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Paper Session III-B - Characterization of Potential ISS/Space Shuttle Environmental Conditions on Growth and Development of R. Sativus: Ground Studies for the Rasta Space Flight Experiment

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Characterization of potential ISS/Space Shuttle environmental conditions on growth and development of *R. sativus*: Ground studies for the RASTA Space flight experiment.

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Using radish as a model system, the RASTA project (Radish Assimilation in Spaceflight Testbed Atmospheres) will be investigating carbon partitioning of salad crops in microgravity. Before this goal can be accomplished, the effects of the unique environment of orbiting spacecraft on growth and development of radish must be characterized so they can be separated from those of microgravity. The environmental conditions on ISS and the space shuttle most likely to effect carbon partitioning in radish are air temperature, CO_2 concentration and atmospheric contaminants. Several radish cultivars were grown in temperatures ranging from 18-30°C at ambient (400 part per million [ppm]) or elevated (1,500, 3,000 and 10,000 ppm) CO_2. The effects of temperature and CO_2 on growth and development of these cultivars were characterized and a high temperature cultivar was identified. In a separate series of experiments, radishes were exposed to different levels of ethylene, a biologically active volatile organic compound, to characterize its impact on radish growth and development over a range of concentrations. With these environmental characterizations, the effect of microgravity on carbon partitioning can be more readily separated from environmental factors coincidental to the spacecraft environment.

Introduction

Crops that require minimal processing prior to consumption are being considered as dietary supplements for short-duration spaceflight missions. The RASTA (Radish Assimilation in Spaceflight Testbed Atmospheres) experiment will study carbon partitioning in radish to characterize the production of the edible portion of plants in the weightless environment. Radish is an example of a crop that is ready for consumption at harvest and combined with the advantage of a short life cycle (21 days), it is an excellent model system for the RASTA experiment. Ground studies are underway to determine the effects of the space shuttle/ISS environment on the growth and development of radish so spacecraft environment effects can be distinguished from microgravity-induced effects. Among the environmental conditions typically found on orbiting spacecraft that may effect plant growth and thus mask the effects of microgravity are elevated air temperature, CO_2 and atmospheric contaminant (eg. ethylene) levels.

Temperatures aboard the space shuttle are typically below 24°C, but excursions to 30°C or higher are not uncommon. Elevated temperatures have profound effects on photosynthesis and plant growth, especially in a cool temperature crop such as radish. As temperatures increase, thermal energy is introduced which affects enzymatic activity, enhances chemical reaction rates and alters membrane permeability (Geigenberger et al, 1998; Huang and Gao, 2000). High temperatures also inhibit photosynthetic CO_2 fixation (by inhibiting Rubisco activity) and damage photosynthetic electron transport (Feller et al, 1998; Geigenberger et al, 1998).

In addition to heat accumulation in this closed environment, atmospheric gases accumulate as well. Two biologically active atmospheric constituents that can accumulate are CO_2 and ethylene. Sources of these compounds in a spacecraft are both biogenic (human respiration, plant and microbial metabolism) and anthrogenic (derived from man-made sources such as off-gassing of materials) in origin (Stutte & Wheeler, 1997). Human respiration, along with other factors in this confined environment, produce CO_2 levels that are typically elevated above 1,500 ppm and are often higher than 5,000 ppm. Because atmospheric CO_2 levels on Earth are expected double in the 21st century, many studies have concentrated on the physiological effects of approximately twice ambient CO_2 on plant growth (Mulholland et al., 1997; Will and Ceulemans, 1997; Murthy et al, 1997) but only a few have examined the effects of super-elevated CO_2 levels (Wheeler et al., 1993; Wheeler et al., 1999).

Ethylene (C_2H_4) is a volatile plant hormone that regulates many aspects of plant development including germination, leaf expansion, flower formation and senescence (Abeles et al., 1992). Ethylene accumulation in this closed environment may have profound effects on plant growth. In addition to chronic exposure created by this closed environment, acute expose may occur as well. In the absence of convective currents that on Earth move ethylene from the immediate vicinity of the plant, ethylene may accumulate adjacent to where it is produced, causing acute exposure. It is important to identify the threshold of ethylene exposure that produces morphological and developmental effects in ground experiments for effective control systems to be implemented.

Before the effects of microgravity on plant growth can be defined, the compounding effects of the spacecraft environment, such as elevated temperature, exposure to ethylene and super-elevated CO_2 levels must first be understood. Once this characterization is made, the effects of microgravity can be better differentiated from those of the spacecraft environment.

Materials and Methods

Temperature and CO_2 exposure studies

For temperature studies, seeds of 20 different cultivars of *Raphanus sativus* were obtained from Burpee (Warminster, PA), Ferry-Morse Seed Co. (Fulton, KY), OSC Seeds (Waterloo, Ontario), Seiger's Seed Company (Zeeland, MI), Park Seed Co. (Greenwood, SC) and Johnny's Selected Seeds (Albion, ME). A cultivar, SORA, obtained from Johnny's Selected Seeds was used for CO_2 exposure studies. Seeds were sown in blocks of Oasis™ (Kent, OH) foam. The blocks were placed in plastic containers and the foam was saturated to 75% of its holding capacity with Hoagland's nutrient solution. The containers were then sealed to maintain high humidity levels through the first 4 days after planting (DAP), after which the covers were removed. The containers were placed in environmentally controlled growth chambers (Percival Scientific, Perry IA). Plants were grown under a diurnal photoperiod (16 h light /8 h dark) with cool white fluorescent lamps (Sylvania, Danvers. MA, product number F15T12). Relative humidity was maintained at 75% and PPF ranged from 180-200μmol•$m^{-2}s^{-1}$. For temperature studies, CO_2 levels were maintained at 1,500 ppm and temperature was maintained at either 18, 22, 26 or 30°C. For CO_2 studies, temperature was maintained at 23°C and CO_2 levels ranged from 400, 1,500 and 10,000 ppm. After 4 DAP, the containers were re-supplied daily with 1X Hoagland's solution to maintain the 75% saturation level throughout the growth cycle. As the plants grew, they were lowered to maintain the same light level at the top of the canopy throughout the growth cycle. Daily water loss was tracked by weighing the containers before and after nutrient replenishment. To distinguish between transpirational water loss and evaporation from the surface of the planting foam, an empty foam block (without plants) was placed in each chamber and the daily water loss from it was tracked as well. Radishes were harvested at 21 DAP. Plant height, root and shoot fresh and dry mass and leaf area were determined. Tissue was then oven-dried at 70°C and dry mass determined.

Ethylene exposure

Radish seeds (cv. Cherry Belle) were sown as described above. The containers were placed into small clear Lexan chambers (76.2 x 96.5 x 91.4 cm). These chambers were installed in a controlled environment chamber (CEC; Conviron, Winnipeg Canada). Plants were grown at 23°C, relative humidity of 75%, 18 h light and 6 h dark photoperiod under 300 μmol $m^{-1}s^{-1}$ PAR with cool white fluorescent lamps. CO_2 levels were maintained at 1,500 ppm. Ethylene concentration was monitored with a Photovac 10S Plus Portable Gas Chromatograph and with a 6890 Plus GC system. A f1ow control system provided independent control of ethylene levels in each of the small chambers in the CEC. Eight different ethylene concentrations (25, 40, 100, 200, 300, 500, 1000 and 10,000 parts per billion [ppb]) were evaluated.

Results

Temperature

Germination and initial growth rates (plant height) were greater at the higher temperatures for all the cultivars tested. Stem elongation at elevated temperature was evident after 7 DAP (days after planting) and continued throughout the growth cycle, leading to generally taller plants at 30°C. Leaf area was greatest between 22 and 26°C but was reduced at 30°C for all cultivars tested. Therefore, although stem elongation led to taller plants at 30°C in some of the cultivars, shoot biomass produced was reduced in all cultivars. This was observed in both shoot fresh and dry weights where maximum levels were reached between 22 and 26°C but were lower at 30°C (Figure 1).

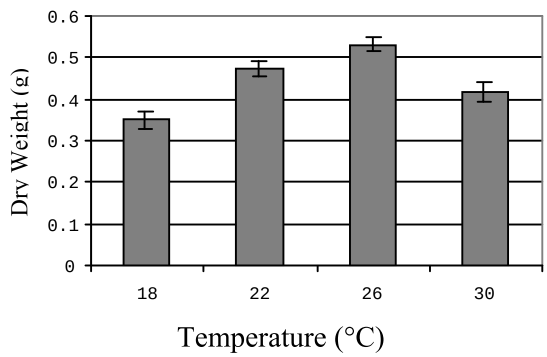

Figure 1: Total biomass production (plant dry weight) at 18, 22, 26 and 30°C. Standard error bars are shown.

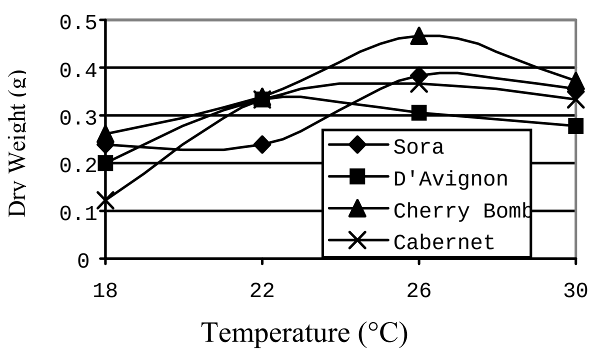

Figure 2: Dry weight of radishes from cultivars that produced a radish at 30°C that was within 80% of the optimum.

Many of the cultivars tested failed to produce appreciable radish swelling at 30°C. These cultivars tended to instead have elongated hypocotyl regions. Of the cultivars that produced a radish at 30°C, radish size and weight were greatest between 22 and 26°C. Four cultivars were identified that produced a radish at 30°C that was within 80% of that optimal radish size (Figure 2). Of these cultivars, SORA (Johnny's Selected Seeds, Albion ME) had the highest germination rate.

Transpirational water loss increased with increasing temperature for all cultivars. For most, transpirational water loss doubled as the temperature was increased from 22°C to 30°C. For some cultivars, this increase in transpiration rate was as high as 300%.

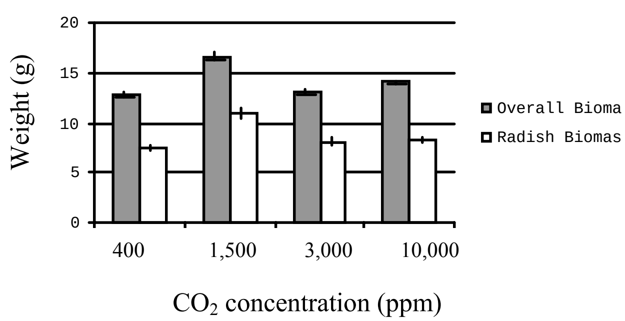

Figure 3: Biomass production and radish weight at different CO_2 levels. Standard error bars are shown.

Elevated CO_2 Exposure

Although plant height was greater at 7 DAP in the plants grown at elevated CO_2, this did not persist and there was no significant differences in plant height between treatments after 21

DAP. In contrast, overall biomass production was significantly affected by CO_2 concentration. Biomass production was greatest at 1,500ppm and reduced to control (400 ppm) levels at 3,000 and 10,000ppm. Similarly, radish size was greatest at 1,500 ppm CO_2 but not significantly different between the control and the 3,000 and 10,000ppm treatments (Figure 3). Harvest index was therefore greatest at 1,500 ppm CO_2 but was not significantly reduced at higher CO_2 concentrations.

Ethylene Exposure

Alterations in plant growth and development became apparent at ethylene concentrations of 40 ppb. Between 40 and 100 ppb, leaves curved downward and stem and root lengths were reduced. These

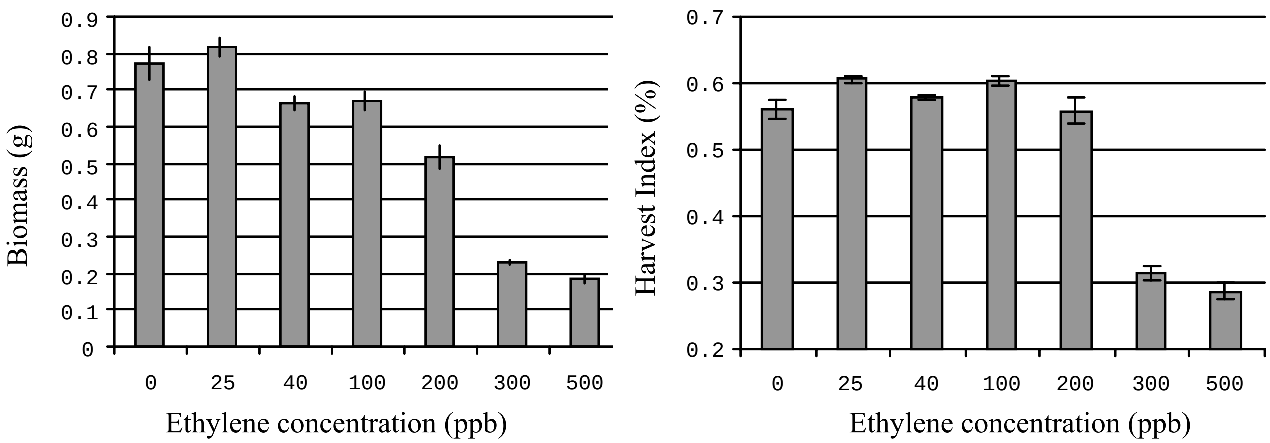

Figure 4: Physiological responses at 21 DAP due to Ethylene exposure.
(Data reproduced from Eraso et al, 2001)

changes in morphology were only evident for the first week of growth. After the first week, there were no statistically significant differences in shoot length associated with ethylene exposure at 40 and 100 ppb. At ethylene concentrations higher than 200 ppb, plants showed chronic injuries which included: reduced biomass, smaller roots, leaf epinasty, leaf curling, increased root hair initiation and delayed hook opening. Plants exposed to even higher concentrations of ethylene (300, 500 and 1000 ppb) showed increasingly severe leaf epinasty, stem and root growth and enlargement inhibition (i.e. harvest index greatly reduced) and formation of roots with a corkscrew shape. As early as 3 days after germination, plants showed inhibited hypocotyl elongation at concentrations >300 ppb. These results are similar to those reported by Vreugdenhil and Bowmeester (1989) with radish and Vreugdenhil and Van Dijk (1989) with potato. Finally, at ethylene concentrations greater than 1,000 ppb, plants exhibit symptoms of acute toxicity, including severe stunting of growth, stem swelling, and failure of leaves to develop.

Discussion

To varying extents, high temperature (30°C) had adverse effects on growth in all radish cultivars. Of the parameters tested in this study, temperatures between 22 and 26°C enhanced growth but temperatures of 30°C reduced growth. The adverse effects of high temperature on shoot growth could be due to increased media temperature since high temperatures directly inhibit root growth and activity. Limited root production and accelerated root death in high temperatures also interrupts synthesis and transport of root-produced hormones (Huang and Gao, 2000; Xu and Huang, 2000). Cytokinin for example, is a shoot growth regulator that is synthesized mainly in the roots and its transport is inhibited by elevated root zone temperature.

Increased temperature also increases evaporative demand because the water capacity of the air increases as temperatures increase (Maherali and DeLucia, 2000). The adverse effects of high temperature on shoot growth are thus compounded because this increased demand is on roots whose function is impaired by the elevated temperature of the growth media. Increased transpiration rates and diminished root activity could lead to negative water and nutrient balances and thus account for the reductions seen in root and shoot weight.

Four of the 20 cultivars tested were tolerant of the effects of elevated temperature. These cultivars showed increased transpiration rates, but less reduction in overall biomass. This suggests that these cultivars are not suffering from accelerated root death. Healthier roots will increase tolerance of elevated temperature because sustained transpiration rates will cool the leaves through evapotranpirative cooling. This tolerance of high temperature makes these cultivars desirable selections for use in the RASTA spaceflight experiment because they would be less affected by the temperature excursions typical of the spacecraft environment.

Another attribute that makes one cultivar more attractive than another is a high and consistent germination rate. One of the high temperature tolerant cultivars, SORA, had germination rates >98% and has been selected as the cultivar for the spaceflight experiment.

The characterization of elevated CO_2 on plant development is important to allow for separation of microgravity effects from CO_2 effects. CO_2 concentrations of 1,500 ppm enhanced growth over control (400 ppm) levels yet when these levels were increased above 1,500 ppm, this enhancement was lost and growth returned to control levels. Therefore, since CO_2 levels typically fluctuate between 1,000 and up to 10,000 ppm and above on the space shuttle and ISS, these may significantly alter radish growth and development. These results highlight the need for good atmospheric control of CO_2 in microgravity-rated plant growth chambers. Other physiological effects that were not investigated in this study may occur due to these super-elevated CO_2 levels (Wheeler et al., 1999) and require further study.

Ethylene exposure also affected growth and development in radish. The threshold level necessary to produce a morphological response (40 ppb) was identified. By knowing this threshold level and understanding the subsequence physiological responses, we will be better able to separate the effects of microgravity on crop growth from those of ethylene. Physiological effects produced at higher ethylene concentrations were characterized as well. Again, an understanding of these effects will enable us to better interpret our data upon the return of the RASTA experiment from space flight. These data also highlight the importance of removing ethylene in microgravity-rated plant growth chambers.

In addition to super-elevated CO_2 and ethylene, other volatile organic compounds have been identified as space shuttle/ISS atmospheric constituents (Stutte and Peterson, 1996). Characterization of the effects of these contaminants on growth and development of *Raphanus sativus L*. are currently underway as part of the RASTA project as well.

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