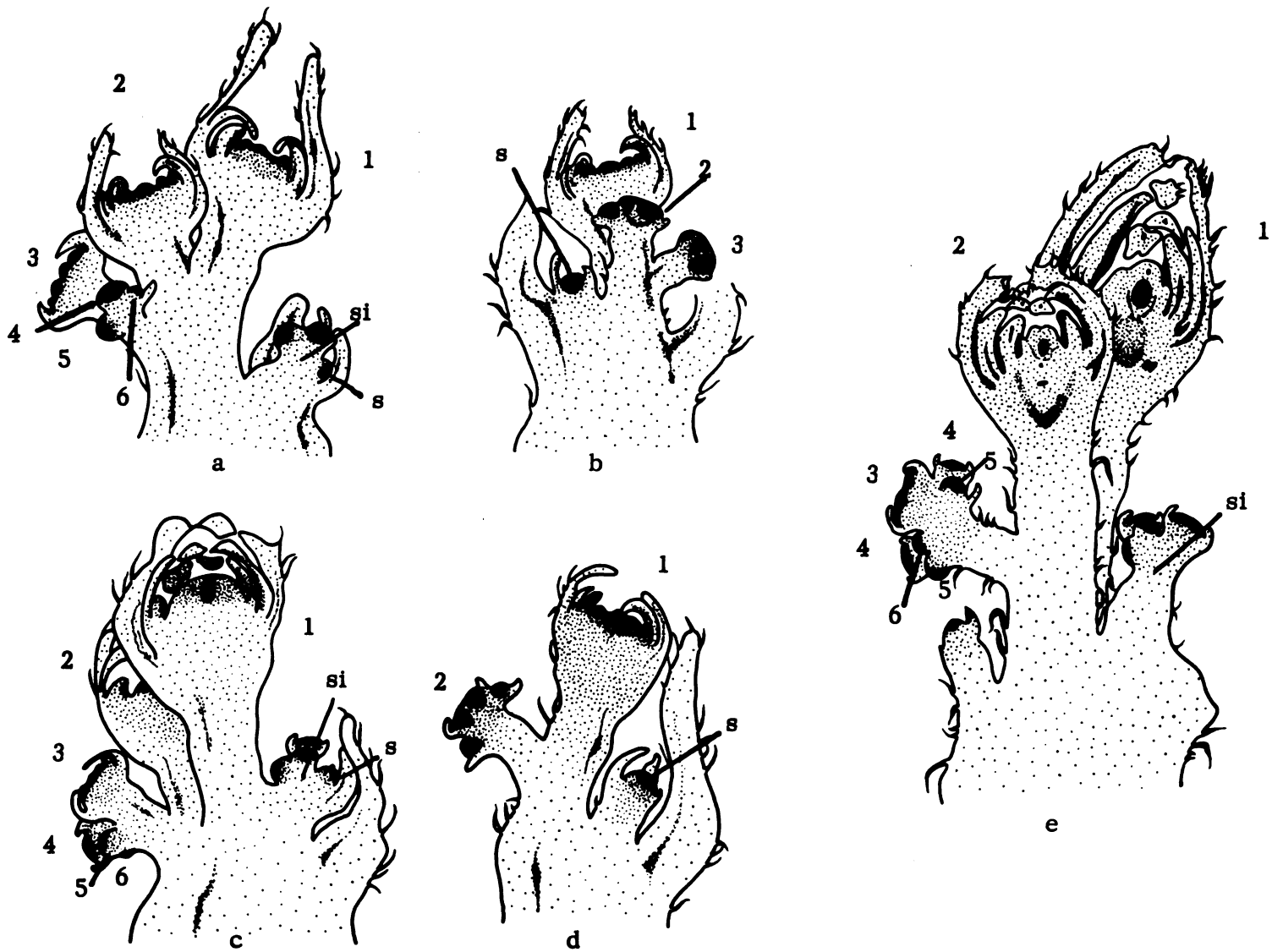


Figure 7. Differentiation of tomato floral primordia.

a, b. Seven days after N-m-tolylphthalamic acid treatment, control and treated meristems, respectively (34X).

c, d. Nine days after treatment, control and treated tomato apical meristems, respectively (25, 49X).

e. Eleven days after treatment, treated meristem (29X).

Key: 1, 2, 3....., sequence of floral primordia formation; s, sympodial bud; si, second inflorescence.

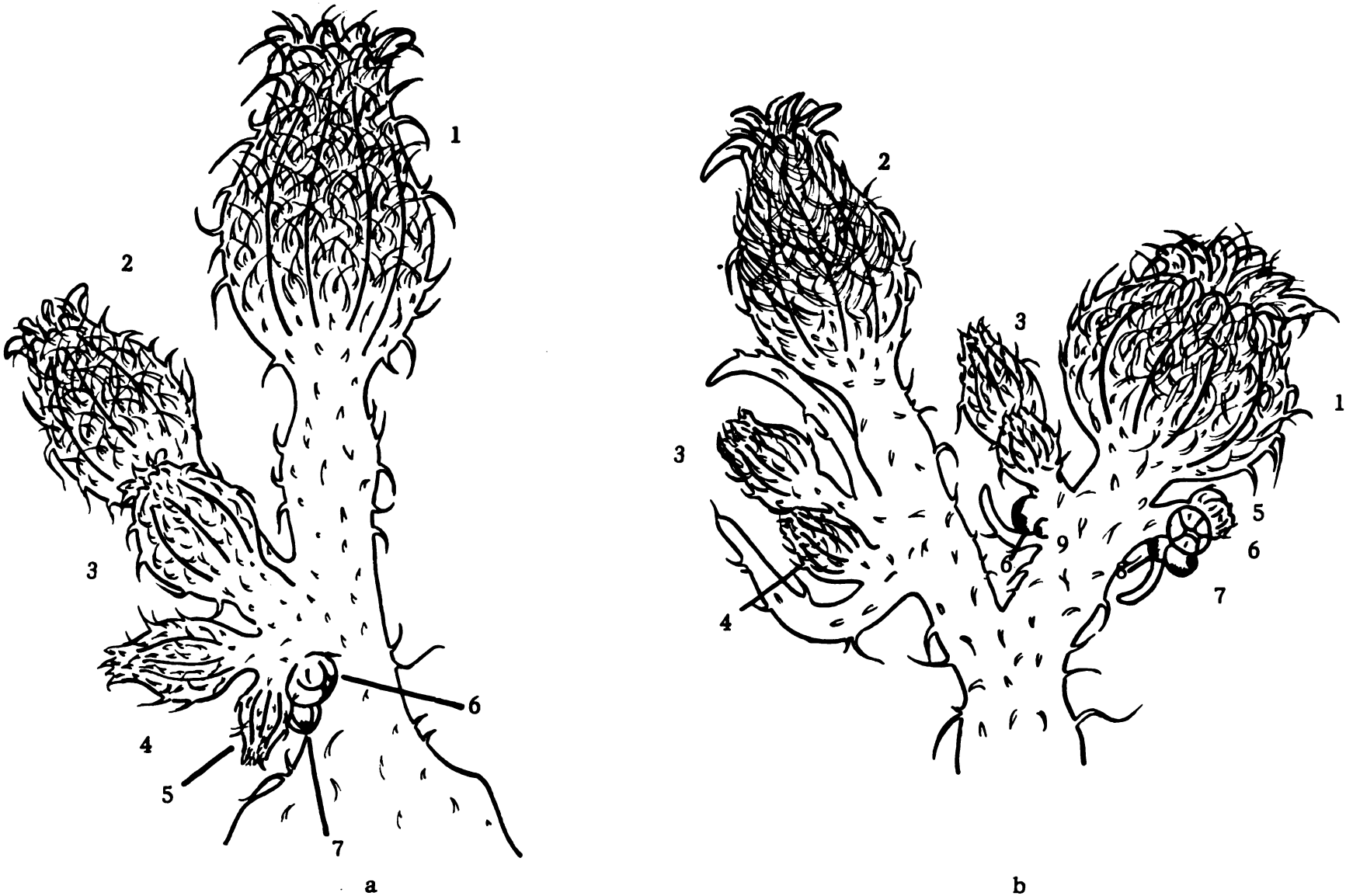


Figure 8. Tomato inflorescence. a, b. Free-hand drawing of non-treated and N-m-tolylphthalamic acid treated tomato inflorescences fifteen days after treatment (33X).

Key: 1, 2, 3..... sequence of floral primordia formation.

C. Defoliation

DeZeeuw (24) has suggested that the young leaves of the sympodial bud inhibit development of the inflorescence. By removing these leaves, he was able to obtain an increased flower number in the first inflorescence. He explains the enhanced flowering obtained with N-m-tolylphthalamic acid as an indirect response resulting from an accelerated maturation effect of the chemical on these leaves. Two possible explanations were given for this phenomenon. First, the young leaves contain a specific inhibitor of flowering. Second, the young leaves may compete successfully over the inflorescence for available auxins or nutrients.

A defoliation experiment was set up in the greenhouse in spring to observe the leaf-inflorescence relationship of the tomato plants. Michigan-Ohio Hybrid seeds were germinated and transplanted to 4-inch pots. Defoliation began at the 2-3 leaf stage (Figure 1, c) by removing:

1. All leaves below the first inflorescence.
2. All leaves above the first inflorescence (sympodial bud).
3. All leaves above (sympodial bud) and below first inflorescence.

Each treatment consisted of 10 single plant replicates. Flower and branch number were counted and observations of plant development of each treatment were made at intervals.

The results presented in Table III and Figure 9 confirm those of DeZeeuw (23, 24); namely, that removal of young leaves above the inflorescence promoted flowering in the inflorescence.

Removing leaves above the inflorescence (sympodial bud), and both above and below the inflorescence increased the flower number in the inflorescence significantly. Removing leaves below the inflorescence had no effect on flower number or branching, but did result in abnormal flower development.

When leaves below the inflorescence were removed, the plants developed very short internodes. As compared to the control plants, cotyledons were twice as large. The diameter of the stem was greater. There was no increase or decrease of node number to the first inflorescence, nor was the time of appearance of the inflorescence hastened or delayed. Development of the inflorescence was incomplete and both peduncle and pedicels failed to elongate. The flowers had elongated sepals and petals, stamens and pistils were retarded. No fruit-set was obtained from these flowers. However, leaves, stems and subsequent inflorescences above the first inflorescence developed normally.

Removing leaves above and below the inflorescence gave plants the same general characteristics as those having only the lower leaves removed. The cotyledons were larger and pedicels and peduncles longer (still shorter than control plants). Polychotomy and increased flower number were observed. A few flowers set fruit. The time of fruit-set, however, was much delayed.

Figure 9. Tomato defoliation experiment. Left to right: upper (sympodial bud) and lower leaves removed, upper (sympodial bud) leaves removed, and lower leaves removed.

TABLE III

The Effect of Defoliation on Tomato Flowering

Leaf Removal	Number of Flowers	Number of Branches
None	5.7	1.0
Lower	5.0	1.2
Upper (sympodial bud)	10.0**	1.4
Upper and lower	10.7**	2.1

**Significantly greater than when no leaves were removed (1% level).

D. N-m-Tolylphthalamic Acid Treatment and Removal of Sympodial Bud

DeZeeuw (24) found no increase in flower number when N-m-tolylphthalamic acid was applied to the cut surface after removal of the sympodial bud. An objection to DeZeeuw's conclusion that N-m-tolylphthalamic acid is effective only in the presence of the sympodial bud is that a single concentration equivalent to 10, 000 ppm (1 percent in lanolin) was employed. That this concentration may have resulted in inhibition of flower development when applied in such close proximity to the floral apex is suggested by some unpublished observations of Teubner and Wittwer (112). Furthermore, if DeZeeuw's hypothesis, that the young leaves of the sympodial bud of the tomato plant produce an inhibitor which retards the development of the subjacent inflorescence, is correct, then simultaneous removal of the sympodial bud and treatment with N-m-tolylphthalamic acid should give an increase in flower number above that obtained by removing the sympodial bud alone.

Michigan-Ohio Hybrid seedlings were sprayed with 0, 50, 100, 200 and 500 ppm N-m-tolylphthalamic acid at the 2-3 leaf stage (Figure 1, c). Seedlings were treated in 20 single plant replications. Sympodial buds were removed from half of the replications at each concentration. Flower and branch number in the first inflorescence from both sets of treatments (sympodial bud removed and non-removed) were counted after the inflorescences had reached full development.

The results presented in Table IV show that when the tomato plants were treated with N-m-tolylphthalamic acid, removal of the sympodial bud showed no statistical increase of flower numbers over the intact plants. Typical increases in flower numbers were obtained on N-m-tolylphthalamic acid treated plants. Thus, the removal of the sympodial bud bears no direct relationship to the effectiveness of N-m-tolylphthalamic acid.

TABLE IV

The Effect of N-m-Tolylphthalamic Acid Treatment and Sympodial Bud Removal on Flower and Branch Numbers in the Tomato First Inflorescence

N-m-Tolylphthalamic Acid (ppm)	Intact Plant		Sympodial Bud Removed	
	Flowers	Branches	Flowers	Branches
0 (control)	5.7	1.1	5.9	1.0
50	10.6**	2.0	12.9*	2.3
100	12.5**	2.2	11.0	2.1
200	10.0	1.8	12.3*	2.0
500	21.0**	4.0	20.9**	4.4
Mean	11.96	2.22	12.60	2.36

*, **Significantly greater than control at 5% and 1% level, respectively.

III. SITE OF ACTION OF N-m-TOLYLPHTHALAMIC ACID

These studies were undertaken with two objectives in mind. The primary concern was to establish a concentration or amount of N-m-tolylphthalamic acid which, applied to the primary (plumule) leaves, would induce a specific flowering response. This was in preparation for absorption and distribution studies employing C-14 labeled N-m-tolylphthalamic acid. The second objective was to determine if direct application to the terminal meristem would induce a greater response than application to other distal parts of the plant. This was of particular interest in view of the report of Teubner and Wittwer (111) that root temperature of tomato seedlings during the low temperature treatment controls flower number.

Michigan-Ohio Hybrid seedlings were treated at the 2-3 leaf stage (Figure 1, c). Single drops of Duraset-20W solution at concentrations of 200, 500 and 1,000 ppm were applied to either the meristem, a cotyledon or a plumule leaf. A 1-milliliter tuberculin syringe, fitted with a No. 27 stainless steel hypodermic needle, was used to apply the solutions, and when held at a 90 degree angle delivered 180 drops per milliliter. The quantity applied to each plant, therefore, was 1.1, 2.8 or 5.6 micrograms for the three concentrations. There were ten single plant replicates of each treatment. Measurements consisted of (1) number of nodes to the first inflorescence;

(2) number of flowers in the first inflorescence; and (3) the number of branches in the first inflorescence. These results are presented in Table V.

A quantity of 1.1 or 2.8 micrograms of N-m-tolylphthalamic acid applied to the meristem gave a significant increase in flower numbers in the first inflorescence. Application of these amounts to either a cotyledon or plumule leaf gave no increase in flower numbers. The reduced response with the 5.6 micrograms on the meristem was probably the result of mechanical difficulties in syringe operation as a consequence of the large amount of talc diluent present in the formulated chemical, Duraset-20W.

No vegetative termination of chemically treated plants was observed in this experiment, nor was the node number subtending the first inflorescence changed.

Failure to achieve a flowering response from application to the cotyledon or plumule leaf was indicative that either the chemical was not absorbed, not transported out of these organs, or that an insufficient quantity had been applied. To obviate the difficulties encountered with the talc diluent of the formulated Duraset-20W, a sample of pure N-m-tolylphthalamic acid was dissolved in a small quantity of saturated sodium bicarbonate and then diluted to the proper concentrations. Five, 10, 20 or 50 micrograms of N-m-tolylphthalamic acid were then applied to one of the plumule leaves of Michigan-Ohio Hybrid seedlings at the 2-3 leaf stage. The results in Table VI indicate

TABLE V

The Effect of Site of Application of N-m-Tolylphthalamic Acid on Tomato Flower Formation in the First Inflorescence

Micrograms Applied	Meristem		Cotyledon		Plumule Leaf	
	Flower (No.)	Branch (No.)	Flower (No.)	Branch (No.)	Flower (No.)	Branch (No.)
0 (control)	6.0	1.0	6.0	1.0	6.0	1.0
1.1	9.5*	1.8	6.6	1.1	5.8	1.0
2.8	11.3*	1.8	7.0	1.1	5.3	1.0
5.6	8.2	1.5	5.3	1.0	6.1	1.0

*Significantly greater than the control at 5% level.

TABLE VI

Effect of N-m-Tolylphthalamic Acid Applied as a Single Drop (0.01 ml) to One Primary (Plumule) Leaf on the Number of Flowers in the First Inflorescence of Tomato

Concentration (ppm)	Micrograms Applied	Flower Number
0 (control)	0	9.3
500	5	10.7
1000	10	13.3*
2000	20	14.7**
5000	50	17.8**

*, **Significantly greater than control at 5% and 1% level, respectively.

that the amount of N-m-tolylphthalamic acid applied to the plumule leaf required to induce a specific flowering response is ten times greater than that required when application is made directly to the meristem.

A second attempt to avoid the difficulties encountered with the talc diluent of the formulated Duraset-20W involved filtration of the suspensions to remove the talc. In this study, several additional methods of application were used and a range of five concentrations, or amounts, were selected which were anticipated to induce a specific flowering response for each method. The results presented in Table VII show inconsistent responses. It is possible that some of the active chemical was removed by filtration.

A final study was conducted of several methods of application with suspensions of the Duraset-20W. Frequent and vigorous shaking of the hypodermic syringe containing the suspension reduced the difficulties from needle plugging previously encountered. The concentrations and/or amounts applied using the various methods, together with the flower numbers in the first inflorescence are given in Table VIII.

It is apparent that drops of varying amounts or concentrations of Duraset-20W suspension directly on the terminal meristem increased flower number in the same manner as an overall plant spray. When only one of the plumule leaves was dipped, the concentrations necessary to elicit a given response were ten-fold higher than with either spraying or meristem treatment.

TABLE VII

The Effect of Method and Site Application of N-m-Tolylphthalamic Acid on Flower Number in the First Inflorescence

Spray Application (ppm)	Entire Plant	Plumule Leaf	Drop Application (micrograms)	Meristem	Plumule Leaf
0 (control)	5.7	5.7	-	5.7	5.7
25	5.9	-	-	-	-
50	6.5	-	0.28	7.2	-
125	9.4**	-	0.70	7.0	-
250	11.1**	6.4	1.40	7.9	-
500	14.2**	9.3*	2.80	9.3	-
1250	-	6.6	7.00	7.6	7.1
2500	-	8.7*	14.00	-	6.4
5000	-	9.7*	28.00	-	7.1
-	-	-	70.00	-	9.6*
-	-	-	140.00	-	12.7**

*, **Significantly greater than control at 5% and 1% level, respectively.

TABLE VIII

The Effect of Method and Site Application of N-m-Tolylphthalamic Acid on Flower Number in the First Inflorescence

Spray Application (ppm)	Entire Plant	Plumule Leaf	Drop Application (micrograms)	Meristem	Plumule Leaf
0 (control)	8.5	8.5	0	8.5	8.5
25	11.6*	-	-	-	-
50	12.6**	-	0.28	12.5**	-
125	14.0**	-	0.70	14.0**	-
250	13.9**	12.3**	1.40	16.4**	-
500	25.3**	13.8**	2.80	18.6**	-
1250	-	12.7*	7.00	23.8**	13.4**
2500	-	13.6**	14.00	-	12.3**
5000	-	14.5**	28.00	-	14.2**
-	-	-	70.00	-	14.0**
-	-	-	140.00	-	19.5**

*, **Significantly greater than control at 5% and 1% level, respectively.

A more direct comparison, where definite quantities were applied to either the meristem of one of the plumule leaves, showed that flowering increases can be obtained from meristem treatment with 1/50 amount needed if the plumule leaf is treated. Eighty percent of the sprayed plants (500 ppm) terminated above the second inflorescence. No node was apparent between the first and second inflorescences. Drops of solution containing 125 and 250 ppm of N-m-tolylphthalamic acid applied to the meristem resulted in 10 percent vegetative termination above the second inflorescence, with 40 percent at 500 and 1250 ppm. There were no nodes between the first and second inflorescences in the 500 and 1250 ppm drop treatments.

A preliminary study in which 10 milliliters of Duraset-20W solutions applied to the soil of pots with tomato seedlings at the 2-3 leaf stage showed no increase in flower number in the concentration range of 25 to 500 ppm N-m-tolylphthalamic acid. In view of the possibility of inactivation by either soil microorganisms or adsorption on the soil colloids, subsequent studies were conducted in which tomato seedlings were grown in solution cultures. At the 2-3 leaf stage of development, sufficient Duraset-20W was added to each 8-liter crock of nutrient solution to achieve final concentrations of 10, 100 and 1,000 ppm of N-m-tolylphthalamic acid. The results of this study, presented in Table IX, clearly show that the concentration employed was excessive.

TABLE IX

The Effect of N-m-Tolylphthalamic Acid in Solution Cultures on Tomato Flower Formation

Concentration in Solution Cultures (ppm)	First Inflorescence			Second Inflorescence		
	Nodes Subtending	Buds	Branches	Nodes Subtending*	Buds	Branches
0 (control)	7.9	6.0	1.1	3.0	5.9	1.2
10	8.6	10.5	2.7	1.6	10.6	2.3
100	8.0	4.6	1.0	1.3	8.0	2.7
1000	All seedlings died two days after treatment					

*Nodes between first and second inflorescences

Statistical analysis showed no significant difference in bud numbers between treated and control plants.

Seedlings treated with 1,000 ppm showed epinasty within an hour after treatment, and none survived longer than two days.

Thickening of nodes was observed at bases of petioles in the 100 ppm treatment. Plants were thin and spindly, and showed intense epinasty. Flower number in the first inflorescence was less than for the controls. Although a slight increase in flower number was observed in the second inflorescence, there was considerable variability and the differences were not significant. Abnormal development of flower buds resulting from a suppression of elongation of sepals, petals, stamens and styles was observed in many plants. Masses of partially differentiated young buds occurred in some of the inflorescences. Fasciation was severe in the developing flowers. Elongation of petals and stamens was retarded, so that the unopened buds were spherical rather than conical in shape and remained green after anthesis. Pistils failed to diverge and occurred in large numbers within a single flower. Vegetative termination was 100 percent above the second inflorescence.

Epinasty was detectable up to the sixth leaf in the 10 ppm treatment. All flower buds had short pedicels and the inflorescence developed as a tight cluster of flowers. Flower numbers in both the first and second inflorescences were greater than in the control. However, there was again considerable variability. Vegetative termination above the second inflorescence was 100 percent.

In a second experiment with concentrations of 0.1 and 1.0 ppm N-m-tolylphthalamic acid the plants showed no epinasty. The flower numbers in the first inflorescence of plants treated with 1.0 ppm were significantly less than non-treated plants (Table X). Although flower numbers were increased in both the first and second inflorescences by 0.1 and 1.0 ppm, the increases were not significant. There was considerable vegetative termination after the first inflorescence -- 50 percent at 0, 67 percent at 0.1, and 33 percent at 1.0 ppm. The extensive vegetative termination was probably a consequence of low night temperature (50°F during early development). The night temperature was adjusted to 60 to 65°F when the first inflorescence became macroscopically visible. As described in a previous experiment, the initial flowers in the first inflorescence showed abnormal development of corolla, stamens and pistils.

TABLE X

The Effect of Addition of N-m-Tolylphthalamic Acid to Nutrient Solution on Tomato Flower Formation

Concentration (ppm)	First Inflorescence			Second Inflorescence		
	Nodes	Buds	Branches	Nodes[1/]	Buds	Branches
0 (control)	7.8	8.6	1.8	3.2	10.3	2.2
0.1	8.1	10.3	2.4	3.2	13.2	2.8
1.0	8.2	5.4**	1.4	3.4	10.9	2.1

[1/] Nodes between first and second inflorescences.

**Significantly smaller than control at 1% level.

IV. ABSORPTION AND TRANSLOCATION OF N-m-TOLYLPHTHALAMIC ACID

It was established in previous studies that application to one plumule leaf required 70 to 140 micrograms of N-m-tolylphthalamic acid to produce a flowering response similar to that obtained with only 1 to 3 micrograms applied directly to the meristem. Since it appeared that the meristem was the primary site of action, application of C^{14} labeled N-m-tolylphthalamic acid to a plumule leaf should delineate the path of transport of the chemical to the meristem and permit determination of the threshold amount required to obtain increased flower numbers. Consequently, studies on the absorption, translocation and the subsequent distribution of N-m-tolylphthalamic acid applied to plumule leaves were outlined.

A. Absorption

Seedlings of Michigan-Ohio Hybrid tomato were treated at the 2-3 leaf stage (Figure 1, c) with varying quantities of Duraset-20W suspension having a concentration of 5,000 ppm N-m-tolylphthalamic acid. Either 30, 60 or 90 micrograms of the chemical were applied to each of the two plumule leaves using a 1 ml syringe fitted with No. 27 needle. The total amount received by each plant was, therefore, 60, 120 or 180 micrograms. Both treated leaves were removed immediately following application (zero time) or

thereafter at intervals of 24, 48, 96, 144 and 192 hours. There were 10 single plant replicates for each concentration at each time interval.

Absorption of the chemical was determined by measuring the amount of N-m-tolylphthalamic acid remaining on the leaf surface at the various intervals after application of the measured quantities. The leaves were washed immediately after removal from the plant with 10 ml of a 1 percent sodium hydroxide solution delivered from a 10 ml syringe fitted with a No. 18 needle. The sodium hydroxide solution served two purposes, to dissolve the unabsorbed residue and to hydrolyze the N-m-tolylphthalamic acid to phthalic acid and m-toluidine. Hydrolysis was complete after 24 hours, the m-toluidine was then diazotized and the chromogenic diazomium product was measured colorimetrically according to modifications of the method described by Hubbard and Smith (52).

Colorimetric determination of m-toluidine:

Reagents:

Sodium hydroxide (saturated)

Hydrochloric acid (concentrated)

Acetic acid (glacial)

Diazonium reagent - prepared by mixing equal volumes of:

(a) 0.125% sodium nitrite solution and

(b) 1.0% sulfanilic acid in 30% acetic acid.

Allow (a) and (b) to interact at least 5, but not more than 30 minutes before using.

Procedure:

The 10 ml sodium hydroxide (1%) leaf washes were held for 24 hours or longer. Five tenths of a ml of concentrated HCl, 0.25 ml of the diazonium reagents and 0.5 ml of glacial acetic acid were added to each vial. The solutions were held for 24 hours to permit diazotization to take place. Forty percent NaOH was added to adjust the pH to 2.8-3.0 using Hydrion pH paper[1] as an indicator. Color intensity was allowed to develop for 7 days, then 3 to 5 drops of concentrated HCl were added to adjust the pH to 1.0-1.5. Optical density (absorbance) of the solution was measured in 1 ml colorimetric tubes at 540 millimicrons with a Bausch and Lomb "Spectronic 20" spectrophotometer.

The percent absorption[2] of N-m-tolylphthalamic acid at the various time intervals is presented in Table XI, together with the flowering responses of these plants subsequent to removal of the treated leaves. The marked discrepancies for absorption of 60 micrograms at 24 hours and of 120 and 180 micrograms at 96 hours were the result of the talc diluent remaining in suspension. This was a difficulty in technique which occurred only rarely and was apparent only after the diazotization reaction was complete. The samples thus involved had a milky appearance which could not be removed by sedimentation or filtration. This increased the optical density reading on the colorimeter

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[1]The pH of the solutions were measured by immersing a strip of Hydrion paper in the solution and promptly comparing with the specimen colors on the Hydrion paper dispenser.

[2]The percent absorption was derived from percent remaining on the leaf surface.

TABLE XI

Rate of N-m-Tolylphthalamic Acid Absorption and Flower Formation in Tomato First Inflorescence

Time (Hrs)	Amount (Micrograms)						
	0	60		120		180	
	Flower (No)	Absorption (%)	Flower (No)	Absorption (%)	Flower (No)	Absorption (%)	Flower (No)
0	7.0	0	7.0	0	7.0	0	7.0
24	7.1	2.8	10.7*	24.7	10.8**	13.4	12.2**
48	8.3	38.0	15.4**(*)	35.5	12.5**	34.8	12.3*
96	8.9	43.7	10.7	26.9	16.5**	5.4	15.2**(*)
144	7.4	73.2	10.5**	74.2	14.0*	62.5	17.1**(*)
192	7.7	85.9	11.4**	71.0	13.7**	78.6	22.3**(*)
Not removed	8.3	-	13.0**(*)	-	17.8**	-	18.6**

*, **Significantly different from 0 time at the 5% and 1% levels, respectively.
(*)Significantly higher than the preceding absorption periods at the 5% level.

and, thereby, decreased the apparent absorption percentage. In the majority of the samples, however, the diazotized solutions were clear. The threshold amount of N-m-tolylphthalamic acid required to induce a significant increase in flower number was approached after 24 hours when 60 micrograms were applied. The flowering response after the same period of absorption was clearly evident with 120 and 180 micrograms. In these cases approximately 25 to 30 micrograms of N-m-tolylphthalamic acid had been absorbed. When flower numbers were compared after different absorption periods, it appeared that the maximum flowering response was reached after a 48-hour period for the 60 microgram application, and at 96 and 144 hour for the 120 and 180 microgram applications, respectively. No effect on flower numbers was evident when the plumule leaves were removed from the non-treated plants.

When absorption data were plotted graphically, typical absorption rate curves were obtained (Figure 10). At dosages of 120 and 180 micrograms the absorption rates were slightly lower, although the amount absorbed was greater than with the 60 microgram dosage. While the absorption periods did not extend for sufficient intervals to permit an exact calculation, the approximate half-time for absorption was 96 hours.

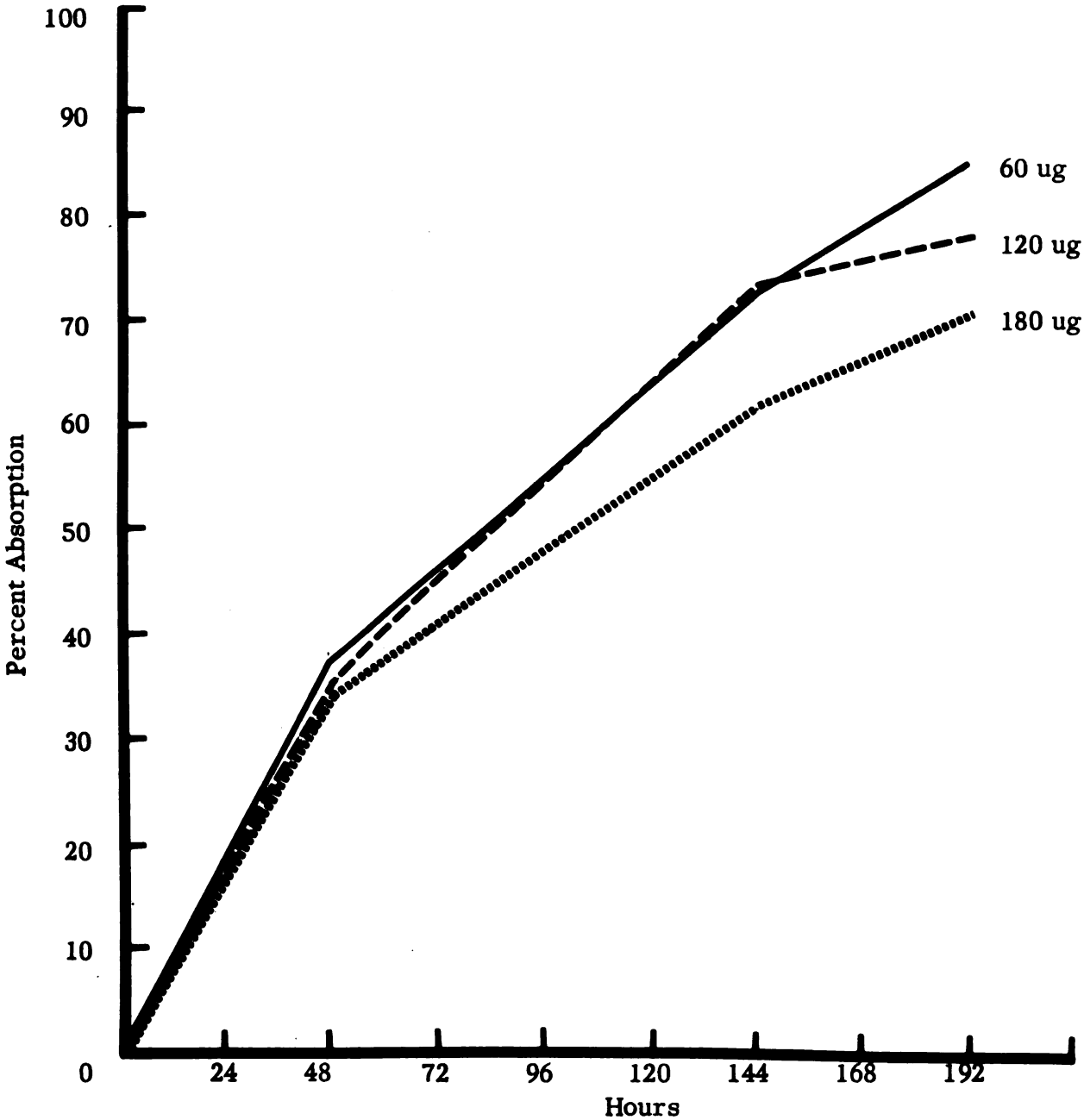

Figure 10. Absorption (percent) curves for 60, 120 and 180 micrograms (ug) of N-m-tolylphthalamic acid by the plumule leaves of tomato seedlings at intervals of 0, 48, 144 and 192 hours. (The intervals of 24 and 96 hours were not plotted due to contamination of samples by the talc diluent as explained in the results).

B. Translocation and Distribution

Michigan-Ohio Hybrid seedlings were treated at the 2-3 leaf stage with carbon 14 labeled N-m-tolylphthalamic acid[1]. The treating solution was prepared by dissolving a weighed sample in a small amount of acetone and diluting to proper volume with distilled water. The concentration of N-m-tolylphthalamic acid was 5, 000 ppm (micrograms per milliliter) and the concentration of acetone did not exceed 5 percent. Aliquots of this solution containing 30 micrograms of the chemical were applied in three drops to one plumule leaf of each plant using a 1-ml syringe fitted with No. 27 needle.

Ten single plant replicates were harvested at intervals of 24, 48, 96 hours, and 96 hours to 10 days[2] after treatment. The treated leaf was washed with 1 percent sodium hydroxide to remove non-absorbed N-m-tolylphthalamic acid and the plants were divided into three parts and extracted with acetone. The following fractions were obtained:

(a) Leaf wash

(b) Treated leaf

(c) Leaves above treated leaf

(d) Stems, epicotyl, cotyledons and hypocotyl

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[1] N-m-tolylphthalamic acid labeled with C-14 at the carboxyl carbon was obtained from Dr. A. E. Smith, Agr. Chem. Res., Naugatuck Chemical Div., U. S. Rubber Co., Naugatuck, Conn. The sample was prepared by reacting equimolar amounts of phthalic-7-C-14-anhydride with m-toluidine in benzene at 30° to 80°C. The resulting N-m-tolylphthalamic acid was, therefore, equally labeled in the free carboxyl group and the carbonyl carbon of the amide group. The specific activity of the sample was 0.2 millicurie per millimole.

[2] Treated leaf was removed after 96 hours while the remainder of the plant was harvested 10 days after treatment.

Harvested plant parts were ground separately in mortar and pestle with 2 ml acetone. The extract was decanted into 50 ml vials, and the tissue re-extracted twice with 2 ml acetone aliquots. Successive portions of the combined acetone extracts were poured into 1-inch cupped stainless steel planchets and dried under an infrared lamp. The vials were rinsed twice with 2 ml acetone aliquots, and this was evaporated in the same planchet. The extracted tissue was counted and found to contain no activity. A one ml aliquot from the NaOH leaf wash was pipetted into planchets for drying. Samples were placed in a dessicator to complete dryness for a minimum of 24 hours.

The samples were counted in a Nuclear model N-5 sample changer with a model D-47 Gas-flow Counter[1] and a Nuclear model 172-ultrascaler. Each sample was flushed with the "Q"[1] gas for 3 minutes before counting.

A sample of N-m-tolylphthalamic acid (C-14) was weighed, dissolved in a definite volume of acetone and aliquots were counted for activity. These data permitted calculation of the amount of N-m-tolylphthalamic acid present in the various samples. One microgram of the labeled chemical gave 435 counts per minute. This represented an overall counting efficiency of 25 percent.

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[1]The D-47 counter was operated in the Geiger plateau region at 1350 volts, with a "micromil" end-window having a density of less than 120 micrograms per square centimeter, and "Q" gas (98.7% helium, 1.3% butane) at a pressure of 3 psi was used. The total absorption loss with "micromil" window and air layer was less than 20 percent for carbon 14 (73).

The results of this study are presented in Table XII. Calculations of the amount of the chemical recovered in the various fractions were based on the activity of the applied N-m-tolylphthalamic acid and it was assumed that no other labeled component was present. These values are given in micrograms and as the percent distribution of the total amount of chemical recovered in all fractions including the non-absorbed portion in the leaf wash.

The recovery of N-m-tolylphthalamic acid (C-14) from the leaf surface indicated a fairly rapid absorption rate comparable to that obtained with the formulated Duraset-20W in the previous study. However, the total amount recovered from both leaf surfaces and in the extracted plant parts indicated an equally rapid disappearance of the absorbed chemical. This was clearly evident from the decrease in percent of the total activity recovered from the leaf surfaces. A decrease both in percent and in actual amount of absorbed chemical present in the treated leaf, indicates either metabolic destruction of the N-m-tolylphthalamic acid or transport out of the leaf at a more rapid rate than absorption occurred. The increase in N-m-tolylphthalamic acid (C-14) found in other plant parts suggest that transport may be at least partly involved. The possibility of accumulation in the roots also exists and this will be examined in a subsequent study. However, the very drastic drop in amount of chemical recovered in other plant parts during the six days subsequent to removal of the treated leaf suggests that more than transport was involved. Furthermore, the greatest

TABLE XII

The Distribution of Various Tomato Plant Parts of 30 Micrograms of N-m-Tolylphthalamic Acid Applied to One of the Plumule Leaves

Plant Parts	Total Recovered Microgram Equivalents*				Distribution of Total Recovered (%)			
	24	48	96	96-10**	24	48	96	96-10**
	Hours				Hours			
Leaf wash	19.80	18.40	16.60	16.60	79.40	84.40	87.80	87.80
Treated leaf	4.90	3.00	1.60	1.60	19.70	13.80	8.50	8.50
Meristem	0.004	0.013	0.013	0	0.02	0.06	0.069	0
Stem and leaves	0.126	0.200	0.479	0.069	0.50	0.92	2.53	0.36
Stem and cotyledons	0.102	0.182	0.224	0.067	0.41	0.83	1.18	0.35
Totals	24.93	21.80	18.91					
In Plant	5.13	3.40	2.31		20.6	15.6	12.2	

*Based on activity recovered in plant part and specific activity of applied N-m-tolylphalamic acid (C-14).
**Leaf wash and treated leaf collected at 96 hour, the remainder at the end of 10 days after treatment. Percentage calculated based on total recovered at 96 hours.

increase in the total amount of the chemical recovered in the plant at 96 hours was in the tissues above the treated leaf, indicating acropetal rather than basipetal movement.

The amount of non-absorbed chemical in the leaf wash at the various time intervals provides an estimate of the total absorption from the original 30 micrograms of chemical applied (Table XIII). Absorption was 34.0, 38.6 and 44.7 percent for 24, 48 and 96 hours, respectively. These values were slightly higher for the 24-hour period and lower for the 96-hour interval than those derived by another proceedure in a previous experiment (Table XI). Of the 10.2 micrograms of chemical absorbed by the plumule leaf in 24 hours, only 5.1 micrograms or 50 percent was recovered in the above ground plant parts. After 96 hours more than 80 percent of the 13.4 micrograms absorbed during this period was not recovered.

N-m-tolylphthalamic acid (C-14) treated tomato seedlings that were allowed to develop inflorescences had significantly greater flower numbers in the first inflorescence than the non-treated plants. The trend of decreasing recovery from the above ground plant parts suggested the possibility that the N-m-tolylphthalamic acid may have been translocated to and accumulated in the roots. An additional experiment was thus carried out using nutrient solution cultures to facilitate recovery of the roots.

Michigan-Ohio Hybrid seedlings were transplanted into 8-liter crocks

TABLE XIII

Absorption and Recovery by Above Ground Parts of Tomato Plant from 30 Micrograms of N-m-Tolylphthalamic Acid (C-14) Applied to One Plumule Leaf (Values Obtained from Data in Table XII)

Time (Hours)	Absorption		Recovery		Losses	
	Micrograms	Percent	Micrograms	Percent	Micrograms	Percent
24	10.2	34.0	5.1	50.0	5.1	50.0
48	11.6	38.6	3.4	29.4	8.2	70.6
96	13.4	44.7	2.3	17.2	11.1	82.8

containing a standard Hoagland nutrient solution. The C-14 labeled N-m-tolylphthalamic acid solution (5, 000 ppm) was prepared by dissolving a weighed sample in the proper volume of 50 percent acetone.[1] Aliquots of this solution containing 71 micrograms were applied in four drops to one plumule leaf of each plant with a syringe and needle. Seedlings were collected in six replicates at each interval. The treated plumule leaf was washed with one percent sodium hydroxide, and each seedling was divided into five parts and extracted with acetone. Radioactivity of the following fractions was determined:

(a) Leaf wash

(b) Treated leaf

(c) Leaves, stem between treated leaf and meristem

(d) Apical meristem

(e) Epicotyl below treated leaf, cotyledons, hypocotyl

(f) Roots

Methods of extraction and counting were the same as described in the previous experiment.

The results presented in Table XIV confirm those of the previous experiment in that the total recovery of N-m-tolylphthalamic acid decreased

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[1] Solubility of N-m-tolylphthalamic acid (5, 000 ppm) was complete in 50 percent acetone. No detrimental effects on tomato seedlings were found from acetone concentrations as high as 60 percent.

TABLE XIV

The Distribution of Various Tomato Plant Parts of 71 Micrograms of N-m-Tolylphthalamic Acid Applied to One of the Pumule Leaves

Plant Parts	Total Recovered Microgram Equivalents*				Distribution of Total Recovered (%)			
	24	48	96	96-10**	24	48	96	96-10**
	Hours				Hours			
Leaf wash	43.80	36.80	32.05	32.05	91.36	89.22	90.49	90.49
Treated leaf	4.50	4.20	3.10	3.10	9.28	10.17	8.76	8.76
Stem and leaves	0.12	0.12	0.12	0.03	0.25	0.29	0.31	0.09
Meristem	0	0	0	0	0	0	0	0
Stem and cotyledons	0.05	0.05	0.05	0.03	0.08	0.12	0.14	0.09
Roots	0.05	0.11	0.11	0.09	0.10	0.27	0.31	0.27
Total	48.51	41.28	35.42					
In Plant	4.71	4.48	3.37		9.71	10.85	9.52	

*Based on activity recovered in plant part and specific activity of applied N-m-tolylphthalamic acid (C-14).

**Leaf wash and treated leaf collected at 96 hours and the remainder at the end of 10 days after treatment. Percentages calculated based on total recovered at 96 hours.

with time. Of the 71 micrograms applied, 27 micrograms, or 38 percent was absorbed in 24 hours. This rapid initial absorption rate, possibly facilitated by the high acetone concentration, was followed by a much slower rate of only 9. 9 percent additional uptake during the following 24-hour interval and an increase of only 6. 7 percent in the next 48 hours (Table XV, Figure 11a, 11b). Recovery of the absorbed chemical, however, was lower than in the preceding study, even though the amounts present in the roots were included. As much as 91. 3 percent of the absorbed chemical was not recovered in the plant. The relatively constant percent of the chemical on the leaf surface indicates that absorption rate in this case was more or less equal to disappearance of the chemical from the plant (Figure 11a, 11b). Furthermore, transport out of the treated leaf was much slower than in the previous study. Only three percent was found in the tissue above the treated leaf, and none was detected in the meristem. At the same time N-m-tolylphthalamic acid (C-14) treated plants that were allowed to develop inflorescence showed no flower number increase in the first inflorescence. As anticipated, a portion of the chemical transported out of the leaf was recovered in the roots. However, in contrast to the rapid disappearance from the above ground parts after removal of the treated leaf, the amount accumulated in the roots was relatively stable (Table XIV, Figure 11b).

An attempt to determine the chemical substances associated with

TABLE XV

Absorption and Recovery by Above Ground Parts of Tomato Plants from 71 Micrograms of N-m-Tolyphthalamic Acid (C-14) Applied to One Plumule Leaf (Values Obtained from Data in Table XIV)

Time (Hours)	Absorption		Recovery		Losses	
	Micrograms	Percent	Micrograms	Percent	Micrograms	Percent
24	27.2	38.3	4.7	17.3	22.5	82.7
48	34.2	48.2	4.5	13.2	29.7	86.8
96	39.0	54.9	3.4	8.7	35.6	91.3

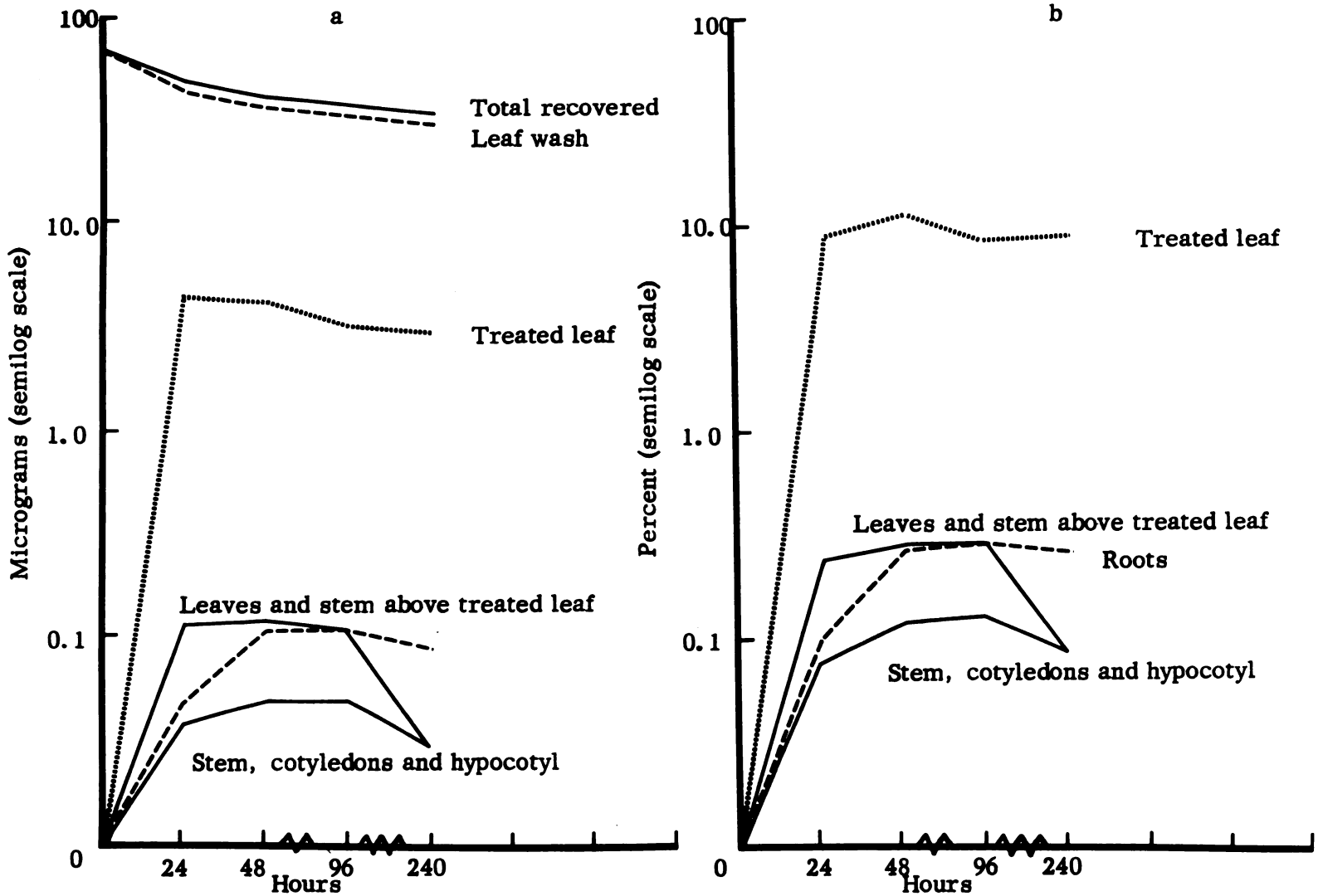


Figure 11. Distribution of C-14 N-m-tolylphthalamic acid equivalents recovered in different parts of tomato plant of various time intervals after treatment of a plumule leaf.

the radioactivity obtained in acetone extracts of the various plant parts was carried out employing paper chromatographic techniques. Preliminary studies were conducted with non-labeled N-m-tolylphthalamic acid, phthalic acid and m-toluidine. These materials were spotted on Whatman No. 1 chromatographic filter paper and developed 6 to 8 hours with a solvent of isopropanol:water:ammonium hydroxide in a ratio of 7:2:1, using an ascending technique. The upper half (solvent front portion) of the chromatogram was sprayed with the diazonium reagent (p. 55) to detect N-m-tolylphthalamic acid and m-toluidine, and the lower half with an acid-base indicator (brom cresol green at pH 4.0-5.6) to detect phthalic acid. The Rf values obtained for each of the compounds are presented below:

Compound	Experiment			
	I	II	III	Mean
N-m-tolylphthalamic acid	0.71	0.63	0.68	0.67
Phthalic acid	0.24	0.25	0.24	0.24
m-toluidine	0.84	0.88	0.88	0.87

Each of the above figures represented an average of six spots. The Rf values obtained by Smith (98) were: N-m-tolylphthalamic acid 0.78-0.84; phthalic acid 0.18-0.20; and m-toluidine 0.90-0.96.

The dried acetone extracts of the treated leaf (from plants grown in winter) collected at 24 hours (Table XIII) were redissolved in acetone,

combined and chromatographed using the above procedure. The chromatogram was counted directly with the D-47 gas flow counter. Fifty percent of the radioactivity originally present in the leaf extract was recovered on the chromatogram and of this over 90 percent was found at Rf 0.70. This suggests that most of the radioactivity present in the plant was as the intact N-m-tolylphthalamic acid. When acetone solutions of N-m-tolylphthalamic acid were allowed to stand for 10 days and then chromatographed, all the activity was at Rf 0.20 indicating hydrolysis had taken place.

Subsequent studies were conducted in which 57 micrograms of C-14 N-m-tolylphthalamic acid were applied to one plumule leaf of each of four tomato seedlings (grown in spring) at the 2-3 leaf stage. The treated leaf was washed with 1 percent sodium hydroxide at 24 hours, and then ground in a mortar and pestle and extracted with acetone. The supernatant acetone extract was decanted from starch grains released during grinding. The starch residue was transferred into a planchet, counted, and identified as starch by reaction with iodine-potassium iodide (IKI) (53). The planchet was found to contain over 90 percent of the activity apparently adsorbed to the starch granules. A modified procedure was employed by filtering the acetone extract rapidly through Whatman No. 1 filter paper using a Büchner funnel and suction flask and collected into a test tube. This was done to avoid excessive adsorption of the chemical through prolonged contact with

the starch granules. The acetone extract was evaporated in a stream of nitrogen gas. The concentrated extract was applied to Whatman No. 1 chromatographic paper. After development, the strip was counted as described above. While the entire strip registered an activity slightly higher than background, indicating the possible presence of $C^{14}0_2$, the only appreciable concentration of activity occurred at Rf 0.65. This corresponded closely to the Rf obtained with a standard sample of N-m-tolylphthalamic acid (C-14).

C. Autoradiography

Autoradiography of the N-m-tolylphthalamic acid (C-14) treated tomato seedlings was carried out for additional evidence of the distribution of the chemical.

Seedlings of Michigan-Ohio Hybrid were transplanted into crocks of nutrient solution. The seedlings were treated at the 2-3 leaf stage with 100 micrograms of C-14 N-m-tolylphthalamic acid (0.285 millicuries per millimole) in 60 percent acetone solution. The solution was applied in six drops to one plumule leaf of each seedling, using a 1 ml syringe fitted with No. 27 needle. The seedlings were harvested in three single plant replicates at intervals of 12, 24, and 48 hours after treatment. The treated area of the plumule leaf was removed with a cork borer to eliminate contamination

and excessive fogging of the film. The intact seedling was mounted between two sheets of 8 by 10 inch botanical drying paper, and the mounts separated by two 8 by 10 inch masonite boards. The mounted plants were placed in a 70° F oven, pressed with a steel weight, and dried for 24 hours.

Each dried plant was carefully removed with a spatula, placed on a clean sheet of botanical drying paper and covered with Saran wrap. The following operations were carried out in a dark room. The mounted plant was placed, face down, in an exposure cabinet on a 8 by 10 inch sheet of Eastman Kodak Blue Brand X-ray film, having emulsion on both sides. Successive plant and film combinations were separated by masonite boards and the entire stack was pressed with a steel weight. The film were developed after 60 days. The autoradiogram (Figure 12) shows the overall distribution pattern of N-m-tolylphthalamic acid within the plant. The chemical apparently translocated in considerable quantities to the meristem. With the exception of the treated leaf and the meristem, there was a rather uniform distribution of the chemical throughout the plant.

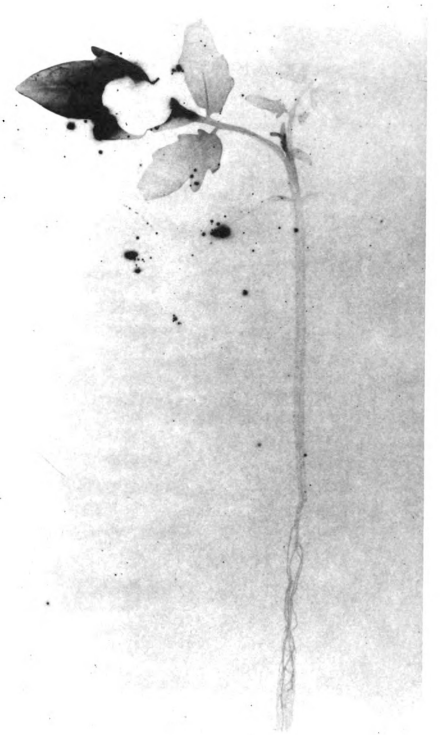
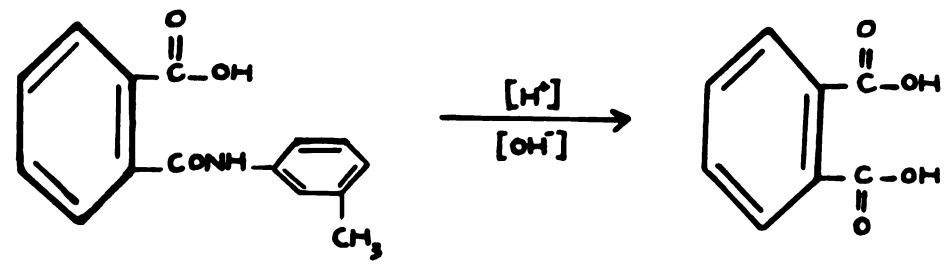


Figure 12. An autoradiogram of a tomato seedling 24 hours after C-14 N-m-tolylphthalamic acid treatment of one of the plumule leaves. (The scattering black spots around the plumule leaf were contaminations by the C-14 N-m-tolylphthalamic acid on the plant mount).

V. HYDROLYSIS OF N-m-TOLYLPHTHALAMIC ACID AND EFFECTS OF THE BREAKDOWN PRODUCTS ON TOMATO FLOWERING

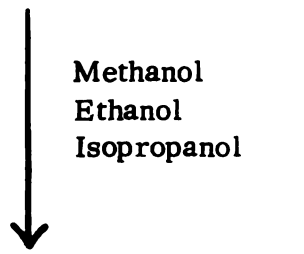
In contrast to many other synthetic growth regulators, N-m-tolylphthalamic acid is quite labile. The amide bond hydrolyses readily between acid (pH 3.0) and alkaline (pH 10.0) conditions to yield phthalic acid and m-toluidine, and cyclizes in alcohol to N-m-tolylphthalimide (Figure 13) (28). Teubner and Wittwer (110) have reported that solutions of N-m-tolylphthalamic acid (200 ppm) stored for 24 to 48 hours had no effect on tomato flower formation. Accordingly, the breakdown of N-m-tolylphthalamic acid at a pH of 6.8 as measured by subsequent flowering activity was studied. Duraset-20W solutions containing 500 ppm N-m-tolylphthalamic acid were prepared at intervals of 1, 2, 4, 6 and 10 days prior to treatment. These together with a freshly prepared solution were applied as a spray to Michigan-Ohio Hybrid plants at the 2-3 leaf stage (Figure 1, c). Ten single plant replicates of each treatment and a control were included in the study. The following were recorded: (1) Number of branches in the first inflorescence; (2) number of flowers in the first inflorescence; and (3) percent of plants that vegetatively terminated.

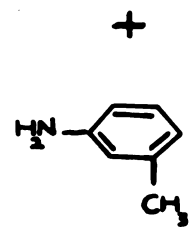
The effect of allowing solutions of N-m-tolylphthalamic acid to age for 1 to 10 days before use on subsequent flowering responses of tomato plants is presented in Table XVI. Only one-third of the activity remained after the



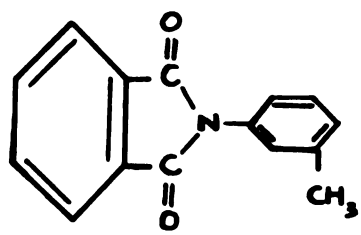
N-m-tolylphthalamic Acid

Phthalic Acid





m-toluidine



N-m-tolylphthalimide

Figure 13. Hydrolysis and cyclization of N-m-tolylphthalamic acid.

TABLE XVI

The Effect of Aging of Solutions of N-m-Tolylphthalamic Acid on Tomato Flower Bud Formation in the First Inflorescence

Age of Solution (Hours)	Bud (No.)	Branch (No.)	Bud Increase (A_t)	Relative Activity (A_t/A_o)	Decay Constant (λ)
0	21.7**	3.9	15.3	1.000	-
24	12.1**	2.3	5.7	0.372	0.041
48	11.4**	2.3	5.0	0.327	0.023
96	8.6	1.4	2.2	0.144	0.020
144	8.1	1.4	1.7	0.111	0.015
240	6.4	1.2	0	0.000	-
Control	6.4	1.0	-	-	-

Concentration of N-m-tolylphthalamic acid at 0 hour was 500 ppm.

$A_o = 15.3$

**Significantly greater than control at 1% level.

compound had been in solution (distilled water) for 1 to 2 days and the activity (in terms of flower formation in the tomato) had completely disappeared by 10 days. Although the data involve a biological response, and are admittedly quite variable, a decay constant (λ)[1] was calculated for those intervals at which some flowering increase was obtained.

The decreasing values of λ indicate a threshold concentration is required to induce a measureable response. However, assuming a mean value of 0.025 hour^{-1}, an estimate of the half-life of N-m-tolylphthalamic acid in solution at pH 6.8 can be calculated from the equation, $T\,1/2 = \frac{0.693}{\lambda}$. The calculated half-life of 27.7 hours corresponds closely to the practical observations made by Teubner and Wittwer (110) of 1 to 2 days.

The previous study assumed that the products of hydrolysis of N-m-tolylphthalamic acid, namely, phthalic acid and m-toluidine, were inactive in the flowering response of tomato. To test the validity of this assumption, Michigan-Ohio Hybrid plants were sprayed at the 2-3 leaf stage with solutions of phthalic acid, m-toluidine, and a mixture of these at equimolar concentrations of 1 and 2 millimoles, which were equivalent of 255 and 510 ppm of N-m-tolylphthalamic acid respectively. Teubner and Wittwer (110) have reported that N-m-tolylphthalimide, which results from splitting out of water and cyclization (Figure 13) is inactive. Since this is also a potential breakdown

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[1] λ is calculated from the formula $\frac{A_t}{A_o} = e^{-\lambda t}$, where A_t is the total flower bud increase from control plant at time t; A_o is the total flower bud increase from control plant at zero time; and e is the base of natural logarithm.

product, it was included in the study at the same molar concentrations. Measurements were recorded for ten single plant replicates of each treatment for numbers of nodes subtending the first and second inflorescences, and the numbers of flower buds and branches in the first and second inflorescences.

It is evident from the results presented in Table XVII that the potential breakdown products: N-m-tolylphthalimide, phthalic acid, m-toluidine or the mixture of phthalic acid and m-toluidine did not produce the flowering response obtained with equal millimolar concentrations of N-m-tolylphthalamic acid. Thus, an intact molecule of N-m-tolylphthalamic acid is necessary for this particular response.

From the biological responses derived in the preceding experiment, the half-life of N-m-tolylphthalamic acid at pH 6.8 was determined as 27.7 hours. According to Smith (98) N-m-tolylphthalamic acid at a wavelength of 260 millimicrons (mu) has three times the optical density of the breakdown components, phthalic acid and m-toluidine. To obtain additional information on the chemical stability of N-m-tolylphthalamic acid, solutions of 5 ppm N-m-tolylphthalamic acid, m-toluidine, phthalic acid and a mixture of m-toluidine and phthalic acid were prepared for spectrophotometric examination using a Beckman DU spectrophotometer. The absorption spectra of these solutions were examined in the range 240-300 mu. Since none of the compounds had absorption peaks, 260 mu was chosen as the wavelength of

TABLE XVII

The Effects of Potential Breakdown Products of N-m-Tolylphthalamic Acid on Flower Formation in the First and Second Inflorescences of the Tomato

Chemicals	Concentration of Applied Solutions (mm)	First Inflorence			Second Inflorescence		
		Nodes Subtending	Buds	Branches	Nodes[1] Subtending	Buds	Branches
None (control)	0	7.5	8.5	1.4	3.0	5.2	1.0
N-m-tolylphthalamic acid	1	8.3	9.6	2.0	2.9	8.3**	2.0
N-m-tolylphthalamic acid	2	7.9	16.0**	3.3	2.9	10.8**	2.4
N-m-tolylphthalimide	1	7.9	8.1	1.4	3.0	5.5	1.0
N-m-tolylphthalimide	2	7.9	8.0	1.5	3.0	5.0	1.0
m-toluidine (m-T)	1	7.4	8.5	1.6	3.0	5.3	1.0
m-toluidine (m-T)	2	8.0	8.2	1.3	3.0	5.2	1.0
Phthalic acid (PA)	1	8.0	7.6	1.2	3.0	5.3	1.0
Phthalic acid (PA)	2	8.0	7.7	1.3	3.0	5.3	1.0
m-T + PA	1 + 1	7.6	9.0	1.5	3.0	5.3	1.0
m-T + PA	2 + 2	8.2	8.6	1.5	3.0	5.3	1.0

[1]Nodes between first and second inflorescences.
**Significantly greater than control at 1% level.

measurement which showed a greater difference in absorption of N-m-tolylphthalamic acid and the breakdown products. The solutions were adjusted to pH 6.8 and the absorbance at 260 mu measured at 24-hour intervals for a period of one week. In contrast to the results reported by Feldman (28) of rapid hydrolysis of N-m-tolylphthalamic acid in the pH range 3.0-6.0 as measured by a decreasing absorbance at 260 mu (98), in this study the absorbance of N-m-tolylphthalamic acid increased slightly during the first three days and then remained constant. Several possible explanations of these observations were considered. The studies reported by Feldman (28) were carried out only in acid media and this would retard oxidation of the free m-toluidine. Alternatively, formation of N-m-tolylphthalimide might account for the decrease in biological activity without appreciable change in absorbance. Finally, an appreciable shift in the absorption spectrum would not be detected by measurement at the single wave length of 260 mu.

Consequently, additional solutions were prepared by dissolving an appropriate amount of each chemical in 1 ml of saturated sodium bicarbonate and diluting to a volume of 100 ml with distilled water to give a one millimolar concentration of each. A 5 milliliter aliquot of each preparation was transferred to a second 100 milliliter volumetric flask and again made to volume. The final concentration was 0.05 millimolar and the pH was 7.0 ± 0.2. The absorbance of all solutions was measured over a wave length range of 240 to

300 mu using a recording Beckman DK-2 spectrophotometer. Results are given in Figure 14a, b for the initial preparations and five day old solutions, respectively. A solution prepared from Duraset-20W was also included at an equivalent concentration, since this material had been employed in the studies reported by Feldman (28). The initially high absorbance of the Duraset-20W solution was apparently the result of suspended diluent which precipitated during the subsequent five day interval. If measured at only 260 mu this would indicate an apparent hydrolysis; however, the shape of the absorption spectrum obviate this conclusion. The pH was measured at the end of five days, and no change was noted. As in the previous studies the absorbance of phthalic acid m-toluidine and the mixture of these remained constant at all wave lengths. This precludes the possible oxidation of m-toluidine or the resynthesis of N-m-tolylphthalamic acid from the breakdown products. The absorption spectrum of N-m-tolylphthalimide is also given and is much lower at all wave lengths than N-m-tolylphthalamic acid. Thus, transformation to the imide is also precluded. As observed in the previous study, the absorbance at 260 mu of the solution of N-m-tolylphthalamic acid increased during the five day period. If N-m-tolylphthalamic acid was being hydrolyzed to phthalic acid and m-toluidine, the shape of the absorption spectrum should assume that of the mixture of these products. Since this is evidence that N-m-tolylphthalamic acid was not hydrolyzed at a neutral pH,

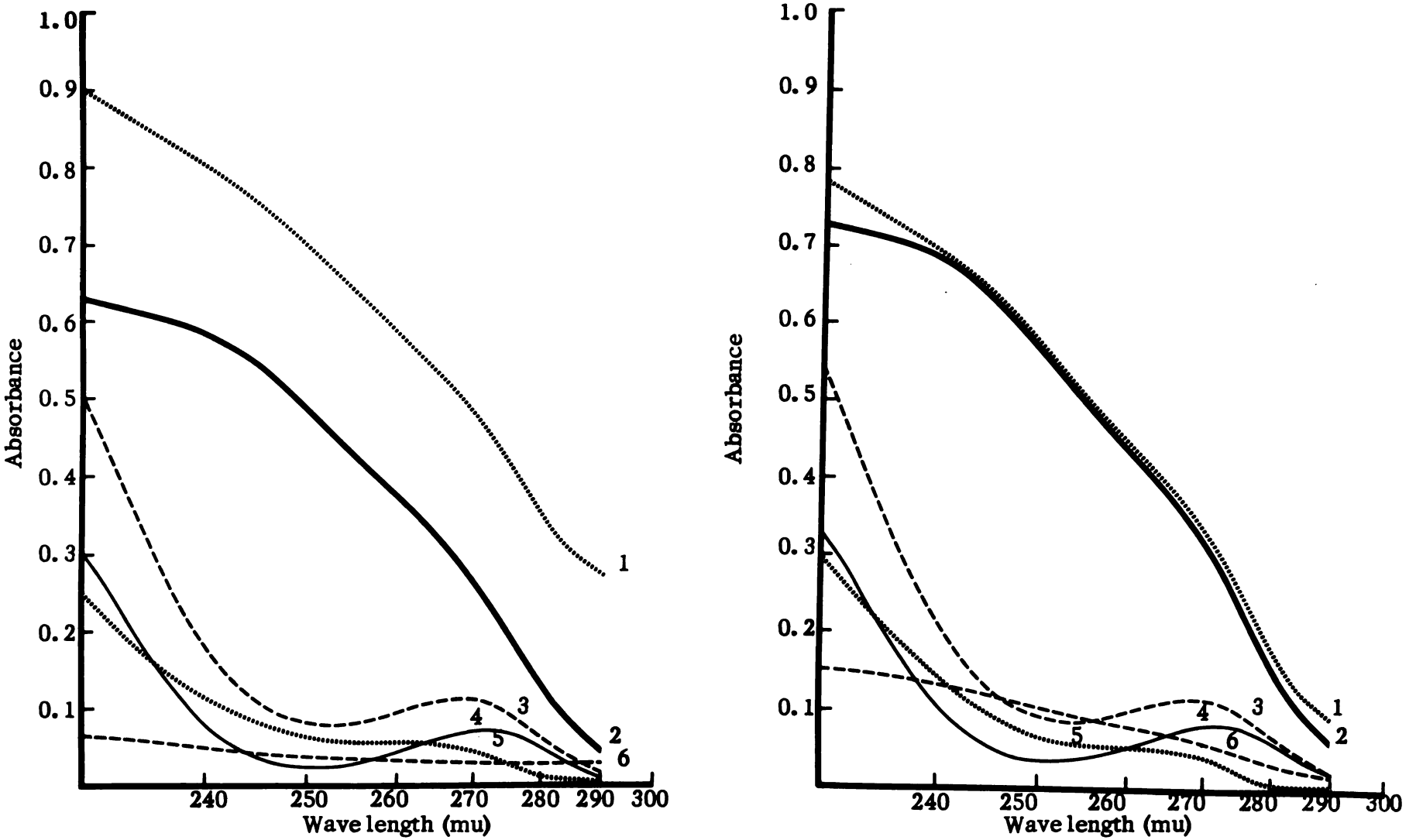

Figure 14. Spectrographs of absorbance of the various chemicals. Order of the chemicals from top to bottom:

1. Duraset-20W
2. N-m-tolylphthalamic acid
3. Mixture of phthalic acid and m-toluidine
4. m-toluidine
5. Phthalic acid
6. N-m-tolylphthalimide

it was thought that the two aromatic ring components (phthalic acid and m-toluidine) with -$\overset{H}{\underset{|}{N}}$- as the center, might exist in two or more distinct spatial relationships through restricted rotation of the amine ring as a consequence of an electron shift from the ring to the nitrogen and semi-restricted rotation of the carbonyl carbon because of the dipole moments of the oxygen atom. Thus, through steric hindrance of the rings, potential stereoisomers with a "pseudo-assymetric" nitrogen atom could exist. The phenomenon of an assymetric nitrogen has been established by Harris (42) and Harris et al. (43). Furthermore, it has been established for a number of synthetic auxins having assymetric carbon atoms that only one of the stereoisomers possesses appreciable auxin activity (117). If during synthesis of N-m-tolylphthalamic acid, an "active" isomer was preferentially produced, then racemization to an "inactive" form could occur in solution. While this would result in a disappearance of biological activity, no change in the absorption spectrum would be apparent. In this event, however, the original solutions should have optical activity as measured by a polarimeter. The optical activity of N-m-tolylphthalamic acid was measured using a modified polarimeter (Saccharimeter). Thus, a N-m-tolylphthalamic acid solution was prepared in acetone. No change of rotation was observed from the solution as compared to acetone alone. This suggests that the chemical is not optically active and that the disappearance of biological activity cannot be explained on the premise that that racemization to an inactive isomer occurs upon aging of solutions of N-m-tolylphthalamic acid.

VI. AUXIN ACTIVITY OF N-m-TOLYLPHTHALAMIC ACID

The original report of growth regulating activity in the N-aryl-phthalamic acids was based on the ability of these chemicals to induce parthenocarpic fruit-set (48). Subsequently, Teubner and Wittwer (113) have pointed out a direct relationship between activity of mono-, di- and tri-chlorophenyl-phthalamic acids in flower formation and induction of parthenocarpy in the tomato. Since activity in parthenocarpic fruit-set of tomatoes has been found by Osborne et al. (86a) to be indicative of auxin activity of synthetic growth regulators, N-m-tolylphthalamic acid was assayed for auxin activity employing a modification of the classical Avena section straight growth test (84) and for parthenocarpic fruit growth in the tomato using a technique developed by Luckwill (72).

A. Avena Straight Growth Test

The Avena coleoptile section test of Nitsch and Nitsch (84) was modified as follows: Dehusked Victory oats (Avena sativa) were placed in a suction flask, evaculated and soaked in water for 2 to 3 hours. The seeds were then planted on a porcelain dessicator plate covered with wet paper towel, and placed in a covered 12-inch diameter evaporating dish in an incubator at 25°C. One day after seeding, the germinating seeds were exposed to 2 hours of red light to retard elongation of the first internode. After three days of incubation,

uniform coleoptiles, 2 to 3 centimeters in length, were cut and sectioned. The apical 2 to 4 millimeter was discarded and subapical 5-millimeter sections were polled in a dish of double distilled water. The plumule leaves within the coleoptile cylinders were removed by threading the sections on a thin glass rod (diameter less than inside of coleoptile, ends polished). Five sections were threaded on each rod and two rods (10 sections) were placed in a stender dish (1 1/2 inch diameter) containing filter paper that had been spotted with a known amount of N-m-tolylphthalamic acid. One milliliter of a phosphate-citrate buffer (pH 5.0) containing 3 percent sucrose was pipetted into the dish. Final concentrations of N-m-tolylphthalamic acid ranged from 10^{-10} to 10^{-4} molar. After 24 hours the sections were measured to the nearest 0.5 millimeter.

The results of the various tests summarized in Table XVIII show considerable variability. In every instance where crystalline N-m-tolylphthalamic acid was used, some "auxin activity" was observed; however, the optimum concentration varied widely. In contrast, the coleoptiles failed to respond to the Duraset-20W formulation. The talc diluent in the formulation may have adsorbed the chemical and prevented the growth response. As Feldman (28) has indicated, N-m-tolylphthalamic acid is readily hydrolyzed to phthalic acid and m-toluidine at pH 3-5, and it was considered that the chemical may have been hydrolyzed during the 24 hour period in the pH 5.0 buffer solution.

TABLE XVIII

The Effect of N-m-Tolylphthalamic Acid on the Growth of Avena Coleoptile Sections

Date of Assay	Molar Concentration							
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	Control
	(Length in millimeters)							
Oct. 1, 1956[1]	7.7	8.3	7.7	9.1*	9.3*	8.6	8.65*	7.9
Oct. 12, 1956[1]	7.1*	6.6	6.9	6.8	6.9	6.9	6.7	6.9
Oct. 18, 1956[1]	6.5	6.4	6.6	6.3	6.5	6.8	6.5	6.4
Nov. 1, 1956[2]	9.2	7.9	8.4	8.1	8.7	8.5	-	8.2
Nov. 5, 1956[1]	7.7*	6.6	6.3	6.6	6.6	6.5	-	6.5
Nov. 12, 1956[2]	6.7	7.2	6.7	6.8	7.1	6.9	6.9	6.7
Mean	7.5	7.2	7.1	7.3	7.5	7.4	7.2	7.1

[1]Crystalline N-m-tolylphthalamic acid

[2]Duraset-20W formulation of N-m-tolylphthalamic acid

*Significantly greater than control

Consequently, a KH_2PO_4 - NaOH buffer, pH 8.1 was employed in two assays. No "auxin activity" was observed, and normal elongation of control sections was retarded. The buffering capacity of the coleoptile sections was such that the pH of the solutions was 4.0 to 4.5 after 24 hours.

An alternative explanation of the inconsistent response obtained with Avena coleoptile sections is the possible hydrolysis of the amide bond between the m-tolyl and phthalic moieties. The occurrence of amidases in the plant is well established (143), although no literature on hydrolysis of aromatic amide bonds by specific amidases has been found.

Consequently, corn coleoptile, Alaskan pea epicotyl and pea root sections were also tested for stimulation of straight growth (66). The first two did not respond to N-m-tolylphthalamic acid, while pea roots elongated significantly over control sections at concentrations from 10^{-8} to 10^{-4} molar.

B. Parthenocarpic Development of Tomato Ovaries

The quantitative method of auxin assay developed by Luckwill (72) using emasculated tomato ovaries was employed in these studies. The rate of fruit diameter increase during the first six days has been shown (72) to be directly proportional to the amount of growth substance applied. Tomato flowers on the first cluster of Michigan-Ohio F_1 hybrid plants were emas-

culated at anthesis. Two ovaries, 2.0 millimeter diameter, were selected and all other flowers and buds removed from the cluster. Three single plant replicates, of two ovaries each, were treated with N-m-tolylphthalamic acid, 3-indoleacetic acid and p-chlorophenoxyacetic acid, at concentrations of 3×10^{-5}, 3×10^{-4} and 3×10^{-3} molar. The ovaries were emasculated on the morning of the day of anthesis and treated in the afternoon by applying 10 lambda (0.01 ml) of N-m-tolylphthalamic acid to the selected ovaries using a micropipette. Final diameters were measured five days after treatment. Values given in Table XIX are means of six measurements.

Although N-m-tolylphthalamic acid was not as active (at 3×10^{-3} concentration) as p-chlorophenoxyacetic acid in fruit setting, its activity was considerably higher than indoleacetic acid as well as p-chlorophenoxyacetic acid at lower concentration ranges. This evidence supports the possibility of auxin activity of N-m-tolylphthalamic acid.

TABLE XIX

The Effect of N-m-Tolylphthalamic Acid, 3-Indoleacetic Acid, and p-Chlorophenoxyacetic Acid on Parthenocarpic Development of Tomato Ovaries

Chemicals	Molar Concentration			
	3×10^{-3}	3×10^{-4}	3×10^{-5}	Control
	(Ovary diameter in mm)			
N-m-tolylphthalamic acid	3.37**	4.82**	2.80	2.53
3-Indoleacetic acid	5.37**	3.07	2.43	
p-chlorophenoxyacetic acid	8.00**	4.70**	2.77	

** Significantly different from control (at 1% level).

DISCUSSION

Morphological Aspects:

There are a number of reports (17, 62, 63, 70, 118, 124, 125, 126, 127, 134, 135) that low temperature exposure during early growth of tomato plants induces increased branching and greater flower numbers in the first inflorescence. Low temperature treatment also reduced the node number to the first inflorescence. In the present study with tomato seedlings exposed to a night temperature of 50 to 55° F beginning at cotyledon expansion, the transition from a vegetative to reproductive apex occurred on the 8th day of cold treatment. In non-cold exposed plants grown at 72° F, the transition occurred two days later. Thus, cold exposed plants not only had two fewer nodes subtending the first inflorescence, but initiation of flower primordia also occurred earlier than on the non-treated plants. Subsequent flowering in cold exposed plants was also earlier if the low temperature was discontinued after initiation. However, the slower rate of leaf initiation indicated a general retardation of growth and if the cold exposure was prolonged to obtain increased flower numbers, then flowering was delayed.

Wetmore (128), after studying apices of Chendopodium album (lamb's quarter), Xanthium saccharatum (cockleburr) and Glycine mox (Biloxi soybean) found that in the vegetative apices of these species, cell division was more frequent in the peripheral region and scarce, or absent,

in the large-cell center region. He found that during transition from a vegetative to reproductive apex, cell division increased in the latent center region, and this changed the topography from the flat vegetative apex to a somewhat rounded flowering apex. Borthwick et al. (9) working with Daucas carota and Phillipson (88, 89, 90) with Bellis perennis, Succia pratensis, and Dipsacus fullomuin, found that the vegetative-reproductive transition in these species occurred when the shape of the apex became conspicuously flattened and widened. In contrast to Wetmore's findings, Lawalree (60) and Phillipson (90, 91) maintained that the reproductive apex restricts its meristematic activity to the peripheral zone, which corresponds to tunica and corpus layers of the vegetative apex. Phillipson (88, 91) and Reeve (94) observed that the cells in the central region of the reproductive apex enlarge and vacuolate prominently and intercellular spaces may appear. Esau (27) interprets the transition as an expression of determinate growth during organization of the floral apex. Axis elongation ceases and, therefore, activity of the corpus layers leading to rib meristem formation is discontinued. In the absence of axial elongation, the flower parts develop in close proximity (27). The present studies of tomato are in close agreement with those on Daucas carota and other species, in which the transition involves a shift from elongation of the axis to a peripheral expansion. The vegetative apex in Figure 2, b illustrates a definite rib meristem in the pro-meristem region, while the repro-

ductive apex in Figure 2, c has no definite pattern of cell organization. Cell division became more prominent in the peripheral region with densely stained cells in the tunica and corpus layers, as the apex became reproductive and the shape spherical and widened. Gorter (34) found the same trend of development in tomato.

At the 2-3 leaf stage (Figure 1, c), the optimum time for N-m-tolylphthalamic acid to induce polychotomy in the first inflorescence, floral primordia had already been initiated. In addition to cell division and expansion of the peripheral cells as in the control apex, the treated primordia elongated, as shown in Figure 6, h. It was pointed out in the results of histological studies (p.33), that there was a six day lapse between initiation of a bud and differentiation of floral parts in the treated apex. Since this lapse was only one day in the control apex, the treated apex can be interpreted as existing in a prolonged "vegetative-reproductive transitional state". Apparently, it possesses the characteristics of both a vegetative and reproductive apex. It is further suggested that because of the vegetative characteristic, the neighboring sympodial bud is retarded in development. As development of the inflorescence advances, floral whorl differentiation begins, and reproductive characteristics become more pronounced. Concomitant with differentiation of floral parts, in both treated and control plants, the suppressed sympodial bud begins active development and eventually assumes a dominant role in vegetative extension.

Sympodial Bud - Inflorescence Relationship:

DeZeeuw (24) on the basis of defoliation studies, suggested that the young leaves of the sympodial bud produce an inhibitor which retards flower development in the subjacent inflorescence. In a subsequent paper he has postulated that N-m-tolylphthalamic acid accelerates maturation of the young leaves and, thereby, alleviates their inhibiting effect (24). A defoliation experiment confirmed DeZeeuw's (24) observation on the stimulation of the subjacent inflorescence by removal of the sympodial bud (Table III, Figure 9). However, histological examinations of meristems from N-m-tolylphthalamic acid treated plants (Figure 7b, d) do not confirm his interpretation that premature maturation of the young leaves of the sympodial bud is involved. On the contrary, the sympodial bud remains in a juvenile or meristematic state for a longer period of time (Figure 6g, h). Consequently, the young leaves above the inflorescence are delayed in initiation and growth. It is possible that this suppression of the sympodial bud could prevent or reduce the production of a flower inhibiting substance by the vegetative tissues and, thereby, indirectly stimulate flower formation. In this event, however, the accelerated maturation of young leaves, which DeZeeuw (24) suggests as the mechanism of N-m-tolylphthalamic acid action, would not be involved.

While there is little doubt that a vigorously developing sympodial shoot can, under certain conditions, retard or suppress flower initiation, an

interpretation is possible which does not involve the inhibition proposed by DeZeeuw (23, 24). It is well established that actively growing organs have the capacity to divert nutrient supplies and thereby retard development of less vigorous adjacent organs. This has been clearly demonstrated by Marre and Murneek with auxin treated tomato ovaries (74), When one or more ovaries in a cluster were treated with a supra-optimal concentration of auxin, growth and accumulation of carbohydrates were depressed in adjacent ovaries treated with an amount of auxin ordinarily sufficient to induce a maximal growth rate in the absence of competing fruits. Unfortunately, the more subtle competition which may exist between juvenile stages of flower primordia and adjacent vegetative organs has not been intensively studied.

Goebel (31) in discussing quantitative correlations said "of the numerous organ initials, many remained undeveloped because the building materials which they need for their development go to others which can attract these materials more powerfully". Murneek (82) and Miller (77) found a negative correlation existing between vegetation and reproduction. Murneek (81) observed curtailment of vegetative growth with maximum set of fruits or seeds. He suggested that the fruits might be drawing the available food supply from the part of the plant nearest to them. Eaton (26) by removing flowering branches from cotton plants obtained four times as much root growth and total growth more than double that of the checks. McCollum (76) noted growing

fruits of cucumber exerted an inhibiting influence upon plant growth until the seed coat of the developing seeds began to harden and mature. Murneek (80), Wittwer and Murneek (133) and Wittwer (131) suggested that accelerated vegetative growth after removal of immature inflorescence and fertilized ovaries was correlated with synapsis (union of chromosomes) and syngany (union of nuclei), respectively. However, the acceleration of vegetation was curtailed later by further fruit development. Apparently then, growth of either vegetative or reproductive organs can be stimulated by removal of the other competing organ.

In contrast to the stimulation of vegetative growth obtained in the above mentioned studies, the results indicate that in indeterminate tomato plants, the developing sympodial bud has a predominate option on food supply and growth elements over the adjacent inflorescence. This is illustrated in Table III where greater flower numbers were obtained in the inflorescence when the sympodial bud, with its young leaves, was removed. Inhibition of flower development, but no reduction in flower number, occurred when all the older leaves were removed and young leaves allowed to remain. Histological examinations indicated that in plants receiving N-m-tolylphthalamic acid, the inflorescence maintained its apical dominance for a longer period of time and consequently, it delayed the development of the sympodial bud and the exertion of apical dominance by its vegetative organs.

Finally, with N-m-tolylphthalamic acid treatment, the presence or absence of the sympodial bud had no direct effect on the number of flowers obtained in the adjacent inflorescence (Table IV). This indicates that N-m-tolylphthalamic acid affects the flowering pattern of tomato plants in two ways: (1) It delays the development of the sympodial bud; and (2) it exerts a direct action on floral multiplication. The second statement is based on the fact (Tables II, III) that N-m-tolylphthalamic acid resulted in greater flower numbers in the inflorescence than the removing of the sympodial bud.

Genetic and Chemical Effects on Flowering:

As indicated by Crane (22), the simple racemose type of tomato inflorescence is due to a single dominant gene. N-m-tolylphthalamic acid treatment apparently alters the expression of this dominance and the resulting inflorescence is polychotomous. This is a temporary phenotypic effect, and polychotomy does not occur in later formed inflorescences of the same plant (110). Wagner et al. (120) has described phenotypic adaptations of this type as products of the interaction of environment and genotype. This adaptation is not inherited unless it, in addition, causes genetic materials to mutate. Since the determinants of the phenotype expression are located in the cytoplasm, the expression of a gene may be considerably altered without the gene itself being altered (120). In the cytoplasm this might involve an enzyme shift, as illustrated by a change in temperature optima in Paramecium auralis;

yeast and bacteria with change of substrate (120). In the case of the tomato however, low temperature treatment of tomato seedlings also induces polychotomous inflorescences (17, 62, 63, 70, 124, 126, 134, 135).

The development of fasciated flowers on many N-m-tolylphthalamic acid treated inflorescences further supports the assumption that the ability to mobilize food reserves is enhanced in the floral primordia enabling them to develop at the expense of the sympodial bud. This is in agreement with the suggestion (129) that "The basic cause of fasciation is a disturbed metabolism, involving excessive nutrients which mobilize energy that must be utilized. The energy once accumulated, must go into growth, and it becomes 'widely' expanded in extravagant, abnormal and unpredictable tissue production."

In genetic studies, fasciation in tomato flowers is found to be a partial recessive characteristic (138). Since N-m-tolylphthalamic acid intensifies fasciation in the tomato inflorescence, the chemical is again apparently affecting the expression of a gene. It is interesting to note that N-m-tolylphthalamic acid permits the expression of two recessive characteristics of tomato plants, polychotomy of inflorescences and fasciation of flowers. Whether the chemical is effecting the expression of a single gene or the wo characteristics are governed by two separate genes, remains to be clarified. Similarly, cold temperature exposure causes tomato flowers to fasciate (124, 126).

Dosage and Site of Treatment on Flowering:

When dosage-response data for several methods of application were compared, direct treatment of the meristem gave essentially the same flowering response as when the entire plant was sprayed. However, a considerably higher dosage to the plumule leaf is required to elicite similar responses. This suggests that N-m-tolylphthalamic acid exerts its action directly on the meristem. Furthermore, direct addition of the chemical to the root media failed to produce any appreciable increase in flower number over a wide concentration range (Tables IX, X), although vegetative termination above the second inflorescence suggests that the sympodial bud was completely inhibited in development. On the other hand, the presence or absence of the sympodial bud apparently has little effect on the increase in flower numbers obtained with N-m-tolylphthalamic acid treatment (Table IV).

Distribution patterns of radioactivity after treatment with C-14 N-m-tolylphthalamic acid showed a decreasing recovery of the chemical from the plants at successively later samplings (Tables XI, XII; Figure 11). In the early periods, most of the activity was detected in the stems and leaves above the treated plumule leaf. Accumulation of radioactivity at later intervals in the roots and the negligible quantities in the meristem might suggest that N-m-tolylphthalamic acid was translocated to the roots and induced the roots to produce another compound which was transported to the meristem and in-

duced flower primordia multiplication. Teubner and Wittwer (111) have found, in differential temperature exposure of tomato roots and tops, that the root temperature was responsible for the increase in flower numbers from cold exposure. These data suggest the production of a substance in the roots which affects differentiation of the flower cluster. From the similarity in responses, it would appear that N-m-tolylphthalamic acid acts in the same manner as the hypothetical substance produced in the roots. Fuller et al. (29) exposed roots of a short day plant, Amaranthus candatus L., to short (7 hours) and long (14 hours) illuminations while the tops received a photo-inductive period of 7 hours. They found a delay in flower initiation and retardation in formation of floral parts. Thus, roots can affect flower initiation and development in photoperiodic studies and may participate in flower formation in vernalized tomato plants. An indirect action of N-m-tolylphthalamic acid involving the roots is less likely in view of the dosage-response effects discussed above and the failure to obtain an increase in flower numbers with additions of the chemical to the root media (Tables IX, X).

If autoradiograms of C-14 N-m-tolylphthalamic acid treated tomato seedlings are examined (Figure 12), there is an apparent concentration of activity in the meristem after 24 hours. The failure to confirm this in the distribution studies may partly be accounted for by the negligible amount of tissue in the meristem portion of plant extract with acetone. Finally, if the

quantity of N-m-tolylphthalamic acid in the meristem for an appreciable flowering response is less than 0.01 microgram, it would not be detectable with the rather low specific activity of the C-14 labeled N-m-tolylphthalamic acid employed in these studies. Partial support for this suggestion can be inferred from the data presented in Table XII. The extracted meristems yielded approximately 0.01 microgram equivalents based on the activity of the applied N-m-tolylphthalamic acid, and plants in this study which were allowed to develop the first inflorescence showed the typical increase in flower numbers. In contrast, no activity was found in the meristems of the plants in a second study (Table XIV) and subsequent flowering of similarly treated plants did not exhibit any increase in flower number.

Biological Activity:

The activity of N-m-tolylphthalamic acid in Avena coleoptile straight growth, although admittedly low, and in stimulation of parthenocarpic development of tomato ovaries suggest that this chemical is an auxin. An important difference from more typical auxins is the possible rapid decomposition into the inactive phthalic acid and m-toluidine through hydrolysis of the amide bond.

This hydrolysis has been found to occur readily under either acidic (pH 3.0-5.0) or basic (pH 10.0) conditions (Figure 13). When the half-life of N-m-tolylphthalamic acid was measured, in solution at pH 6.8, using the

flowering response as an assay, the value of 27.7 hours obtained was considerably lower than expected from the spectrophotometric data presented by Feldman et al. (28). Attempts to measure this hydrolysis spectrophotometrically at pH 6.8 were not only unsuccessful (Figure 13), but produced some inexplicable anomalies.

The widespread occurrence of amidases such as urease, asparaginase, glutaminase in plants (106, 142), suggests the possibility that enzymatic hydrolysis of the amide bond may also have been involved. Furthermore, Small (97) had found that tomato protoplasm has a pH of 4.6, which could result in a rapid non-enzymatic hydrolysis of the amide bond.

An indication of the very short biological half-life of N-m-tolylphthalamic acid is inferred from the data presented in Tables XIII and XV. There was a 50 percent loss of activity during the first 24 hours in one study and more than 80 percent loss during the same period in the later study. Apparently, the intact N-m-tolylphthalamic acid is not only rapidly hydrolyzed but the C-14 labeled phthalic acid is decarboxylated at an even more rapid rate and the evolved $C^{14}O_2$ lost to the surrounding atmosphere. This was confirmed in a subsequent study where chromatography of the acetone extract of the treated leaf revealed that most of the activity present was as the intact N-m-tolylphthalamic acid. Thus, the activity recovered in the various plant parts is a reasonably valid estimation of the amount of N-m-tolyphthalamic acid present at the various intervals.

The data presented by DeZeeuw (24) show that low concentrations of α-naphthaleneacetic acid (0.05 to 5.0 ppm in lanolin) applied to the cut stem after removal of the sympodial bud increased flower numbers in the subjacent inflorescence. In contrast, N-m-tolylphthalamic acid was reported to reduce flower numbers when applied in this manner. However, only a single concentration of 10,000 ppm (in lanolin) was employed. Similarly, a concentration of 500 ppm naphthaleneacetic acid reduced flower numbers. Teubner and Wittwer (110) have also observed that excessively high concentration of N-aryl-phthalamic acids applied in close proximity to the floral meristem reduced flower numbers even in the presence of sympodial bud. An indication of this inhibitory effect can be seen in Table V. The stimulation in flower production which DeZeeuw (24) obtained by applying 10,000 ppm N-m-tolylphthalamic acid to a cotyledon is in close agreement with the results presented in Table VI, which show increased flower numbers when concentrations above 1,000 ppm were applied to a plumule leaf. Finally, Teubner and Wittwer (110, 111) have reported that indoleacetic acid will cause increased flower bud formation, but that subsequent development of these buds is retarded and they absciss before full development. This effect of auxins in bud development has been described by Hemphill (45).

It would appear, therefore, that the enhanced production of flowers obtained with N-m-tolylphthalamic acid is indeed an auxin induced response

but that the lability of this and related chemicals preclude the secondary and, often adverse effects on bud development found with more typical auxins. The actual concentration of auxin required at the meristem would appear to be of the order of 0.01 microgram or less. This is in close agreement with the report of Kramer and Went (56) that the apical meristem with adjacent young leaves produces diffusible auxin in amounts of 0.001 microgram equivalent of indoleacetic acid. These workers found that stem tips from 10 day-old tomato seedlings produce only one-tenth this amount, but this rapidly increases to a maximum during the following three weeks. This same period is closely associated with differentiation of the flower buds (Figures 6, 7, 8). A more pertinent observation by Kramer and Went (56) was that exposure of young seedlings to low temperatures (46° F) doubled the auxin production by the stem tip as compared to seedlings maintained at 72° F. These same conditions lead to both earlier initiation (Table I) and increased flower production (124, 126, 134).

In conclusion, it may be stated that flower formation in the tomato appears to be an auxin controlled response, with both promotive and inhibiting effects obtained from applied auxins. In this respect, N-m-tolylphthalamic acid apparently functions as an auxin, and this property of the chemical is confirmed by its activity in stimulating parthenocarpic development of tomato ovaries and Avena coleoptile straight growth. Typical auxins, such as

indoleacetic acid produce a similar stimulation of multiple flower bud primordia, but apparently persist in the meristem at levels sufficient to cause bud abortion. The unique action of N-m-tolylphthalamic acid in causing increased flower numbers over a wide range of concentrations is evidently related to the lability of the amide bond, which results in a relatively short biological half-life. This property of biological lability in an auxin or plant growth regulator should permit a control of many other morphogenetic responses in plants which heretofore has not been possible.

SUMMARY

Histological examination of the tomato apical meristem at cotyledon expansion revealed that only the two primary leaves had been initiated. The subsequent development of apical meristems of plants grown at 72° F and 55° F night temperature was followed. The cold exposed plants initiated fewer leaves before the apex entered the transition from vegetative to reproductive. In control plants this corresponded to the 2-3 leaf stage of development, and occurred 10 days after cotyledon expansion. The transition was two days earlier in the cold exposed plants than those grown at the warmer temperature.

Application of N-m-tolylphthalamic acid produced polychotomous inflorescences in treated plants, provided the apical meristem had reached the transitional vegetative to reproductive state. In such treated meristems the flower primordia remained in a juvenile state for a longer period of time, developed multiple meristematic regions, and each of these eventually matured as a simple floral raceme. It appeared that this accelerated meristematic activity and the associated increase in nutritional demands suppressed the development of the sympodial bud, although a direct action of the chemical on the sympodial bud is not precluded. There was a day lapse between the initiation of a flower bud and the differentiation of its floral whorl in non-

chemically treated meristems. This period was extended to six days in N-m-tolylphthalamic acid treated meristems. The non-treated meristem completed its flower bud initiation seven days after vegetative to reproductive transition of the apex.

The defoliation studies indicated the presence of young leaves (leaves formed after the inflorescence) reduced the flower numbers in the adjacent inflorescence. The presence or absence of the sympodial bud had no significant effect on flower numbers in the adjacent inflorescence when the tomato plants were treated with N-m-tolylphthalamic acid.

The terminal meristem appeared to be the primary site of action of N-m-tolylphthalamic acid. When several methods of application were compared, a single drop to the apical meristem produced a response equivalent to spraying the entire seedling. Application to plant parts at points distal to the meristem required successively higher dosages to elicit comparable flowering responses. Root application of N-m-tolylphthalamic acid over a wide range concentration to plants through solution culture did not increase the number of flowers.

The distribution of carbon-14 labeled N-m-tolylphthalamic acid applied to a plumule leaf showed considerable accumulation of activity in the roots, but the concentration per unit mass of tissue was far below that accumulated in the apical meristem as shown by autoradiograms. Recovery

of total activity applied in two separate experiments was only 50 and 20 percent, respectively, in 24 hours.

The data presented indicate a very short biological half-life of N-m-tolylphthalamic acid. Chromatography of extracts of treated plant tissue showed that most of the radioactivity present was as the intact N-m-tolylphthalamic acid. This indicates the rate of decarboxylation of the hydrolyzed product, phthalic acid, is as high or higher than the hydrolysis of the amide bond between phthalic acid and m-toluidine in the intact molecule of N-m-tolylphthalamic acid. These products of hydrolysis were completely inactive in the flowering response.

The activity of N-m-tolylphthalamic acid in stimulating both Avena coleoptile straight growth and parthenocarpic fruit development indicated that it is an auxin. This characteristic plus the fact that low concentrations of typical auxins will also cause increased flowering in the tomato suggest that flowering in the tomato is an auxin controlled phenomena.

It would thus appear that differentiation of meristematic tissue in the inflorescence into either flower buds or branches is dependent on both the concentration of auxin and the supply of food materials. Increased auxin concentrations or food supplies apparently favor a branched inflorescence, although excessive levels of auxin may inhibit this or subsequent development of the flower buds. N-m-tolylphthalamic acid probably acts as an auxin in causing branching and increased flower numbers.

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