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Morphological Evaluation of Apical Meristem Decline in Greenhouse-grown Tomato Transplants and the Effect of Mineral Nutrition on Its Occurrence

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Abstract. **Tomato (*Lycopersicon esculentum* Mill.) transplants can be affected by an intermittent physiological problem manifested by loss of apical meristem function and retarded growth rates, referred to herein as apical meristem decline (AMD). Production losses associated with this condition can be substantial. Similar abnormal and arrested development of the shoot apex has been observed in a number of other species, and referred to as blindness, budlessness, toplessness, blindwood, and bud abortion. A developmental study using scanning electron microscopy was conducted in 'Agriset' tomato during an occurrence of AMD to evaluate and compare normal and afflicted plants. The AMD condition was associated with cessation of leaf primordia development and lack of flower initiation. The shoot apex of plants with AMD remained vegetative compared to normal plants which at the same age had well-differentiated flower primordia. No evidence of abortion, die back, or necrosis of the shoot apex was observed. The effects of mineral nutrient additions on symptom development varied with year. In year 1, N fertilization reduced the incidence of both AMD and retarded bud growth (i.e., the percentage of normal plants increased from 29% to 97% with N applications). Preplant applications of P, alone or in conjunction with $CaCO_3$ and trace elements, also ameliorated AMD. In year 2, AMD was observed only at very low levels, i.e., 4% or less, and mineral nutrition had no apparent effect on AMD or normal plant number.**

Loss of apical meristem function or apical meristem decline (AMD) occurs in tomato (Lycopersicon esculentum) seedlings, and can be a significant problem for the vegetable transplant industry. Affected plants exhibit retarded growth and cessation of terminal growth. When transferred to the field, plants not identified with AMD undergo an additional set back during pruning procedures which essentially remove most active (lateral) meristematic tissue. Surveys of commercial plantings in the southeastern United States show the incidence to range from 10% to more than 90% of the production within an afflicted area (Vavrina, 1993). In Florida alone, tomato production is valued at $588 million (Florida Agricultural Statistics Service, 2002). The tomato transplant industry is appreciable with a production of more than 520 million tomato transplants annually and an estimated value of $15 million (Vavrina and Summerhill, 1992). Production losses associated with AMD can be substantial, particularly in plants cultivated during the fall–winter seasons when the condition is most prevalent. The fall Florida tomato industry requires >65×10^6 transplants (U.S. Department of Agriculture/National Agricultural Statistics Service, 1999).

Abnormal, incomplete, or arrested development of the apical meristem has been reported in a number of other crops including brassicas (*Brassica* L. sp.) (Wurr et al., 1996), cauliflower (*Brassica oleracea* L.) (Salter, 1957), baby's breath (*Gypsophila paniculata* L.) (Hicklenton et al., 1993), rose (*Rosa hybrida* L.) (Horridge and Cockshull, 1974; Moe, 1971; Nell and Rasmussen, 1979a, 1979b), and geranium (*Pelargonium ×hortorum* L.H. Bailey) (Armitage and Kaczperski, 1992). This condition has been variously referred to as blindness, budlessness, toplessness, blind-wood, and bud abortion. Cessation of shoot growth and lack of visible flower production are characteristic of this condition. Leaves may be fewer in number, distorted, thick, fleshy, and/or stem-like (Armitage and Kaczperski, 1992; Moe, 1971; Salter, 1957). The condition may yield a multistemmed plant with decreased growth. In flowering crops such as roses (*Rosa* L. sp.), affected shoots are not marketable and must be removed (Nell and Rasmussen, 1979b).

Critical studies describing the morphological condition of the shoot apex in plants with AMD are limited. A structural evaluation of the organization of the shoot apex in plants with AMD is fundamental to understanding the underlying causes of this condition and central to the development of protocols to alleviate its occurrence. Due to the erratic appearance of AMD, it is difficult to conduct definitive cause-and-effect studies in that severe treatments may fail to produce any appreciable level of the disorder (Wurr et al., 1996). However, in the course of conducting plant nutrition studies in tomato, AMD arose and showed some positive relationships to fertilization regime. Therefore, the objectives of this study were to 1) evaluate and compare apical meristem development in tomato transplants with normal growth and AMD using scanning electron microscopy (SEM), and 2) document the effect of fertilization regime on frequency of AMD.

Materials and Methods

Developmental studies. Seedlings of 'Agriset' tomato (PetoSeed, Saticoy, Calif.) for the SEM study were obtained from a commercial transplant grower in Naples, Florida in 1997. The seeds were sown into Styrofoam plug flats (242 cells per tray; 25 cm^3 per cell) with a 7 peat : 3 vermiculite medium (by volume) containing a standard commercial nutrient charge (P, lime, fritted trace elements). Seeds were sown from late October to early December 1997 which is a peak period for the appearance of AMD. Plants were grown in an open-sided, double layer polyethylene covered greenhouse under conditions of natural light and ambient temperature. Fertigation was applied to plants two to three times per week, depending on weather conditions, as 350 $mg \cdot L^{-1}$ of a 20N–4P–12K formulation (Miller Chemical and Fertilizer Co., Hanover, Pa.).

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Plants that exhibited normal development or AMD were selected for microscopic evaluations of the shoot apex. Normal plants had successively developing immature leaves at the shoot apex. Plants designated as having AMD were identified by delayed or inhibited leaf development at the shoot apex. Samples were collected from plants at five different ages (i.e., 18, 28, 35, 42, or 56 d after sowing). Five to six shoot tips per age and condition were harvested. Older leaves were removed, and the shoot apex was carefully excised. Tissues were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2, dehydrated through a graded ethanol series, and critical point dried through CO_2. Afterwards, samples were further dissected as needed, removing leaf primordia to expose the shoot apex. Samples were mounted on aluminum stubs using double-sticking tape, sputter-coated with gold/palladium, then viewed with a scanning electron microscope (model 505; Phillips, Mawah, N.J.).

Nitrogen fertilization. AMD occurred at appreciable levels during two fall seasons in experiments designed to determine N requirements for fresh-market tomato transplants grown in a subtropical environment (Vavrina et al., 1998). Fourteen-day-old tomato seedlings were subirrigated daily with a 25% Hoagland's solution (Hoagland and Arnon, 1950) containing N (NH_4NO_3) at 0, 15, 30, 45, 60, or 75 $mg \cdot L^{-1}$ for 5 weeks. The treatments were replicated four times in a randomized complete block design. The experiment was conducted on two different years (1992 and 1993). Following treatment schedule, plant sampling and field setting, 50 or 100 (depending on year) of the remaining transplants were assessed visually for the frequency of AMD or retarded bud growth. AMD was designated as either no new leaf formation or severely limited development. Retarded growth was designated as anything exhibiting a new leaf, but suggesting an out of phase growth relationship when compared to the subtending leaf.

Preplant nutrient addition. Similarly, in an experiment designed to evaluate the need of mineral nutrient additions (lime, P, fritted trace elements) to a transplant plug mix, AMD arose again (Vavrina, 1997). About 14 d after medium amendment and seeding, tomato transplants were put on a fertigation regime of twice weekly applications of 300 $mg \cdot L^{-1}$ of a 20N–8P–12K fertilizer (Miller Chemical and Fertilizer Co.). Upon completion of the experiment (5 weeks), plant number within the flats varied due to various sampling procedures. However, all remaining plants from three replications of a randomized complete block design were counted (range 100 to 141) and were separated into fractions with AMD, retarded growth, or normal growth. The fractions were converted to percentage to normalize the data and subjected to analysis of variance with mean separation by Fisher's protected least significant difference (LSD) (SAS Institute, Inc., 1989). The number of true leaves present, assessed as fully formed leaves, was also analyzed statistically to determine if the onset of AMD could be tied to a physiological age/nutrition parameter.

Results and Discussion

Normal seedling growth in tomatoes was characterized by a continuous and successive production of developing leaves.

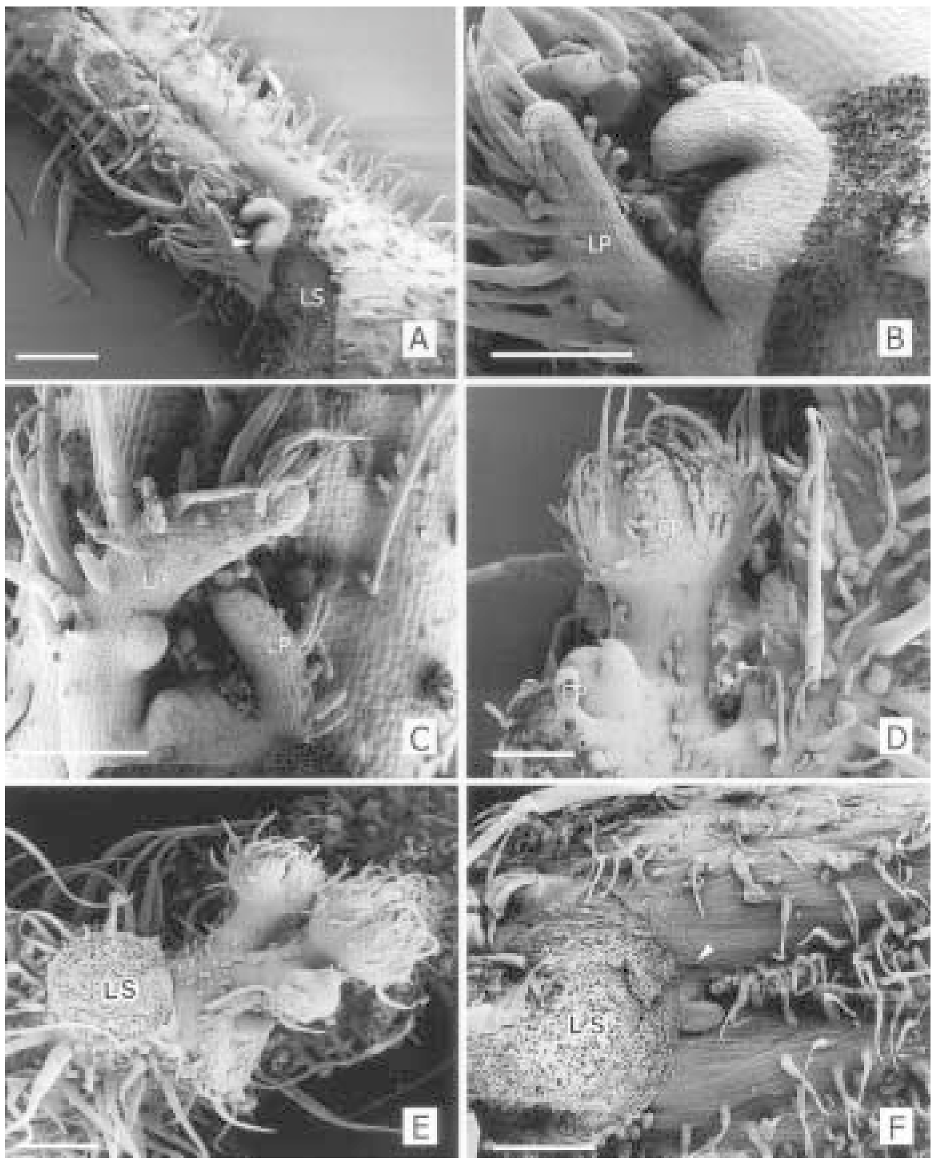

Fig. 1. Scanning electron micrographs of shoot apical areas from 'Agriset' tomato seedlings with normal growth or apical meristem decline (AMD). (**A**) Shoot apex (point) from a normal plant, 18 d after sowing, is shown surrounded by subtending leaf primordia. An older leaf has been removed to expose the apex (LS = leaf scar). The base of the next-older leaf extends behind the shoot apex (arrow). (**B**) Higher magnification of the shoot apex shown in **A**. The shoot apex is vegetative and subtended by three leaf primordia (LP). Trichomes are prevalent on the abaxial surface of the older primordium. (**C**) Shoot apex of a plant 18 d after sowing which exhibited AMD. The shoot apex is vegetative (LP = leaf primordium). (**D**) Shoot meristem from a normal plant 35 d after sowing. The shoot meristem is reproductive with differentiated flower primordia (FP). (**E**) Shoot apex of a normal seedling 42 d after sowing. Numerous flower primordia are visible within the inflorescence. (LS = leaf scar). (**F**) Shoot apex from a plant with AMD 56 d after sowing. The apical meristem (arrow) is vegetative and subtending leaf primordia are poorly developed. The meristem is enclosed within older, developed leaves. A leaf scar (LS) and adhering leaves are visible. (Scale bars: **A**, **E**, **F** = 400 μm; **B**, **C**, **D** = 200 μm).

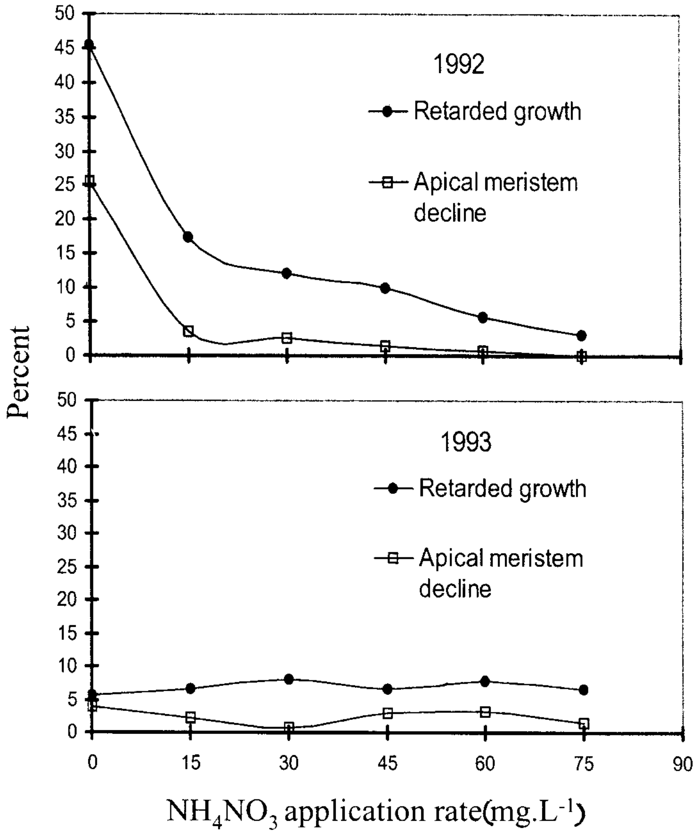

Fig. 2. Effect of N (NH_4NO_3) nutrient applications on the occurrence of AMD and retarded growth in 'Agriset' tomato transplants. (**A**) Observations in 1992, data based on 100 randomly selected transplants. (**B**) Observations in 1993, data based on 50 randomly selected transplants.

Plants with AMD exhibited a cessation of growth and retarded development of smaller leaves. In some cases, developing leaves in affected plants were distorted, and in later stages leaves were sickle-shaped with purple pigmentation.

The shoot tips of normal tomato seedlings 18 d after sowing were typically subtended by developing leaves and leaf primordia (Fig. 1A). Immature leaves near the shoot apex were unexpanded, and had numerous, elongate trichomes. Normal plants at this age characteristically had vegetative, nonreproductive, dome-shaped shoot apices (Fig. 1B). The apical meristem was surrounded by successively older leaf primordia. The shoot apical meristems in plants with AMD of the same age were similarly vegetative (Fig. 1C), with leaf primordia subtending the shoot apex. Although normal and AMD plants exhibited differences in gross morphology related to leaf development, the apical meristems of the two groups of plants showed no marked differences in size or configuration in young plants.

In normal tomatoes seedlings, the shoot apex became reproductive 35 to 42 d after sowing. Flower primordium development varied within an inflorescence (Fig. 1D). Younger primordia had only a differentiated floral apex or had early sepal primordia initiated peripherally around the apex. Older flowers had well-differentiated sepals that enclosed the developing floral apex. At 56 d after sowing, normal plants had inflorescences with numerous flowers at various stages of development (Fig. 1E). In contrast, the meristems of seedlings with AMD remained vegetative at all sampling dates. The vegetative apex from a plant 56 d after sowing is illustrated in Fig. 1F. The shoot apex retained a rounded dome-shaped appearance. Older leaves and a few undifferentiated primordia subtended the apex. Intermediate-staged leaves generally were absent.

In tomato, the condition of AMD appears to be associated with cessation of leaf primordia development and lack of flower initiation. We saw no evidence of abortion, die back, or necrosis of the shoot apex. In contrast, Horridge and Cockshull (1974) evaluated flower initiation and development in glasshouse rose by dissecting the apex of lateral shoots. They postulated that production of blind wood was caused by events occurring after flower initiation, i.e., the abortion of developing flowers, because all actively growing buds appeared to initiate flowers. Similarly, AMD has been attributed by others to death or degeneration of the growing point (Armitage and Kaczperski, 1992; Wurr et al., 1996) or abortion of the flower at an early stage of development (Hubbell, 1934; Moe, 1971). However, these previous studies did not evaluate shoot apex morphology microscopically. Our results concur more with those of Nell and Rasmussen (1979a) who evaluated rose shoot tips using SEM. They likewise found that blind shoots were in the vegetative state compared to flowering shoots of the same age that had differentiated floral initials.

Lack of floral development observed microscopically in tomato plants with AMD concurs with observations of plant growth in the field. Suppression of terminal growth in AMD plants is associated with lack of fruiting at that location. Subsequent fruiting on affected plants occurs on lateral suckers. Sucker removal, like that applied during conventional pruning of normal tomato plants, results in further loss of yield in plants with AMD. Retention of some sucker growth on plants with AMD allows fruiting development on lateral shoots (Vavrina, 1993).

The effect of N addition on the occurrence of AMD and retarded growth in tomato transplants varied with year (Fig. 2). In 1992, AMD and retarded growth were very prevalent with >72% of the plants exhibiting either disorder (Fig. 2A). Nitrogen application had a pronounced effect. Application at the lowest concentration tested (i.e., 15 $mg \cdot L^{-1}$ NH_4NO_3) dramatically reduced the occurrence of AMD and retarded growth by 7- and 2.6-fold, respectively. Nitrogen when applied at 75 mg ammonium nitrate/L resulted in a 100% and 93% decrease in plants that had AMD or that exhibited retarded bud growth, respectively. In early work by Hubbell (1934), blind-shoot formation in rose decreased with an increase in soil nitrates. Chemical analysis indicated that blindness was associated with high percentages of noncolloidal N and insoluble carbohydrates while flowering shoots contained high percentages of reducing sugars. In the current study, tomato transplants in 1993 exhibited a lower incidence of bud decline compared to that seen in 1992 (Fig. 2). Apical meristem decline and retarded bud growth was little affected by N addition.

Preplant mineral nutrient studies indicated that P may play a role in alleviation of AMD in tomato (Table 1). Addition of P, whether alone or in combination with $CaCO_3$ and/or with trace elements, significantly reduced the incidence of plants with AMD. However, decreases in AMD were associated with a significant increase in the incidence of plants with retarded bud growth. A small, but significantly higher number of normal plants was associated with the addition of P as superphosphate or in combination with trace elements. Thus, preplant P addition mitigated, but did not normalize inhibited shoot growth in tomato seedlings. In the present experiment, liquid P (10 $mg \cdot L^{-1}$) was supplied twice weekly beginning ≈14 d after seeding. Therefore, the P amelioration effect on AMD may have been the result of either early application or a higher rate. Phosphorus addition also

Table 1. Effect of preplant nutrient additions on the occurrence of 'Agriset' tomato plants that exhibited apical meristem decline (AMD), retarded bud growth, or normal growth.

Nutrient addition	Rate	AMD %	Retarded growth (%)	Normal (%)	True leaves (no.)
Control	No additions	55.1 ab[z]	44.2 bc	0.7 c	2.0 d
Superphosphate	42.0 g/28.4 dm^3	4.6 d	89.4 a	6.1 ab	3.2 a
$CaCO_3$	67.2 g/28.4 dm^3	65.4 a	34.6 c	0.0 c	2.0 d
Trace[y]	0.8 g/28.4 dm^3	36.1 bc	63.0 b	0.9 c	2.0 d
$CaCO_3$/Trace	Combined	35.6 c	63.1 b	1.3 c	2.2 cd
P/Trace	Combined	6.2 d	85.5 a	8.3 a	3.0 ab
$CaCO_3$/P/Trace	Combined	4.0 d	93.5 a	2.5 bc	2.7 abc
$CaCO_3$/P	Combined	3.3 d	92.9 a	3.8 bc	2.5 bcd

[z]Mean separation (n = 100 to 141) within columns by Fisher's protected LSD, $P \leq 0.05$.
[y]Trace elements = 3% Cu, B, Mn, Mo, Fe, and Mg.

had a tendency to increase the number of true leaves which was likely associated with the reduced occurrence of plants with AMD. Superphosphate and P/trace additions produced significantly more leaves than all treatments without P. A decrease in the number of leaves in blind rose shoots was reported similarly by Moe (1971) who described a loss of the uppermost four leaves.

Occurrence of AMD is intermittent and variable from season to season. Thus, studies to induce the condition and/or evaluate control protocols have been problematic. This was the case in N studies where application effects varied with year. The occurrence of AMD has been attributed to various causes. Most frequently cited as causative factors are low/fluctuating temperatures (Armitage and Kaczperski, 1992; Moe, 1971; Mounsey-Wood, 1957; Salter, 1957; Smith, 1953) and low levels of irradiance (Moe, 1971; Nell and Rasmussen, 1979b; Wurr et al., 1996). Additional factors mentioned include photoperiod (Mounsey-Wood, 1957), over watering (Armitage and Kaczperski, 1992), mineral nutrition (Armitage and Kaczperski, 1992; Hubbell, 1934), competition for assimilates (Wurr et al., 1996; Zeroni and Gale, 1989), and low position of cane pruning (Moe, 1971).

Incidence of AMD is generally expressed in the fall in the Florida transplant industry and therefore suggests the interaction of photoperiod (Mounsey-Wood, 1957). In addition, this period is characterized as having high levels of irradiance and day time temperatures warranting daily irrigations. Perhaps during this time, growers continue to irrigate according to summer production schedules and hence apply too much water (Armitage and Kaczperski, 1992). Excessive irrigation heightens leaching from the soil and, in the mainly overhead-irrigated industry, possibly fosters mineral nutrient stress. Nuances in irrigation and fertilization schemes may account for the erratic appearance and expression severity of this syndrome, although other causes are likely at play as well.

Results of the current study verify that AMD in tomato transplants is due to lack of floral initiation and development which is furthermore associated with suppressed overall activity of the shoot apex. A reevaluation of factors that affect the initiation of leaf primordia and reproductive development may provide insight into strategies to minimize AMD. Nutritional supplements, namely N and P, may be useful to alleviate its occurrence and warrant further study.

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