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# Root Respiration and Phosphorus Nutrition of Tomato Plants Grown at a 36 °C Root-zone Temperature

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***Abstract.*** **Growth of tomato (*Lycopersicon esculentum* Mill.) plants decreases at root-zone temperatures (RZTs) >30 °C, but no research has been conducted on the effects of changes in root respiration on P acquisition at supraoptimal RZT. We monitored the changes every 3 to 5 days in root respiration, root surface phosphatase activity, and P acquisition of 'Jet Star' tomato plants grown in Hoagland's no. 1 solution held at 25 and 36 °C RZT for 19 days. Root respiration rate in plants grown at 25 °C increased linearly from RZT initiation to day 12, but there was no difference in respiration between days 12 and 19. Root respiration at 36 °C, however, increased from RZT initiation to day 8 and then decreased. Shoot P concentration and root phosphatase activity for plants grown at 25 °C did not change during the experiment. Shoot P concentration for plants at 36 °C, however, linearly decreased over time, and root phosphatase activity linearly increased over time. Decreased shoot growth and demand for P along with decreased root respiration after day 8 probably resulted in the decreased P uptake and shoot P concentration in plants grown at 36 °C RZT.**

Economically important temperate horticulture crops often are subject to root-zone temperatures (RZTs) >30 °C. For example, RZTs ≥35 °C have been reported for tomato plants (*Lycopersicon esculentum* Mill.) grown under plastic mulches (Tindall et al., 1991). According to Tindall et al. (1990), the optimum RZT for Burpee 'Big Boy Hybrid' tomato growth and P uptake is 25 °C. They observed decreased P uptake in Burpee 'Big Boy Hybrid' tomato plants held at 32.2 and 36.7 °C RZTs, but tissue concentrations of P were not deficient (Tindall et al., 1990).

Ion uptake against a concentration gradient requires energy in the form of ATP generated from root respiration (Cooper, 1973; Johnson, 1990, Marschner, 1995). Factors, such as oxygen concentration, carbohydrate supply, and temperature, that affect root respiration also influence ion accumulation. Root respiration rate initially increases with increasing RZT unless limited by accumulation of respiratory products, lack of oxygen or respirable substrate, or inactivation of enzyme systems (Hagan, 1952).

Previous research conducted on tomato root respiration at different RZTs has focused on the short term effects of supraoptimal RZTs (Janes et al., 1988) instead of the long-term effects that are of more ecological importance. No research has been conducted on correlating changes in tomato root respiration and P acquisition every few days for plants held at a supraoptimal RZTs for >1 week. Furthermore, no research has been conducted on the effects of RZTs on the nonspecific surface acid phosphatase activity of tomato roots. Although the precise role of the external phosphatase has not been determined, it might be a phosphate transport agent, the phosphatase activity being incidental, or it might hydrolyze polyphosphates, P-esters, and other organic P compounds in the medium (Bieleski, 1973; Boutin et al., 1981; Juma and Tabatabai, 1988b). Increased phosphatase activity has been used as an enzymatic marker of P deficiency (Bieleski, 1973; Boutin et al., 1981). Our objective was to investigate the changes in root respiration, root surface phosphatase activity, and P acquisition every 3 to 5 d in tomato plants grown at 25 and 36 °C RZT for ≈3 weeks.

## Materials and Methods

'Jet Star' tomato seeds (Green Barn Seed Co., Deephaven, Minn.) were germinated under mist in a greenhouse for 9 d in grade 16 silica sand (Unimin Corp., LeSeuer, Minn.). Seedlings were transferred singly to containers equipped to control RZT (Graves and Dana, 1987) when they had two true leaves. Seedlings were grown in a continuously aerated Hoagland's no. 1 solution (Hoagland and Arnon, 1950) with 0.1 mM FeEDDHA [ethylenediamine di (*o*-hydroxyphenylacetic acid)]. The nutrient solution was adjusted to pH 5 with 0.05 N NaOH and was replaced every 5 d.

The experiment was conducted in January 1995, and repeated in February 1995, in a greenhouse (27 ± 5 °C day and night air temperature and 50% to 85% relative humidity) at Iowa State Univ. (Ames). Natural irradiance of 69 to 162 $W \cdot m^{-2}$ was supplemented by two 400-W high-pressure sodium lamps that provided 70 $\mu mol \cdot s^{-1} \cdot m^{-2}$ of photosynthetically active radiation from 0600 to 2100 HR (values reported according to guidelines by Krizek, 1982).

RZT treatments of 25 and 36 °C were initiated when plants had three to four true leaves and had a fresh mass of 2 ± 0.5 g. Eight plants per RZT treatment were harvested at RZT initiation and 6, 12, 16, and 19 d later in the January replication. The plants grew faster in the February replication. To ensure that tomato plants were harvested at the same developmental stages as in the January replication, the plants were harvested sooner in the February replication at 6, 10, 14, and 17 d after RZT initiation. The pots were arranged in a completely randomized design. To have enough root material to make physiological measurements, roots were combined from two randomly chosen plants per RZT treatment to give four replications for each temperature and day combination. Shoots of the combined root samples also were combined to give four replications per RZT and day combination.

Harvested roots were rinsed three times in deionized–distilled water, and 3-cm-long root tips were cut in a 0.5 mM $CaCl_2$ solution. Root tips were blotted dry and weighed into samples for root

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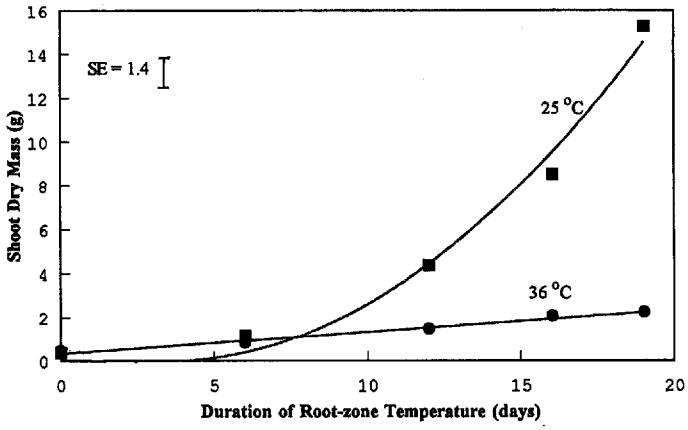


Fig. 1. Increase in mean shoot dry mass of 'Jet Star' tomato plants exposed to a 25 °C (■) or 36 °C (●) root-zone temperature (RZT) for 19 d. Values are means of four replications. Equations of the lines are $y = 0.33 - 0.34x + 0.06x^2$, $r^2 = 0.97$ for 25 °C; $y = 0.23 + 0.11x$, $r^2 = 0.83$ for 36 °C.

respiration and root surface phosphatase measurements.

Root respiration was measured by using a single-valve differential respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.). The reaction vessels were lined with three layers of Whatman no. 1 filter paper that was moistened with 0.8 mL of Hoagland's no. 1 solution. Root samples of 200 mg were placed on the filter paper in the reaction vessel. The reaction vessel was maintained at 25 ± 0.3 °C by using a circulating heated water bath. Oxygen consumption was measured in the presence of 20% KOH (w/v) in the center well of each reaction vessel. Respiration rate was measured immediately after root excision for 1 h and was expressed as $O_2$ consumed on a fresh mass basis ($\mu L \cdot g^{-1} \cdot h^{-1}$).

Root surface acid phosphatase activity was determined by the method of Boutin et al. (1981), Dick and Tabatabai (1986), and Juma and Tabatabai (1988a, 1988b). Activity was determined by the amount of p-nitrophenol (PNP) released when 250 mg of 3-cm-long root tips were incubated with 4 mL of modified universal buffer (pH 5) and 1 mL of buffered 5 mM p-nitrophenyl phosphate (pH 5) for 1 h at 37 °C. The reaction was stopped by adding 4 mL 0.5 M NaOH and 1 mL of 0.5 M $CaCl_2$. Samples were filtered through Whatman no. 1 filter paper, and the intensities of the yellow filtrate were measured on a spectrophotometer (Spectronic 21; Milton Roy Co., Rochester, N.Y.) at 420 nm. Contents of p-nitrophenol were determined from a calibration curve.

Shoots at each harvest date were dried for 72 h at 67 °C, weighed, and ground with a Wiley mill (Arthur H. Thomas Co., Philadelphia) to pass through a 40-mesh screen. Elemental composition of shoots was determined by dry ashing 250 mg of shoot tissue and dissolving the ash in 1:1 (v/v) HCl. Shoot P concentration was determined by the ammonium molybdate colorimetric method (Jackson, 1958).

Response of shoot dry mass, root respiration rate, root phosphatase activity, and shoot P concentration to growth time at a 25 or 36 °C RZT were analyzed by using analysis of variance and regression procedures (SAS Institute, Cary, N.C.). Results were not different for the January and February replications. Because sampling dates did not coincide for January and February, data from two replications were not combined and only January results are presented.

## Results

Shoot dry mass was different (Table 1) with a mean of 5.9 g at 25 °C and 3.5 g at 36 °C. Shoot dry mass of plants at 25 °C increased from a mean of 0.34 g at initiation to 15.3 g at day 19, whereas shoot dry mass of plants at 36 °C increased from 0.42 g at initiation to 2.2 g at day 19 (Fig. 1). Root respiration did not differ with RZT, but there was a significant interaction between day and RZT (Table 1). Root respiration ($O_2$ consumed on a fresh mass basis) for plants grown at 25 °C increased linearly from 21 $\mu L \cdot g^{-1} \cdot h^{-1}$ at RZT initiation to 94 $\mu L \cdot g^{-1} \cdot h^{-1}$ at day 12, but there was no difference in respiration from day 12 to 19 (Fig. 2). Root respiration at 36 °C, however, increased from RZT initiation to day 8 and then decreased (Fig. 2).

Mean shoot P concentration (determined on a dry mass basis) also was different (Table 1) with a concentration of 9.7 $g \cdot kg^{-1}$ at 25 °C and 5.4 $g \cdot kg^{-1}$ at 36 °C. Shoot P concentration in 25 °C-treated plants was not different over time, but shoot P concentration in 36 °C-treated plants decreased linearly from 8.1 $g \cdot kg^{-1}$ at RZT initiation to 3.1 $g \cdot kg^{-1}$ at day 19 (Table 2). Phosphatase activity in roots at 36 °C increased linearly for the duration of the experiment, but phosphatase activity in roots at 25 °C did not change during the experiment (Fig. 3). The mean phosphatase activity (PNP released on a fresh mass basis) was different (Table 1) with 1.6 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ at 36 °C and 1.1 $\mu mol \cdot mg^{-1} \cdot h^{-1}$ at 25 °C.

## Discussion

According to Marschner (1995), ion uptake is more temperature dependent than respiration. Although changes in root carbohydrate content and subsequent root respiration affect ion absorption, other long-term changes (e.g., changes in membrane properties and feedback regulations) due to prolonged exposure of roots to supraoptimal RZTs also influence nutrient acquisition (Marschner, 1995). Our results comparing changes in shoot growth, root respiration, shoot P concentration, and root surface phos-

Table 1. Analysis of variance of the effects of a 25 versus 36 °C root-zone temperature (RZT) on 'Jet Star' tomato shoot dry mass, root respiration, shoot P concentration, and root surface acid phosphatase activity.

| | | Shoot dry mass | | Respiration | | Shoot P concn | | Phosphatase | |
|---|---|---|---|---|---|---|---|---|---|
| Source | df | MS | $P > F$ | MS | $P > F$ | MS | $P > F$ | MS | $P > F$ |
| Replication | 3 | 6.2 | 0.5332 | 231.08 | 0.1760 | 0.017 | 0.1615 | 0.05 | 0.2422 |
| RZT | 1 | 111.9 | 0.0276 | 371.78 | 0.1043 | 2.400 | 0.0002 | 1.02 | 0.0062 |
| Error A | 3 | 6.9 | | 69.85 | | 0.005 | | 0.02 | |
| Day | 4 | 74.7 | 0.0001 | 5260.18 | 0.0001 | 0.142 | 0.0003 | 0.24 | 0.0071 |
| Day × RZT | 4 | 49.3 | 0.0001 | 1802.72 | 0.0041 | 0.043 | 0.0515 | 0.10 | 0.1304 |
| Error B | 18 | 3.3 | | 231.75[z] | | 0.014 | | 0.05 | |

[z]Degrees of freedom for respiration Error B is 10.

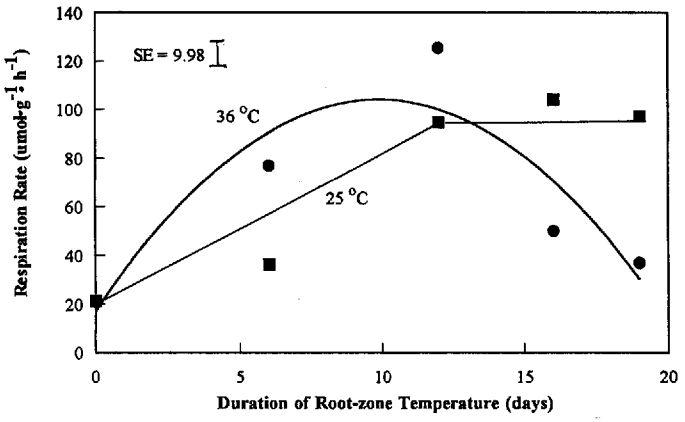


Fig. 2. Changes in mean root respiration ($O_2$ consumed on a fresh mass basis) of 'Jet Star' tomato plants exposed to a 25 °C (■) or 36 °C (●) root-zone temperature (RZT) for 19 d. Values are means of four replications. Equations of the line are $y = 13.7 + 6.1x$, $r^2 = 0.90$ for 25°C for days 0 to 12; $y = 17.0 + 17.6x - 0.89x^2$, $r^2 = 0.80$ for 36°C.

phatase activity in tomato plants grown at a 25 or 36 °C RZT support the idea that decreased shoot growth and demand for P along with decreased root respiration probably resulted in decreased P uptake in plants grown at 36 °C.

Tomato plants grown at a 25 °C RZT had an average increase in shoot dry mass of 11.0 g between days 12 and 19, but tomato plants grown at 36 °C had an average increase in shoot dry mass of 0.7 g between days 12 and 19 (Fig. 1). Gent and Enoch (1983) suggested that growth is limited at supraoptimal RZTs by a shortage of carbohydrates. Photosynthates transported to the root may be used for growth, respiratory, and/or exudation processes (Lambers, 1987). Of the 15% to 30% of the photosynthates respired by roots, 10% goes toward maintenance costs of roots (Lambers, 1987). Maintenance respiration increases with increasing RZTs to maintain protein turnover, cellular and ionic gradients, and physiological adaptations to high RZTs (Amthor, 1984; Lambers, 1987). Increased maintenance respiration accompanied by either no change or a decrease in growth respiration could account for the observed decreased tomato shoot growth.

We suspect that the initial increase in root respiration from RZT initiation to day 12 in plants grown at 25 °C was in response to increased metabolic activity associated with plant growth (Fig. 2). However, when plant tissue reaches maturity, its respiration rate will remain constant or decrease slowly (Taiz and Zeiger, 1991). This explains the lack of change in root respiration rate in plants at 25 °C from days 12 to 19. No steady-state respiration rate was reached in plants grown at 36 °C. Root respiration rate increased from day 0 to 8 and then declined (Fig. 2). It is possible that the decline in root respiration after 8 d in plants at 36 °C was due to a lack of respirable substrate and/or inactivation of respiratory enzymes. Many enzymes are labile at 40 to 50 °C, and enzyme-catalyzed reactions are sensitive to changes in RZT. Janes et al. (1988) incubated cultured 'Vendor' tomato roots at temperatures ranging from 10 to 50 °C for 30 min, and they observed that the highest root respiration rates were at 40 and 45 °C. However, they also observed decreased respiration rates of cultured 'Vendor' tomato roots grown for 7 d at 33 °C compared to roots grown for 7 d at 28 °C. This led them to speculate that tomato respiratory enzymes could function at RZTs above optimum but that long-term exposure of these enzymes to supraoptimal RZTs may damage enzyme functioning (Janes et al., 1988).

Table 2. Change in shoot phosphorus concentration (determined on a dry mass basis) of 'Jet Star' tomato plants grown at a 25 or 36 °C root-zone temperature (RZT) for 19 d. Values are means of four replications.

| Duration of RZT (d) | RZT (°C) 25 | 36 |
|---|---|---|
| | Shoot P concn (g·kg$^{-1}$) | |
| 0 | 8.1 | 8.1 |
| 6 | 11.2 | 7.4 |
| 12 | 10.6 | 4.3 |
| 16 | 8.7 | 3.9 |
| 19 | 10.1 | 3.1 |
| Significance | NS | Linear |

[NS]Nonsignificant response; L = linear. There were no significant quadratic responses.

Although shoot P concentration in plants held at 36 °C decreased over time, shoot P concentrations were not deficient (Table 2). The reported sufficiency range for P concentration in tomato shoots is 2.0 to 12 g·kg$^{-1}$ (Epstein, 1972). Shoot P concentrations in plants at 25 °C also were within the reported sufficiency range. Metabolic energy from root respiration is expended to absorb phosphate actively into the root across a concentration gradient, therefore changes in root respiration will influence phosphate acquisition (Johnson, 1990). Another effect of long-term exposure to supraoptimal RZT on ion uptake is a feedback regulation via demand. For example, depression of ion uptake in maize plants at low temperatures was attributed to a lack of demand for nutrients in the shoot (Marschner, 1995). When shoot growth of maize was increased, so were the uptake rates of nitrate and potassium (Marschner, 1995).

Bieleski (1973) reported that plants grown in P-deficient media had increased phosphatase activity associated with lower tissue P concentration. Boutin et al. (1981) observed that tomato plants grown in a medium without P had lower root and shoot P concentrations and a higher phosphatase activity than plants grown in a medium with P. Our findings show that phosphatase activity also increased in response to a RZT of 36 °C. The reason for this increase is unclear but could have been a direct effect of RZT or a response to the low P concentrations in the plants resulting from the heat stress. Our study was conducted with nonsterile plants so the activity of the rhizospheric microflora may explain part of the

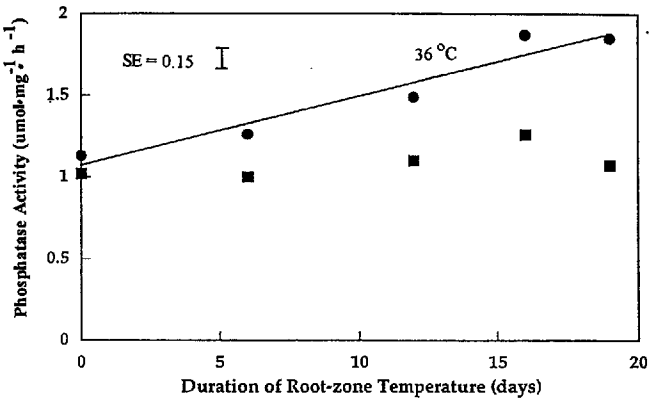


Fig 3. Changes in root surface phosphatase activity(p-nitrophenol released on a fresh mass basis) of 'Jet Star' tomato plants exposed to a 25 °C (■) or 36 °C (●) root-zone temperature (RZT) for 19 d. Values are means of four replications. Equation of the line is $y = 1.0 + 0.05x$, $r^2 = 0.56$ for 36 °C. Phosphatase activity at 25 °C was not different over time and did not show a linear or quadratic response.

observed phosphatase activity (Paul and Clark, 1989). The increased phosphatase activity at 36 °C could have resulted from increased microflora activity in response to increased root exudation at 36 °C. Root exudates are a major source of carbon and are usually rapidly decomposed by microorganisms (Paul and Clark, 1989). Direct injury to roots from RZT extremes may be revealed through loss of membrane integrity and increased electrolyte leakage/exudation (Calkins and Swanson, 1990; Levitt, 1980). Increased electrolyte leakage was observed as the 30-min incubation temperature of excised roots of *Ilex crenata* Thunb. 'Rotundifolia' increased from 30 to 55 °C (Ruter and Ingram, 1991). Additional research should address the relationship between increased phosphatase activity and P acquisition under heat stress as well as the carbohydrate status of roots and shoots.

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