Cultural Conditions Contributing to Vine Decline Syndrome in Watermelon

Srinivas N. Makam, Wendy Ann Peer, Joshua J. Blakeslee, and Angus S. Murphy¹

Department of Horticulture and Landscape Architecture, Purdue University, Horticulture Building, 625 Agriculture Mall Drive, West Lafayette, IN 47907-0391

Additional index words. *Citrullus lunatus*, Alanap, NPA, aminopeptidase, α -naphthylamine, 1,1'-azonaphthylene, ANA, *Arabidopsis*, MWVD

Abstract. Since the mid-1980s, a syndrome known as mature watermelon vine decline (MWVD) has had a serious effect on watermelon (*Citrullus lanatus* Thunb.) crops in Southern Indiana. As efforts to identify a pathogen responsible for MWVD have been unsuccessful, we have examined cultural conditions that might contribute to the syndrome. Field conditions were simulated in greenhouse pot trials to assess the impact of one or more factors on watermelon growth. Alone, low organic matter, soil acidity, black plastic mulch, and liming did not significantly affect root fresh weight; however, when these conditions were combined, root fresh weight was significantly reduced. Alanap-treated watermelons in combination with simulated cultural conditions resulted in further reduction of root fresh weight and had symptoms similar to MWVD. Watermelon plants grown in Alanap-treated, aged soil (from the previous year's experiments) under combined deficient cultural conditions demonstrated increased symptoms of MWVD and susceptibility to the pathogens *Rhizoctonia* and *Pythium* spp. Alanap, *N*-1-naphthylphthalamic acid (NPA), is a preemergent herbicide that functions as an inhibitor of auxin efflux and is widely used by watermelon farmers to impede obnoxious weeds. Metabolism of Alanap *in planta* involves aryl amidases (aminopeptidases) that also function in defense responses. We hypothesize that negative cultural practices are likely to inhibit defense responses and watermelon resistance to residual Alanap, leading to MWVD. We suggest that MWVD incidence is increased by certain common cultural conditions and that the incidence of MWVD can be reduced by altering these cultural practices.

A syndrome known as mature watermelon vine decline (MWVD) has been described in watermelon crops of southwestern Indiana since the mid-1980s (Egel, 2000). In 2000, MWVD accounted for 20% of the yield losses in the region. Similar sudden wilt syndromes have been described in cucurbit crops in other parts of the US, Mexico, Central America, Spain and Israel (Horlock and Akem, 2004). However, in Indiana, MWVD-like effects have not been reported in other cucurbit crops. Initial symptoms of MWVD include wilt and collapse in patches of affected vines at the time of vine initiation, followed by defective fruit ripening and sunburn. Roots of MWVD plants are stunted, contain fewer secondary roots, and develop lesions which apparently provide sites for opportunistic infection of common soilborne pathogens such as *Rhizoctonia* and *Pythium* spp. Pathogen infection of watermelon roots eventually results in a variety of symptoms including wet-rot or crater-like dry rot (Egel et al., 2000). Interestingly, seedlings inoculated

with either isolated fungi or the dried, ground roots from infected plants failed to produce MWVD symptoms (Egel et al., 2000). Although a number of pathogens implicated in MWVD have been identified, the failure to consistently identify a single pathogen in MWVD roots, combined with the association of MWVD with plastic mulch culture in both fumigated and nonfumigated fields (Egel et al., 2000), suggests that cultural conditions contribute substantially to the syndrome.

A number of cultural factors may contribute to MWVD. In Indiana, MWVD is restricted to watermelon cultivars such as Royal Sweet, which was developed for enhanced disease resistance, but is not found in cantaloupes or older, deep-rooted watermelon varieties, such as Black Diamond (Egel et al., 2000). Certain transplantation techniques, black plastic mulching, drip irrigation, low soil organic matter content, and low soil pH have all been proposed to contribute to MWVD. A striking similarity between roots of MWVD plants and *Arabidopsis thaliana* plants treated with high concentrations of Alanap (*N*-1-Naphthylphthalamic acid) (Murphy and Taiz, 1999a) suggests that the use of Alanap as a preemergent herbicide on watermelon crops might also contribute to the syndrome. A slow oxidative coupling product (1,1'-azonaphthylene, ANA) of hydrolyzed Alanap has been shown to form in Alanap-resistant species (Murphy and Taiz, 1999a). ANA was found to inhibit aminopeptidase activities (Murphy and Taiz, 1999a, 1999b) essential to plant wounding and defense responses (Gu and

Walling, 2000). As the dark, near-anaerobic, wet conditions required for formation of ANA are duplicated in black plastic mulch cultivation, we hypothesize that Alanap compounds the cultural deficiencies that lead to MWVD. Here we present an analysis of the effects of various cultural conditions on the development of MWVD symptoms as well as the impact of Alanap on symptom development.

Materials and Methods

Watermelon growing conditions and sample collection. All experiments were conducted in a computer-controlled greenhouse (Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Ind.), 26 °C day/23 °C night, relative humidity of 60% day/50% night. Day length and light intensity were maintained for a minimum of 12 h at 120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with sodium vapor lamps. 'Royal Sweet' watermelon plants were top-watered daily and fertilized twice a week as indicated. For black plastic mulch treatments, watering was applied underneath the black plastic mulch [0.15-mm (6-mil) polyethylene]. Pots were placed on benches using a randomized complete block design. All experiments consisted of 20 plants per trial (one plant per pot) and were repeated three times. For transplantation, seedlings were grown in Metromix 360 (Scotts, Indianapolis, Ind.) for 3 weeks in either # 804 2 × 2 cell packs or 30-cm conetainers, then transplanted to 18927-cm³ pots containing 70 sand : 30 clay + 2% ground peat for 4 weeks. Default soil mix in 18927 cm³ (5 gallon) pots was altered as noted. At the end of 8 weeks (11 weeks in the case of direct seeded plants), root samples were collected, examined, washed, blotted, dried and weighed.

Fertilizer recipe. Plants were fertilized with Peters all purpose plant food with micronutrients (The Spectrum Group, St. Louis, Mo.). Nutrients were supplied at one-fourth teaspoon per 3758.4 cm³ of the 20N–8.8P–16.6K commercial fertilizer formulation (0.5% Mg, 0.02% Bo, 0.05% Cu, 0.1% Fe, 0.05% Mn, 0.0005% Mo, and 0.05% Zn) twice a week. Adjustment of pH to range 5.7 to 6.0 and alkalinity reduction was achieved by injection of 93% sulfuric acid (Ulrich Chemical, Indianapolis) into watering lines at 0.08 cm³·L⁻¹. Additional sulfuric acid was added for acid treatments where noted.

Treatments. Alanap (3.4 mg/pot) was delivered as a spray once before transplanting and again at vine initiation. Alanap was applied as per label instructions. Cell packs were coated with CuO in latex paint (100 $\mu\text{g}\cdot\text{mL}^{-1}$) when used. Fertilizer was amended with magnesium chloride (1000 ppm MgCl₂) and/or manganese chloride (100 ppm MnCl₂) where indicated. 1000 ppm calcitic lime or 1000 ppm dolomitic lime were used where noted. For aged soil experiment, soil from previous year's control (+Alanap) experiments was collected, bagged in black plastic, and stored outside over winter and spring for weathering.

HPLC analysis. Soil samples (5 g) from pot trials and MWVD-affected fields (history of MWVD) at the Purdue Southwest Indiana Agricultural Farm were extracted in 2 cm³

Received for publication 26 Aug. 2004. Accepted for publication 24 Nov. 2004. We thank Daniel S. Egel and Chris Gunter for soil sample collection and information regarding MWVD in SW Indiana as well as Andreas Westphal for examining tissue samples for pathogens. We also thank Vic Lechtenberg and Ray Martyn for valuable insights into the history of MWVD. Funding for this research was provided by Purdue Agricultural Research Programs and USDA–NRI.

¹To whom reprint request should be addressed; e-mail murphy@purdue.edu.

Table 1. Treatments used to determine cultural and soil factors contributing to mature watermelon vine decline.

Cultural conditions	Treatment ^z															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Transplantation	+	+	+	+	+						+					+
Organic matter			+		+	+					+					+
Black plastic mulch			+		+		+				+					+
Mn toxicity			+		+			+	+		+					+
pH			+		+					+	+					+
Lime			+		+					+		+	+	+	+	+
Deficient condition		+				+	+	+		+				+	+	
Alanap		+	+		+	+	+	+		+	+	+	+	+	+	+

^z1) Conetainer, 2) cell pack, 3) cell pack + CuO, 4) direct sowing, 5) 2% organic matter, 6) low organic matter, 7) black plastic mulch, 8) MnCl₂, 9) MnCl₂ + MgCl₂, 10) acid water, 11) house water, 12) calcitic lime, 13) dolomitic lime, 14) no lime, 15) new soil, and 16) Alanap-treated aged soil.

of 80% acetonitrile in water for 1 week under constant shaking and spun in centrifuge at 500 g . The supernatant was collected and analyzed by HPLC (765 HPLC Compact Pump System, Alcott Chromatography; Norcross, Ga.) using a 5% to 95% acetonitrile/water linear gradient acidified with 0.01% trifluoroacetic acid and UV detection at 255 nm. Genuine standards were used to calculate normalized values.

Aminopeptidase (AP) enzyme assay. AP activity in 8-week-old watermelon roots was assayed using Tyr-AMC and Leu-AMC substrates as described previously (Murphy and Taiz, 1999a; Murphy et al., 2002).

Statistical analyses. Statistical significance was determined using Student's t test and one way ANOVA adjusted by Bonferroni post-test correction for multiple dataset, significance set at $P < 0.05$. All results are given as mean values $\pm$ standard deviations ($n = 20$; $n < 20$ in case of treatment mortality; three replications). Data sets were analyzed using the SigmaStat software package (Point Richmond, Calif.).

Results and Discussion

Soils in southwestern Indiana that have a history of MWVD are in low organic matter,

Fig. 1. Effects of cultural conditions on watermelon root growth. (A) Effect of initial growth conditions on watermelon root growth. Plants were transplanted from conetainers, 2 $\times$ 2-cell packs with or without CuO (100 μ g mL⁻¹), or directly sown into 18927-cm³ pots. 20 plants per trial, three replications. Error bars are standard deviations of mean root fresh weights. Statistical significance was determined by ANOVA followed by Bonferroni posthoc analysis; ** $P \leq 0.01$. (B) Effect of Alanap treatment combined with various cultural conditions on watermelon root growth. Alanap-treated plants were grown under conditions as indicated; 20 plants per trial, three replications. Error bars are standard deviations of mean root fresh weights. Statistical significance from controls was determined by ANOVA followed by Bonferroni posthoc analysis; * $P \leq 0.05$, ** $P \leq 0.01$. (C) Effect of Alanap treatment and aged soil on watermelon root growth. Alanap-treated plants were grown in new soil or aged soil (from previous year's experiments) under conditions indicated. In case of treatment mortality (14 plants in aged soil, no CuO, black plastic mulch, low OM, no lime), total number of viable plants were considered for statistical analysis. Other conditions varied as indicated in methods. 10 plants per trial, three replications. Error bars are standard deviations of mean root fresh weights. Statistical significance from controls was determined by ANOVA followed by Bonferroni posthoc analysis; ** $P \leq 0.01$.

Fig. 2. Documentation of MWVD-like symptoms under various cultural conditions. (A) Roots of control watermelon ('Royal Sweet') (left). Roots of watermelon with black plastic mulch have reduced root growth (right). The scale is the same for both plants. (B) Control watermelons have numerous light-colored roots (left). Watermelons grown with black plastic mulch, low OM, no lime, acid water, 100 ppm $MnCl_2$ have root browning and necrotic lesions symptomatic of MWVD (right). (C) Watermelon roots grown in aged (previous year's Alanap-treated soil) have MWVD-like symptoms: severe necrotic lesions, root browning and slimy, purple-stained roots. (D) Alanap-treated watermelons grown in new soil show no signs of MWVD (left). Alanap-treated watermelons grown in aged soil show MWVD-like symptoms (right). (E) Roots of Alanap-treated watermelons grown in aged soil (black plastic mulch, low OM, no lime) show symptoms of MWVD: severe necrotic lesions, root browning, extensive purple staining of roots, and slimy roots. (F) Roots of Alanap-treated watermelons grown in cultivated soil with a history of MWVD from SW Indiana show symptoms of MWVD: severe necrotic lesions, root browning, extensive purple staining of roots, and slimy roots.

acidic, low in Mg^{2+} , high in Mn^{2+} , and receive Alanap treatments yearly. Black plastic mulch is also used to suppress weeds and maintain soil moisture. Cultural practices and soil conditions that may contribute to MWVD symptoms were assessed singly and in combination in greenhouse pot trials. A treatment table is presented in Table 1.

Direct seeding vs. transplantation. Watermelon root fresh weight was compared between direct seeding and transplanting from conetainers, cell packs, and cell packs treated with CuO to reduce root circling (Struve, 1993; Watt and Smith, 1999). After 11 weeks of growth, average root fresh weight of direct-seeded watermelons and watermelons transplanted from conetainers or CuO-treated cell packs were not significantly different as shown in Fig. 1A ($P > 0.5$). Root fresh weight of watermelons transplanted from untreated cell packs

was significantly less than the other treatments ($P = 0.005$). Transplanted watermelons have shallow, but more extensive roots, compared to direct seeded plants (Elstrom, 1973). Although rooting patterns were shown to be similar for both methods (Eigsti, 1971), transplants have been shown to establish more quickly and root length density in the upper 30 cm of the soil was greater than direct seeded watermelons (NeSmith, 1999). As transplantation from cell packs treated with CuO represents the most economical and practical seeding method, subsequent experiments utilized transplants from CuO-treated cell packs, except where noted.

Organic matter (OM) amendment. Soils from southwestern Indiana watermelon fields are generally low in OM ($<0.5\%$); and OM enhances soil aeration, nutrition, and water retention (Acquaah, 2002). Increasing the OM

content to 2% did not statistically increase root fresh weight ($6.24 \text{ g} \pm 1.73$) compared to untreated controls ($5.53 \text{ g} \pm 1.61$) ($P > 0.05$). Therefore, deficiencies in OM alone are not likely to contribute to MWVD.

Black plastic mulch. Black plastic mulch is used by farmers to conserve moisture and curtail weed growth. After 11 weeks of growth, the average fresh weight of watermelon roots grown under 0.15 mm black plastic mulch (6-mil polyethylene) ($3.61 \text{ g} \pm 1.57$) was less than the controls ($5.88 \text{ g} \pm 1.45$), but not statistically significant ($P = 0.1$). Two-thirds of the root fresh weight occupied the upper 7.5 cm of pot soil in black plastic mulched plants, while two-thirds of the root fresh weight was in the upper 15 cm of the pot in control plants as shown in Fig. 2A. Therefore, black plastic mulch did not statistically reduce watermelon root growth and alone is not likely to contribute to MWVD.

Manganese (Mn) toxicity. As soils in southwestern Indiana are often acidic, Mn^{2+} toxicity could contribute to inhibition of watermelon root growth. Acid pH increases Mn^{2+} solubility and phytoavailability which results in toxicity (Hue and Mai, 2002). Mn^{2+} toxicity is characterized by competition with Mg^{2+} uptake and utilization: Mn^{2+} can compete with cation uptake, displace Mg^{2+} enzyme co-factors, displace Ca^{2+} in cell membranes and enhance axillary shoot growth (Marschner, 1995). Additionally, root aminopeptidase activities involved in defense responses are sensitive to Mn^{2+} (Murphy and Taiz, 1999b). As Mn^{2+} is present in most soils used for watermelon growing, amelioration of Mn^{2+} toxicity with Mg^{2+} was tested by measuring watermelon root growth after treatment with 100 ppm $MnCl_2$ or 100 ppm $MnCl_2$ plus 1000 ppm $MgCl_2$. After 11 weeks, watermelons treated with both Mn^{2+} and Mg^{2+} had significantly greater root fresh weight ($8.16 \text{ g} \pm 1.42$) than untreated controls ($4.71 \text{ g} \pm 1.06$) ($P = 0.02$). Symptoms of Mn^{2+} toxicity, e.g., darkening of leaf veins, interveinal chlorosis, leaf cupping, or necrotic blotching of foliage, were not observed in either treatment.

pH effect. Treatment with acid water (pH 5.1) was used to determine the effects of pH on watermelon growth. When irrigated with water pH 5.1, pot soil pH ranged from 5.5 to 6.0, thus mimicking acidic soil conditions common to Indiana watermelon growing regions. Additionally, low soil pH has been shown to inhibit both the activity of defense-related root aminopeptidases and the *in planta* hydrolysis of Alanap (Murphy and Taiz, 1999a,b). Acid pH (≤ 4.5) has been shown to severely affect productivity (Sundstrom et al., 1983) and watermelon root growth which cultivar dependent (Liu et al., 1994). At the end of 11 weeks, soil acidification did not significantly decrease watermelon root fresh weight ($5.39 \text{ g} \pm 1.58$) compared to controls ($5.94 \text{ g} \pm 1.45$) ($P > 0.05$). This suggests that acidic soil alone does not contribute to MWVD.

Treatments with calcitic and dolomitic lime. Treatment with dolomitic lime (calcium magnesium carbonate) simultaneously increases soil Ca^{2+} , Mg^{2+} , and pH levels while treatment

Table 2. HPLC analysis of soil samples from Southwest Indiana Purdue Agricultural Watermelon Cultivation Farm with a history of mature watermelon vine decline (MWVD). Field soil was analyzed for Alanap and breakdown products. Treatments are as indicated. Values are means $\pm$ SD. Alanap-treated aged soil from pot trials had Alanap breakdown products: $5.4 \pm 0.23 \mu\text{g}\cdot\text{g}^{-1}$ dry weight α -naphthylamine (α -NA) and $1.4 \pm 0.39 \mu\text{g}\cdot\text{g}^{-1}$ dry weight 1,1'-azonaphthylene (ANA); Alanap itself was not detected. Untreated soils had no Alanap breakdown residues. NPA = *N*-1-naphthylphthalamic acid (Alanap), NPI = naphthylphthalimide, and AONA = 1,1' azoxynaphthylene.

Treatment	Dry wt ($\mu\text{g}\cdot\text{g}^{-1}$)		
	α -NA ^z	NPA ^z + NPI ^z	ANA ^z + AONA ^z
Field	18.31 ± 6.83	0.688 ± 0.33	0.451 ± 0.42
Heated 50 °C	16.47 ± 6.37	0.515 ± 0.30	0.405 ± 0.41
Heated 60 °C	0	0.06 ± 0.03	0.07 ± 0.05
Autoclaved	0	0	0

with calcitic lime (calcium carbonate) only increases soil Ca^{2+} and pH. Treatment with calcitic lime resulted in growth that was not different from untreated controls ($P > 0.05$). Consistent with results from the $\text{Mn}^{2+}/\text{Mg}^{2+}$ amendment experiments, 1000 ppm dolomitic lime amendment increased root fresh weight ($7.71 \text{ g} \pm 1.45$) compared to calcitic lime ($5.82 \text{ g} \pm 2.03$) and controls ($5.26 \text{ g} \pm 1.45$), but the increase was not statistically significant ($P = 0.1$). Liming to raise the soil pH results in decreased Mn^{2+} toxicity, and is essential for normal watermelon growth (Hue and Mai, 2002). Dolomitic lime was used post-transplantation for all subsequent experiments except where noted.

Combined deficient conditions. Cultural conditions demonstrated to have a negative impact on watermelon root growth were tested in order to determine whether or not such combinations resulted in a synergistic negative effect. Watermelons grown with black plastic mulch, without lime, 100 ppm MnCl_2 and acid water exhibited a 4-fold decrease in root fresh weight ($2.13 \text{ g} \pm 2.86$) compared to controls ($8.78 \text{ g} \pm 1.29$) ($P = 0.02$). The watermelons also displayed several MWVD symptoms, including increased necrotic lesions and root browning as shown in Fig. 2B.

Alanap treatment with varying conditions. The effects of Alanap (3.0 mg/pot) on watermelon growth were tested alone and in conjunction with other cultural conditions. After 11 weeks, Alanap treatment alone did not significantly affect root fresh weight ($7.82 \text{ g} \pm 2.27$) compared to controls ($8.49 \text{ g} \pm 1.88$) ($P > 0.05$), although some necrotic lesions and a small amount of root browning were observed (data not shown).

Alanap treatment combined with either no additional OM, black plastic mulch, or no lime reduced the root fresh weight of watermelons compared to the Alanap treatment alone ($P = 0.05$, $P = 0.09$; $P = 0.03$, respectively) as shown in Figure 1B. Alanap treatment combined with transplantation from cell packs without CuO, low OM, black plastic mulch, acid water, and no lime significantly decreased in root fresh weight 4-fold ($P < 0.005$; Fig. 1B). Under these conditions, inhibition of Alanap amidohydrolase (aminopeptidase) activity and retention of the Alanap breakdown product α -naphthylamine (α -NA) in soil micelles (Li et al., 2001) would be expected to promote plaque formation and lesions on the root surface. Varying degrees of necrotic lesions and root browning were observed when any negative cultural conditions

were combined with Alanap treatment. These MWVD symptoms were aggravated when Alanap treatment was combined with black plastic mulch, either alone or in acidic soil. Alanap treatment, therefore, appears to have a negative impact on root growth only when other negative cultural factors are present.

Alanap treatment in aged soil from previous year's experiments. Previously, ANA, the colored compound produced by oxidative coupling of Alanap breakdown products, was shown to severely reduce root growth and cause discoloration of *Arabidopsis* roots (Murphy and Taiz, 1999a, 1999b); furthermore, the presence of ANA in soils or media was shown to immobilize Alanap and prevent its further hydrolysis. Alanap-treated soils from the previous year's pot trials were stored over the winter and the aged soil used to determine whether a similar residual effect would be observed. In Alanap-treated watermelons, root fresh weight was not statistically different in watermelons grown in aged soils compared to those grown in new soil ($P = 0.1$) as shown in Fig. 1C, but roots had signs of browning and necrotic lesions as shown in Fig. 2C. Alanap-treated plants grown in new or aged soil showed severe MWVD symptoms when combined with other treatments: untreated cell packs, no lime, low OM, and black plastic mulch. In the combined conditions root fresh weight was significantly reduced in both new and aged soil compared to controls ($P < 0.01$; $P < 0.005$, respectively) (Fig. 1C). Under these combined conditions, 14 of 30 plants grown in aged soil were dead at the end of 11 weeks; representative declining plants are shown in Fig. 2D. These plants exhibited MWVD symptoms, e.g., severe necrotic lesions, root browning, extensive NPA/ANA staining of roots, and distorted branches near the root-shoot transition zone, similar to that observed when plants were grown in Alanap-treated cultivated soil from southwestern Indiana with a history of MWVD, as shown in Fig. 2E and F. These effects are also similar to those observed in Alanap-treated *Arabidopsis* (Murphy and Taiz, 1999a).

No specific pathogen was consistently identified in plants displaying MWVD symptoms, and Alanap residues and breakdown products were detected in cultivated soils with a history of MWVD, as shown in Table 2. Alanap hydrolysis products were detected by HPLC analysis in these cultivated soils even when heated to 50 °C (Table 2). Heating the cultivated soil to 60 °C resulted in the loss of α -NA by volatilization and also reduced the formation of ANA. Autoclaving eliminated all traces

of both compounds (Table 2). It is not clear, however, whether heat treatments of soils from MWVD-affected fields eliminated pathogens, Alanap breakdown products, or both.

The amounts of Alanap residues in Alanap-treated aged soil from pot trials were less than that measured in that measured in the field samples. This demonstrates that Alanap residues can persist in the field soil and accumulate over time, as the fields are treated yearly with Alanap.

At low soil pH, a 15-fold increase of the uncharged species of α -NA ($\text{pK}_a = 3.92$) was observed, leading to increased sorption and retention of α -NA in the soil (Li et al., 2001). Similarly, low soil organic matter has been shown to contribute to the long term persistence of the Alanap breakdown product α -NA in soil micelles (Li et al., 2001). Black plastic mulching with subfilm irrigation promotes the slow degradation of Alanap by generating low oxygen conditions which promote the formation of ANA (Murphy and Taiz, 1999a,b), which is resistant to metabolism by soil flora. This conjugated product inhibits not only Alanap arylamidase activity *in planta*, but also inhibits plasma membrane aminopeptidases that have been implicated in both auxin responsive growth and wound responses (Gu and Walling, 2000; Murphy et al., 2000, 2002). Recently, enzymatic resistance by aminotransferases to watermelon downy mildew has also been reported (Taler et al., 2004). The exact type of aminopeptidase most affected by Alanap treatment of watermelon roots could not be determined by enzymatic assays, although the overall aminopeptidase enzymatic activity in Royal Sweet roots was found to be less than that observed in both Eclipse musk melon and Black Diamond watermelon (data not shown).

Conclusion

Our data support the hypothesis that a number of commonly used cultural practices, when combined with Alanap use, contribute to mature watermelon vine decline. The effects of these treatments are apparently cumulative and, in some cases, synergistic. Watermelons grown under poor cultural conditions in aged soils which had been treated with Alanap during the previous year exhibited the most MWVD symptoms. The occurrence of pathogens in affected plants appears to be opportunistic. Field trials addressing the cultural practices investigated are required to develop remedies for MWVD-impacted watermelon growers.

Literature Cited

- Acquaah, G. 2002. Horticulture principles and practices. 3rd ed. Prentice Hall, Upper Saddle River, N.J.
- Egel, D., K. Rane, R. Latin, and R.D. Martyn. 2000. Mature watermelon vine decline: A disease of unknown etiology in southwestern Indiana. Plant Health Progress. 27 Dec. 2000. Plant Mgt. Network, St. Paul, Minn.
- Egisti, O.J. 1971. A comparison of mature triploid watermelon systems of roots and crown from direct field seeded plants and transplants. Hort-Science 6:288.
- Elmstrom, G.W. 1973. Watermelon root development affected by direct seeding and transplanting.

- HortScience 8:134–136.
- Gu, Y.Q. and L.L. Walling. 2000. Specificity of the wound-induced leucine aminopeptidase (LAP-A) of tomato activity on dipeptide and tripeptide substrates. *Euro. J. Biochem.* 267:1178–87.
- Horlock, C. and C. Akem. 2004. Sudden wilt of melons (watermelon, rockmelon and honeydew). Dept. Primary Industries and Fisheries (DPI&F) note, Queensland Government. 21 Jan. 2004. <http://www.dpi.qld.gov.au/horticulture/11648.html>.
- Hue, N.V. and Y. Mai. 2002. Manganese toxicity in watermelons as affected by lime and compost amended to a hawaiian acid oxisol. *HortScience* 37:656–661.
- Li, H., L.S. Lee, J.R. Fabrega, and C.T. Jafvert. 2001. Role of pH in partitioning and cation exchange of aromatic amines on water saturated soils. *Chemosphere* 44:627–635.
- Liu, A.J., G. Latimer, and R.E. Wilkinson. 1994. Effect of pH on seedling growth of six cultivars of watermelon. *J. Plant. Nutr.* 17:537–548.
- Marschner, H. 1995. Mineral nutrition of higher plants, p. 39, 333. 2nd ed. Academic, San Diego, Calif.
- Murphy, A. and L. Taiz. 1999a. Localization and characterization of soluble and plasma membrane aminopeptidase activities in *Arabidopsis thaliana* seedlings. *Plant Physiol. Biochem.* 37:431–443.
- Murphy, A. and L. Taiz. 1999b. Naphthylphthalamic acid is enzymatically hydrolyzed at the hypocotyls-root transition zone and other tissues of *Arabidopsis thaliana* seedlings. *Plant Physiol. Biochem.* 37:413–430.
- Murphy, A., W.A. Peer, and L. Taiz. 2000. Regulation of auxin transport by aminopeptidases and endogenous flavonoids. *Planta* 211:315–324.
- Murphy, A., K. Hoogner, W.A. Peer, and L. Taiz. 2002. Identification, purification, and molecular cloning of N-1-naphthylphthalamic acid-binding plasma membrane-associated aminopeptidases from *Arabidopsis thaliana*. *Plant Physiol.* 128:935–50.
- NeSmith, D.S. 1999. Root distribution and yield of direct seeded and transplanted watermelon. *J. Amer. Soc. Hort. Sci.* 124:458–461.
- Struve, D.K. 1993. Effect of copper treated containers on transplant survival and regrowth of four tree species. *J. Environ. Hort.* 11:196–199.
- Sundstrom, F.J., R.L. Edwards, R.J. Constantin, and D.W. Wells. 1983. Influence of soil acidity on watermelon leaf tissue mineral concentration and yield. *J. Amer. Soc. Hort. Sci.* 108:734–736.
- Taler, D., M. Galperin, I. Benjamin, Y. Cohen, and D. Kenigsbuch. 2004. Plant *eR* genes that encode photorespiratory enzymes confer resistance against disease. *Plant Cell* 16:172–184.
- Watt, K. and I. Smith. 1996. The effect of copper tray treatment on lodgepole pine (*Pinus contorta* Dougl.) seedlings and their root growth potential after transplanting. *Proc. For. Nursery Assn. Brit. Colombia Meetings*.