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Leaf photosynthesis and transpiration of two leek cultivars with differing pseudostem pungency levels

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Abstract: The effects of temperature on leaf photosynthesis and transpiration were investigated for two leek (*Allium porrum* L.) cultivars that showed differing pseudostem pungencies, when grown in a controlled greenhouse environment. Basal sections of 20 week old cv. Tadorna were shown to be nearly four times as pungent as those of cv. Ramona. Greenhouse climate data indicated 37% of weekly average temperatures were above 25°C, approaching the temperature at which leek growth is inhibited. Due to the environmental influence of temperature on tissue pungency, leaf gas exchange was measured at 20°C and 27°C to see if the gas exchange characteristics of the cultivars differed as well. The net carbon exchange rate (NCER) and leaf transpiration of cv. Ramona remained relatively steady over the photoperiod at both 20°C and 27°C, but were reduced by the increased temperature. In comparison, NCER and leaf transpiration of cv. Tadorna were greatly reduced by elevated temperature during the morning and early afternoon periods. Gas exchange of mature and immature leaf tissue was also measured. Immature leaves of cv. Tadorna had lower NCER and displayed a significant decline in photosynthesis and transpiration during the photoperiod. Despite having reduced productivity under the greenhouse conditions, Tadorna displayed higher tissue pungency upon maturity.

Keywords: allium, photosynthesis, transpiration, flavour, oxidative stress, space program

Introduction

Alliums such as, onions, garlic and leek, are known for their pungent flavour and represent important culinary crops worldwide. Research over the past century has focused on understanding the biochemical nature of pungency and the environmental factors that influence its development and intensity, often ignoring aspects of primary metabolism (Brewster, 1994). Most recently, mission specialists at NASA have expressed a need to incorporate alliums into current bioregenerative life-support systems (BLS) and the closed environment systems (CES) utilized in space plant-growth facilities (Salisbury and Bugbee, 1988). NASA's Breadboard project has developed an optimized menu requiring the cultivation of at least two *Allium* species, (*Allium cepa*, *A. fistulosum*) in CES (Waters et al., 2000). Subsequently, the research herein has taken the unique opportunity to begin developing a model *Allium* species, by first examining the limitations on productivity brought on by CES cultivation. Leeks (*A. porrum* L.) were selected as a model species with which to develop subsequent models of other alliums in CES. The long flat leek leaves allow for easy measurement of leaf gas exchange which would be impossible to perform on the tubular leaves of *A. cepa* L. or *A. fistulosum* L.

There are few publications in the literature describing the gas exchange of leeks or alliums in general. One such paper by Roberts and Walters (1988), investigated the photosynthesis of leek leaves infected with the rust, *Puccinia allii* Rud. This study measured the photosynthesis of discrete leaf regions as functions of Rubisco activity or oxygen evolution under saturating CO₂ conditions and varying light levels. Although rates of photosynthesis were reported, they are difficult to compare to standard NCER under similar conditions because they were expressed on a fresh weight basis rather than by leaf area per unit time.

It was the primary purpose of this investigation to measure the gas exchange of leek leaves under controlled conditions and compare measured values to general reports of monocot C₃ species. During a consecutive CES growth experiment, it was noticed that the greenhouse temperatures remained elevated for an extended period due to a ventilation malfunction during the growth season. As a result, a secondary purpose was

to determine the effects of elevated temperature on the photosynthesis and leaf transpiration of both leek cultivars, assessments of productivity were conducted at an optimal (20°C) and inhibitory (27°C) growth temperature.

In addition to the primary gas exchange characteristics one parameter of pungency was monitored. The pungency of any typical allium is a product of genetic variability and environmental influence (Randle, 2000). Distinctive flavour agents arise from an enzymatic breakdown of alk(en)yl-L-cysteine sulfoxides (ACSO's) known as the flavour precursors. ACSO's react with alliinase (E.C. 1.1.1.27) when tissues are damaged and cytoplasmic and vacuolar contents mix. The reaction yields, ammonia, pyruvic acid and sulfenic acids, that further react to produce thiosulfinate derivatives (Schwimmer and Mazelis, 1963; Block 1985). It is known that the relative pungency of allium crops can be increased by conditions such as high temperature and drought stress (Brewster, 1997). Therefore, it was hypothesized that the elevated greenhouse temperatures may have increased the overall pungency in both cultivars. Traditionally, pungency is estimated by measuring the amount of enzymatically produced pyruvate (Schwimmer and Weston, 1961). In this investigation, pyruvate was determined via a colourmetric assay (DNPH), enzymatic hydrolysis (LDH) and liquid chromatographic method (HPLC) (see Materials and Methods). By subtracting a background pyruvate concentration, a relative pungency reading was determined.

Materials and methods

Leek cultivation

Leeks were grown in the greenhouse at the University of Guelph, during the summer of 2001. Seedlings were started in the greenhouse on April 30, 2001. Two cultivars were selected for their moderate thrip resistance; cv. Tadorna (Stokes) and cv. Ramona (Bejo). Seedlings were transplanted into soilless promix, 2 seedlings per six inch pot, June 9. Greenhouse conditions were set to 20°C day, 15°C night, under a 12 hour photoperiod. An ebb and flow hydroponic system watered the crop twice per week with a modified Hoagland's solution, approx. 4:10:4 (N:P:K). The trial was sprayed with Lorsban for thrips when needed. Growth was monitored by fresh weight as well as leaf

area and morphology: leaf number, leaf length and pseudostem width (full results not reported here).

Gas exchange

The primary gas exchange of mature 4th or 5th leaves was monitored over a 12 hour photoperiod using a leaf cuvette method (Jiao et al., 1996; Leonardos and Grodzinski, 2000) between the 15th and 20th week of growth. Greenhouse grown leeks were transferred to a CES growth chamber and acclimated to the desired conditions over night (50% RH, 20°C or 27°C). Artificial illumination ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) was provided by three high pressure sodium lamps. Prior to illumination, leaves were gently clamped into the leaf cuvettes, approximately 30 cm from the leaf tip. Rates of photosynthesis and transpiration were then calculated as functions of enclosed leaf area, light intensity, CO₂ concentration, relative humidity and temperature. Comparisons of mature (3rd) and immature (8th) leaf tissues were also conducted. Newly emerging leaves were considered immature tissue. In all trials, each data point represents the mean of a five leaves with standard error.

Pungency evaluation

Relative pungency readings were measured using an enzymatic assay (LDH) (Schwimmer and Mazelis, 1963; Rabinkov et al., 1994), a colourmetric method (DNPH) modified from Schwimmer and Weston (1961) and a liquid chromatography protocol developed from Yoo and Pike (2001). Each separate assay provided a measure of background tissue pyruvate levels as well as enzymatically formed pyruvate from the catalysis of flavour precursors (ACSO's). Pseudostem tissue samples (~1 g, 1 cm from basal plate) were collected during the 20th week of growth and bisected longitudinally, to allow pairing of background and foreground pyruvate determinations. A crude estimate of ACSO concentration was determined by subtracting out the background pyruvate concentration.

Results and discussion

Leek growth and pseudostem pungency

Greenhouse climate data revealed that the mean weekly noon greenhouse temperature was $22.9^{\circ}\text{C} \pm 0.9$ (s.e.) over the entire growth season. However, during the first five

months of growth and during the period that pungency was assessed, this weekly noon average was $24.4^{\circ}\text{C} \pm 0.9$ (s.e.). Overly high seasonal temperatures and a ventilation malfunction resulted in elevated greenhouse temperatures for most of the growing season. A closer examination of climatic data showed that 37% of the weekly noon temperature averages were greater than 25°C , 67% above 20°C . During this overly warm period, crude estimates of ACSO levels were made using three assays for the enzymatically produced pyruvic acid. The pseudostem regions of Tadorna were nearly four times as pungent as those of Ramona (Table 1), as assessed by all three methods. ACSO's are manufactured in the leaves and are slowly exported to the pseudostem region as the allium matures (Lancaster et al., 1989). Immature leaf tissues of onion seedlings have been shown to be of greater pungency than more mature tissue (Lancaster et al., 1986). Generally pungency decreases once maturity is reached (Lancaster et al., 1989). In this study, it was unclear if the differences in cultivar pungency were the result of genetic differences or an effect of elevated temperatures.

Under stressful conditions that promote oxidative damage to tissues, such as high temperature, gas exchange is inhibited and levels of antioxidants such as glutathione and ascorbate are increased (Hall and Rao, 1999). Glutathione is an intermediate in the synthesis of ACSO's (Lancaster et al., 1989). It may be speculated that higher glutathione levels might induce or boost ACSO production. In this sense, pungency responds to environmental stresses that cause oxidative damage to photosystems. Additional experiments are currently underway which will elucidate any connection between oxidative stress and pungency development.

The CES conditions resulted in leeks that had fresh weights three (cv. Ramona) to six (cv. Tadorna) times lower than field grown counterparts by the end of the season (data not shown). Narrow pseudostems and fewer leaves as compared to a field trial, resulted in a two (cv. Ramona) to four (cv. Tadorna) times reduction in leaf area (data not shown). Rhee et al. (1998) reported similar results for greenhouse grown leeks that took six months to reach maturity and never achieved field diameter. However, Ramona performed better under CES conditions and consistently showed morphological characteristics with greater magnitude, e.g. leaf number, pseudostem width. To

investigate further, leaf gas exchange rates were measured for both cultivars under CES conditions.

Primary gas exchange

Gas exchange rates were measured at two temperatures under select CES conditions to determine if elevated greenhouse temperatures could have been a factor in the observed growth suppression. Rates were measured at 20°C and 27°C, representing the optimum growth and the growth suppression temperatures for leeks (Brewster, 1994). Both cultivars experienced an overall reduction in gas exchange at 27°C. Integrated over the entire photoperiod, Ramona displayed a 19.1% reduction in carbon fixation and a 9.7% reduction in leaf transpiration, Tadorna showed a 14.2% reduction in carbon fixation and an increase of 1.9% in leaf transpiration at the elevated temperature. The contrasting effects of increased temperature on the photosynthesis (NCER) and leaf transpiration of the two leek cultivars can be seen in Figure 1. Cultivar Ramona was able to maintain nearly steady NCER and leaf transpiration at 20°C, approximately 15 to 16 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and 2.2 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. At 27°C the NCER dropped to values between 12 to 13 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Leaf transpiration was also reduced - typical values were in the range of 1.9 to 2.1 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, showing a modest peak during the noon and 4pm period. In contrast, cv. Tadorna had initial NCER values of 14 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 20°C, which declined slowly over the day to around 12 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Leaf transpiration of Tadorna followed a similar trend, 2.0 down to 1.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. At 27°C, the initial NCER ($\sim 10.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was greatly decreased but the leaves were able to recover by the end of the day to a rate comparable to the 20°C plants. Leaf transpiration followed a similar trend, rising from 1.8 to 1.9 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ by the end of the photoperiod. A direct comparison of the two cultivars revealed that Ramona fixed 13.2% more carbon than Tadorna at 20°C, and 8% more at 27°C.

The photosynthesis and leaf transpiration of immature and mature leaf tissue were compared (Fig. 2). Immature leaves (8th leaf) were observed to be lacking a fully developed waxy cuticle. They tended to be pale yellow-green in colour and flaccid as compared to the rigid mature leaves that had fully developed waxy cuticles and were dark blue-green. Mature leaf tissue maintained a steady rate of photosynthesis and leaf

transpiration during the 9 am to 3 pm period, $\sim 14 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ respectively. Gas exchange values of immature leaf tissue declined from ~ 11 to $6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and 2 to $0.8 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ over the same time period.

Overall, the NCER reported for the leeks in this study are comparable to other monocot C_3 species measured under similar conditions. Lawlor (1987) reports a range of carbon fixation rates between 8 and $30 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for typical C_3 plants. The leek cultivars assessed in this study fall about the middle to lower end of this range. However, Ramona maintained steady gas exchange throughout the day even at the elevated temperature and fixed 8% more carbon at the inhibitory growth temperature than Tadorna. This could explain the morphological differences observed between the two cultivars. Overall, the elevated greenhouse temperatures inhibited the growth of Tadorna more than Ramona. When greenhouse temperatures were high, Tadorna spent the greater part of the day photosynthesizing at a much reduced rate. In a study by Rhee et al. (1998), immature leek leaves were shown to have underdeveloped waxy cuticles which promoted drying of the leaf tips when directly exposed to an aerial environment. This could explain the sharp decline in leaf transpiration and NCER of immature leaf tips observed displayed in Figure 2.

Conclusion

In summary, the results reported here represent the first detailed examinations of the effects of temperature on the leaf photosynthesis and transpiration of leeks. In addition, this is also a step towards establishing *A. porrum* L. as a model species with which to study other alliums desired in the space program. If a relationship exists between oxidative stress to photosynthetic tissues and induction of antioxidant pathways and ACSO production, this study represents a first step in establishing a relationship between C fixation and flavour production because it outlines photosynthetic activity in a model *Allium* species grown in controlled environments. Such a relationship may lead to new allium cultivation practices that could control crop pungency.

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Tables

Table 1. Comparison of pseudostem tissue pungency by cultivar and pyruvate assessment method of two leek cultivars at 20 weeks of age.

Cultivar	LDH Enzymatic Assay for Pungency ($\mu\text{mol/g}$ FW $\pm$ s.e.)	DNPH Colourmetric Assay for Pungency ($\mu\text{mol/g}$ FW $\pm$ s.e.)	HPLC Assay for Pyruvic Acid ($\mu\text{mol/g}$ FW $\pm$ s.e.)
Tadorna	3.516 ± 0.883	4.193 ± 0.887	3.984 ± 1.147
Ramona	1.054 ± 0.348	1.097 ± 0.263	0.9669 ± 0.3092

Figures

Fig. 1. Photosynthesis and Transpiration of Leek Leaves at 20°C (○ Tadorna, □ Ramona) and 27°C (● Tadorna, ■ Ramona).

Fig. 2. Photosynthesis and Transpiration of Mature and Immature Leek Leaves, cv. Tadorna, ($\circ$ Mature, $\square$ Immature).