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Effects of guttation prevention on photosynthesis and transpiration in leaves of *Alchemilla mollis*

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Abstract

The ecophysiological function(s) and consequences of guttation, a phenomenon by which water is exuded by and accumulated as droplets along the leaf margins under high humidity in many plants that grow in wet soil, has been poorly studied and remains largely unknown. Thus, leaf gas exchange and chlorophyll fluorescence were examined, using two experimental approaches, in *Alchemilla mollis* plants under conditions that promoted guttation and those that prevented this phenomenon. Although results were variable, depending on the experimental approach, prevention of guttation effected reductions in photosynthesis and transpiration, as well as photochemical activity measured with fluorescence techniques. These findings lend partial support for a previously hypothesized function of guttation: prevention of excess water in leaves, yet they contradict those of several other studies. More work is required in order to adequately understand the function of guttation.

Additional key words: chlorophyll fluorescence; gas exchange; hydathodes; leaf morphology; transpiration.

Introduction

Following nights when soil moisture is plentiful and atmospheric humidity is high, the leaf margins of many plants exude and accumulate droplets of water (Moore *et al.* 1998, Raven *et al.* 1999). This phenomenon, guttation, is characteristic of a phylogenetically and morphologically diverse group of plants. The formation of water droplets in guttation is the result of plant internal water forced out of epidermal pores, hydathodes, scattered along the leaf margins (Haberlandt 1884, Frey-Wyssling and von Rechenberg-Ernst 1944, Belin-DePoux 1969, Fahn 1979, Taiz and Zeiger 2006, Salisbury and Ross 1992). Hydathodes are ontogenetically related to stomata (Stevens 1956, Dieffenbach *et al.* 1980), yet hydathode pores are apparently permanently open and cannot close (Martin and von Willert 2000). Hydathodes are solitary or can occur in groups and are usually located at vein endings along the edges of leaves (Belin-DePoux 1969, Fahn 1979, Stevens 1956). Guttation usually occurs when stomata are closed and root pressure forces xylem water out of the leaf *via* the hydathodal pores (Salisbury and Ross 1992, Taiz and Zeiger 2006). In the few plants examined, relatively small amounts of water are lost during guttation (Janes 1954). The functional significance of guttation has been the subject of only a small number of studies and remains unclear (Singh and Singh 2013). Two studies provide evidence that guttation may play a critical role in protecting the plant from attack by pathogens and herbivores. Grunwald *et al.* (2003) compared guttation in barley plants infected by bacterial pathogens with uninfected plants and found that proteins in the guttation liquid of uninfected plants may prevent infection of the leaves by the pathogens. Likewise, Koulman *et al.* (2007) suggested that guttation in some grasses might provide a mechanism for the mobilization and deposition on leaf surfaces of compounds that deter herbivory by mammals and insects. Also using grasses, Kerstetter *et al.* (1998) claimed that guttation may play a negative role in the function of these plants. They reported that peroxidases, transported from

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Abbreviations: C_i – leaf internal CO_2 concentration; E – leaf transpiration rate; F_v/F_m – intrinsic efficiency of energy conversion by PSII; F_v'/F_m' – efficiency of energy conversion by PSII in the light; g_s – leaf stomatal conductance; HVPD – high-VPD treatment; LVPD – low-VPD treatment; P_N – net CO_2 exchange; q_N – nonphotochemical quenching; q_P – photochemical quenching; VPD – vapor pressure deficit.
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the roots to the leaves, are eventually, after deposition on leaves *via* guttation, returned to the soil, where these enzymes may reduce the availability of nitrogen to the plants. Numerous other detrimental consequences of guttation are described in an extensive review by Ivanoff (1963; also *see* Curtis 1943). Finally, Feild *et al.* (2005) suggested, based on experiments with *Chloranthus japonicus*, in which guttation was prevented by occlusion of the leaf-margin hydathodes with a thick, impermeable liquid, that guttation was necessary to prevent supersaturation of the leaf mesophyll with liquid water, thereby inhibiting leaf photosynthesis by preventing CO_2 uptake.

To summarize past work on the potential function(s) of guttation, several studies provide evidence for a beneficial, protective role against pathogen and/or herbivore attack, whereas Kerstetter *et al.* (1998) claim a detrimental role of this phenomenon in reducing soil nutrient availability, while Curtis (1943) and Ivanoff (1963) provide numerous lines of evidence that guttation serves a negative role in plant function. Finally, Feild *et al.* (2005) suggested that hydathodes are important in allowing guttation to occur, which, in turn, allows photosynthetic gas exchange to proceed normally. Because the potential function(s) of guttation, a common and fascinating phenomenon in a wide variety of plants, has been so infrequently investigated, and the few existing studies provide contradictory evidence for beneficial *vs*. detrimental effects of this phenomenon, more work to understand this phenomenon is clearly needed. Furthermore, the study by Feild *et al.* (2005) does not directly address the role of guttation, but, instead, the role of the hydathodes when guttation occurs. In addition, the experimental approach used in the latter study was artificial and would never occur in a plant in a natural setting.

Given the above, the general goal of the current study was to investigate whether guttation affects leaf function, particularly photosynthesis, in plants. Specifically, the goal of this study was to determine if leaf photosynthesis is affected by prevention of water droplet formation on leaf margins of *A. mollis*. This study differs from that of Feild *et al.* (2005) in the manner by which guttation was prevented. Whereas Feild *et al.* (2005) prevented guttation by an unnatural blockage of the hydathodes, guttation (*sensu lato*) was prevented in the current study by altering atmospheric conditions around the leaves, *i.e.*, increasing atmospheric vapor pressure deficit (VPD).

Materials and methods

Plants: Young plants (approx. 5 cm in height) of *A. mollis* (Buser) Rothm. (Rosaceae) were purchased from a commercial nursery in Lawrence, Kansas 2–4 weeks prior to experimentation, or were grown from seeds obtained from a horticultural supply company for 14 weeks prior to use. Plants were grown in plastic pots (15–20 cm in a diameter) containing standard greenhouse soil in the University of Kansas greenhouse. Approximate environmental conditions in the greenhouse were: 1,000 μmol (photon) m^{-2} s^{-1} of maximum PPFD, 13-h photoperiod, 25/20°C typical day/night air temperatures, low VPD, and moist soil (pots were watered every other day).

Experiments took place in a growth chamber under these environmental conditions: approximately 400 μmol (photon) m^{-2} s^{-1} of average PPFD at plant height, 12-h photo- and thermoperiod, day/night air temperatures of 25/20°C, and day/night air VPD of 2.37/1.05 kPa.

Experimental procedure: Five days prior to photosynthetic measurements, plants were moved from the greenhouse to the growth chamber and watered. At the end of the light period on each of the next four days, all plants were watered until their pots dripped, then three plants were placed in each of two 5-gallon plastic buckets lined with wet paper towels along the inner walls (low-VPD treatment, LVPD), and three plants were placed in each of two dry buckets (high-VPD treatment, HVPD). Lids were affixed to all buckets for the entire night period, then were removed prior to lights-on in the growth chamber. The VPD inside the two buckets containing plants with lids affixed, measured with *Fischer Scientific* (St. Louis, MO, USA) 433 MHz *Cable Free Pro14-648-52* temperature/relative humidity sensors and a remote recorder, were 0.47 and 1.28 kPa in the wet-towel and dry buckets, respectively. Each morning, the amount of guttation visible on the margins of all leaves was visually scored and recorded.

Most leaves in the LVPD treatments guttated to varying degrees, whereas very few leaves in the HVPD treatment guttated. For physiological measurements, leaves with the most consistent and greatest amount of guttation from the former group of plants were compared with leaves that never showed evidence of guttation from the latter group of plants. In both cases, only mature leaves of average size were used.

In the first experiment described above, the physiology of guttation leaves and nonguttation leaves from six different plants were compared (three per VPD treatment), as each plant was given only one treatment. In the second experiment, the same procedures described above were followed; however, six plants were given the HVPD treatment for four days, then physiological measurements were made, after which the same plants were given a LVPD treatment for four days, then measured again.

Photosynthesis and fluorescence measurements: Leaf gas (CO_2 and H_2O vapor) exchange and chlorophyll *a* fluorescence were measured on the day after the four days of VPD treatments. Dark measurements were made 0.5–1 h before chamber photoperiod start; light measurements were made in the middle of the light period. All measurements were made on a 2 × 2 cm portion (avoiding the central vein) of attached leaves of plants inside the

growth chamber using a *LI-COR Portable Photosynthesis System* (*LI-6400*, Lincoln, NE, USA). Environmental conditions inside the *LI-6400-02B* leaf cuvette were: 800 μmol(photon) m^{-2} s^{-1} PPFD (90% red, 10% blue diodes; earlier determined to be a saturating PPFD for CO_2 uptake), 25°C block temperature, 2.53 kPa VPD, and 400 μl(CO_2) l^{-1}. Air flow rates were 500 μmol s^{-1} in the light and 300 μmol s^{-1} in the dark. Data for each leaf were collected only after the Sample and Reference infrared gas analyzers were electronically equalized, and the measured data were stable. Gas-exchange parameters were measured three times for each leaf, and the means of these three measurements were used to calculate plant treatment means (n = 3 or 6 plants; one leaf per plant).

Light- (F_v'/F_m', q_P, q_N) and dark (F_v/F_m) fluorescence parameters were measured with the *LI-COR LI-6400-40 PAM* instrument under the cuvette environmental conditions described above (except F_v/F_m was measured in the dark similarly as dark gas exchange data). The default settings and calculations of this PAM system were used (*e.g.*, van Kooten and Snel 1990; Krause and Weis 1991), and data (three measurements per leaf) were collected only after values stabilized.

Quantification of guttation water: A small piece of tissue paper was weighed before gently absorbing all guttation droplets along the margin of a leaf of each of three plants in the LVPD treatment shortly after lights-on, then the tissue paper was immediately re-weighed. Care was taken to avoid physical disturbance of the plant during removal of the bucket lid and during droplet collection. Following droplet collection, the leaf was excised, weighed, and its surface area measured with a *LI-3000 Portable Area Meter* (*LI-COR*, Lincoln, NE, USA). Leaf mass and area were also determined for a leaf lacking any visible guttation from three plants in the HVPD treatment.

Statistical analysis: Means of the plants in the two VPD treatments were compared using the Student's *t*-test. Thus, sample sizes were three or six plants (one leaf each), and, when the data did not pass normality or homoscedasticity tests, the nonparametric *Mann-Whitney´*s *U*-test was used (Sokal and Rohlf 1981). Statistical significance was inferred when $P \leq 0.05$. Analyses were performed using the software program *Statistics 20* (*SPSS Inc*., New York, USA).

Results

The amount of water lost by an individual leaf of *A. mollis* in the LVPD treatments used here was considerable (18%) when expressed on a leaf mass basis (Table 1), but appeared quite minor (3.9%) when expressed on a leaf area basis and compared with midday transpiration rate (E) measured in these plants (Table 1).

Neither gas-exchange or fluorescence parameters of leaves that guttated differed from those of leaves that did not guttate when different plants (n = 3) were compared in the two treatments (Figs. 1, 3); however, when the same plants (n = 6) were compared after exposure to the two VPD treatments, net CO_2 exchange (P_N) and transpiration rate (E) were considerably higher in leaves that guttated, relative to those that did not (Fig. 2). Furthermore, F_v/F_m, F_v'/F_m', q_P, and q_N of leaves that guttated were also higher than the values of leaves that did not guttate, but, again, only when the same plants were compared (Fig. 4).

Table 1. Mean morphological features and water losses *via* guttation (night only) and transpiration (day only) for *Alchemilla mollis* leaves. n = 3. *Transpirational water loss was estimated as 12-h water loss, based on a transpiration rate of 1 mmol m^{-2} s^{-1} (*see* Figs. 1,2).

Morphology and water loss	Mean ± SD
Guttational water loss per leaf [g]	0.027 ± 0.006
Leaf mass [g]	0.150 ± 0.019
Number of leaf serrations	71.33 ± 18.01
Number of leaf serrations with guttation	56.67 ± 16.77
Leaf area [cm^2]	8.29 ± 0.62
Water loss per leaf serration [g]	0.001 ± 0.001
Water loss per leaf area [g cm^{-2}]	0.003 ± 0.001
Transpirational water loss [g cm^{-2} d^{-1}]*	0.078

Discussion

Alchemilla mollis (Rosaceae) is a herbaceous, rhizomatous perennial native to mountainous, mostly forested regions of eastern Europe (*e.g.*, Carpathians, Siriu Mountains, Caucusus, and Turkey; Neblea and Alexiu 2011). Its typical habitat comprises permanently wet, shallow, sandy, mesic to hydric lithosols. Such hydric habitats are typical of many plants that exhibit substantial amounts of guttation fluid on their leaf margins (Moore *et al.* 1998, Raven *et al.* 1999).

The amount of water lost *via* guttation by leaves of *A. mollis* is similar to amounts lost by leaves of other taxa exhibiting guttation (*see* references above), although different experimental approaches used to determine guttational water losses preclude precise quantitative comparisons. Guttational water losses by the leaves of *A. mollis* appear minor when compared to transpirational water losses. It is based on assumptions that no guttation water is lost during the day (Moore *et al.* 1998, Taiz and Zeiger 2006), and that leaf transpiration rates measured at midday here can be extrapolated to the entire light period.

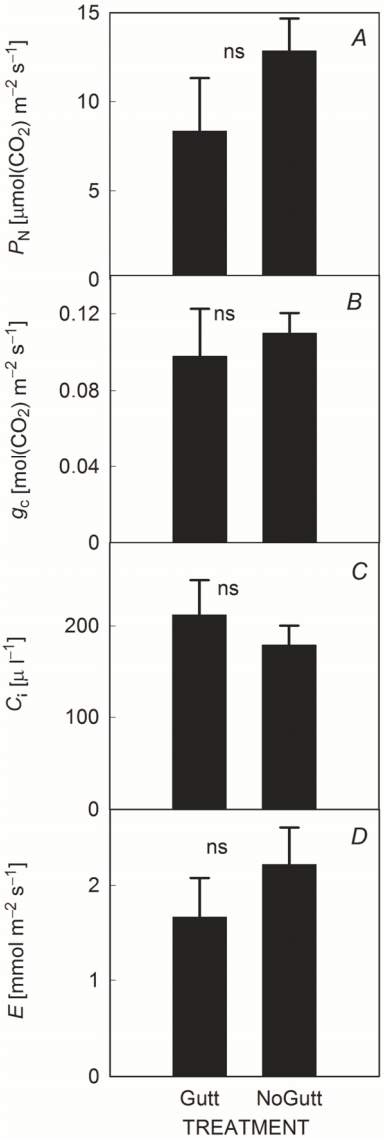

Fig. 1. *A*: Mean (*capped lines* extending from the bars are SD; n = 3 plants) net CO_2 exchange rate (P_N), *B*: stomatal conductance (g_s), *C*: internal CO_2 concentration (C_i), and *D*: transpiration rate (E) in leaves of *Alchemilla mollis* under conditions that promote (Gutt) and prevent (NoGutt) guttation. Statistical results reflect comparisons between means of three different plants in the two treatments. No pair of means is significantly different (NS; P>0.05).

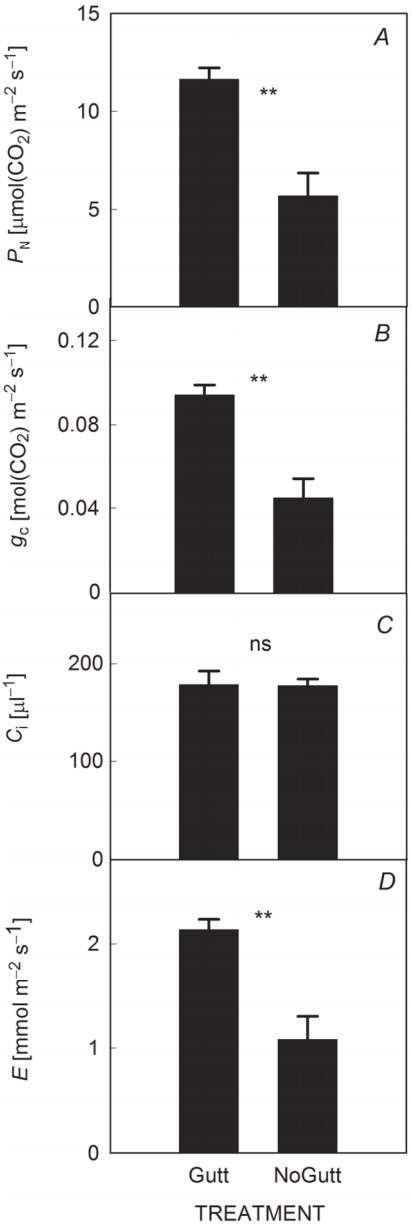

Fig. 2. *A*: Mean (*capped lines* extending from the bars are SD; n = 6 plants) net CO_2 exchange rate (P_N), *B*: stomatal conductance (g_s), *C*: internal CO_2 concentration (C_i), and *D*: transpiration rate (E) for leaves of *Alchemilla mollis* under conditions that promote (Gutt) and prevent (NoGutt) guttation. Statistical results reflect comparisons between the same six plants in the two treatments. NS – means are not significantly different (P>0.05); ** – means are significantly different at P<0.01.

The results of this study differed between the two experimental approaches used. Although photosynthetic measures of gas exchange and fluorescence did not differ between leaves of plants under conditions promoting guttation and leaves of different plants for which guttation was prevented, physiological differences were observed when leaves of the same plants were compared following the same treatments applied sequentially rather than simultaneously. Because more plants per treatment were compared in the second experimental approach, and the same plants were compared, reducing variability in the data, the remainder of the discussion considers only the results obtained using the latter experimental approach. Furthermore, the trends observed in the results from the first experiment were generally similar to those of the second, despite the lack of significant differences between the treatment means. Any conclusions, however, must be tempered by the different findings found in the two experimental approaches used here.

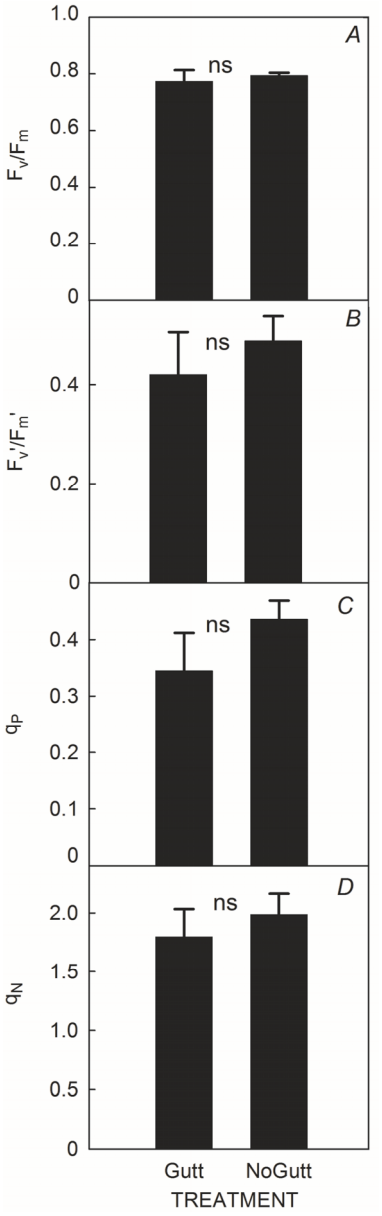

Fig. 3. *A*: Mean (*capped lines* extending from the bars are SD; n = 3 plants) intrinsic photochemical efficiency of PSII (F_v/F_m), *B*: photochemical efficiency of PSII in the light (F_v'/F_m'), *C*: photochemical quenching (q_P), and *D*: nonphotochemical quenching (q_N) in leaves of *Alchemilla mollis* under conditions that promote (Gutt) and prevent (NoGutt) guttation. Statistical results reflect comparisons between three different plants in the two treatments. No pair of means is significantly different (NS; P>0.05).

Prevention of guttation by the leaves of *A. mollis* reduced P_N and transpirational water vapor loss. Because stomatal conductances (g_s) were lower in these plants, yet leaf internal CO_2 concentrations (C_i) were the same as those measured in plants with leaves that exhibited guttation, it can be concluded that the lower P_N measured in the nonguttating leaves were most likely a result of a combination of stomatal closure and a decreased

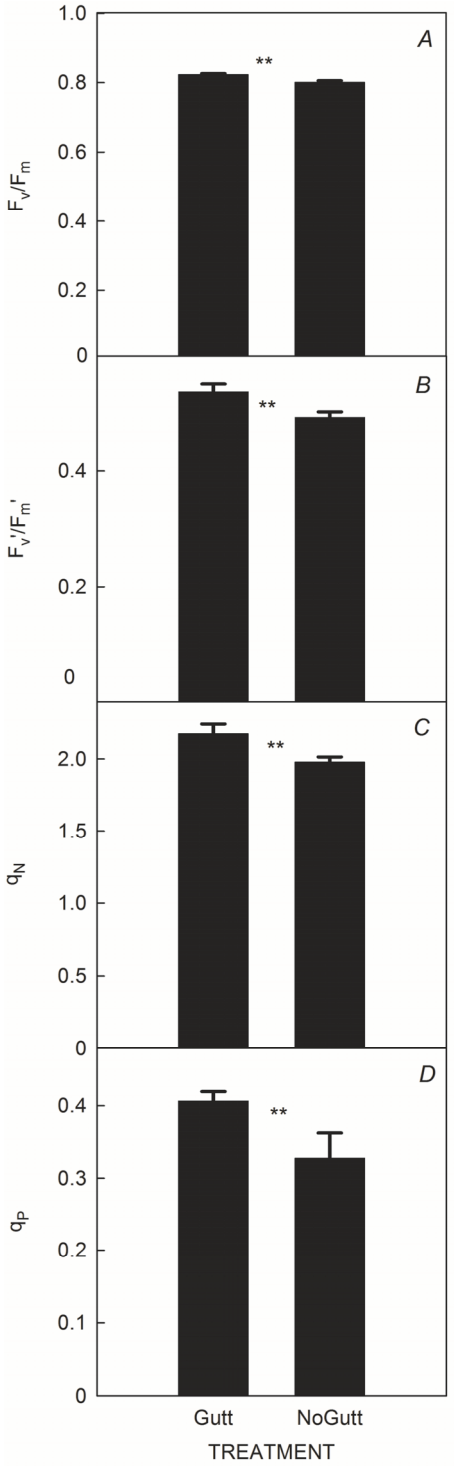

Fig. 4. *A*: Mean (*capped lines* extending from the bars are SD; n = 6 plants) intrinsic photochemical efficiency of PSII (F_v/F_m), *B*: photochemical efficiency of PSII in the light (F_v'/F_m'), *C*: photochemical quenching (q_P), and *D*: nonphotochemical quenching (q_N) for leaves of *Alchemilla mollis* under conditions that promote (Gutt) and prevent (NoGutt) guttation. Statistical results reflect comparisons between the same six plants in the two treatments. NS – means are not significantly different (P>0.05); ** – means are significantly different at P<0.01.

biochemical capacity for CO_2 fixation in these leaves (Farquhar and Sharkey 1982). In support of the latter, both the intrinsic (dark-measured) and simultaneous (light-measured) efficiency of the conversion of absorbed light energy into biochemical activity by PSII were slightly, but significantly, lower in nonguttating leaves than in leaves allowed to guttate. Also, photochemical activity (q_P) and dissipation of excess absorbed energy *via* thermal dissipation (q_N) were lower in the nonguttating leaves.

In the current study, "guttation" is defined in the broadest sense, inclusive of water absorption by the roots, movement to the leaves *via* the stem, movement through the leaves to the hydathodes on the leaf margins, and accumulation of water droplets at the tips of the leaf serrations. As a result, it is possible that most of these processes occurred in the *A. mollis* plants studied here, yet water did not accumulate at the leaf tips in the plants exposed to the LVPD treatment. Therefore, the (eco)-physiological significance of the findings presented here reflect this broad interpretation of guttation.

Midday transpiration rates of guttating leaves of *A. mollis* were nearly two times higher than those of nonguttating leaves. Prevention of guttation in this species resulted in declines in leaf photosynthetic activity, in part a result of an effect on the bio/photochemistry of the photosynthetic apparatus. These findings support, in part, those of Feild *et al.* (2005), despite the radically different experimental approaches used in the two studies (*see* above). For example, no hydration-caused darkening of the marginal leaf tissue occurred in the present study. The latter is important when comparing the results of the two studies because it is reasonable to assume that the internal "flooding" of the leaf tissue in the study of Feild *et al.* (2005) effected an inhibition of the photosynthetic apparatus, whereas the declines in photosynthetic capacity observed in the present study cannot be ascribed to the same cause. In contrast, the claim that guttation plays a detrimental role in leaf function (Curtis 1943, Ivanoff 1963) is not supported by the findings of both Feild *et al.* (2005) and the current study, although comparing the results of the latter two studies with those of previous studies is complicated by the short-term nature of the experiments of the current study and that of Feild *et al.* (2005) *vs*. the longer-term examinations of guttation effects on plant function in the studies reviewed by Ivanoff (1963).

In conclusion, although guttational water movement and accumulation of fluid on the leaf margins affects the physiology of leaves in a positive manner, it is clear that much more work remains before this phenomenon and its effects are adequately understood.

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