Cranberry Gas Exchange under Short-term Hypoxic Soil Conditions

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Abstract. **Cranberries were grown under controlled environmental conditions to determine the effects of soil waterlogging on cranberry gas exchange in three growth stages and to investigate the recovery time after waterlogging. Photosynthesis declined by 28% after the first day of waterlogging at the bud elongation stage and was 46% lower after the fifth day. At the flowering stage, the reduction in photosynthesis started to be significant only after the fifth day, whereas no reduction was observed at the fruit development stage. Stomatal limitations were responsible, in part, for the observed decrease in photosynthesis since stomatal conductance (g_S) declined by 68% and 45% after the fifth day of waterlogging during bud elongation and flowering, respectively. After water drained away in the saturated treatments, leaf photosynthesis remained lower than in the unsaturated control treatment for 1 to more than 10 days at the bud elongation stage. Our results demonstrate that short-term hypoxic soil conditions can alter cranberry gas exchange depending on plant growth stage, and suggest that adequate drainage or control of the water table depth is required to avoid the negative effects of soil waterlogging on cranberry yield.**

The cultivated cranberry (*Vaccinium macrocarpon* Ait.) is a perennial plant native from North America (Eck, 1990). Its productivity is maximized when soil water potential in the root zone (at ≈10-cm depth) is maintained between –3.0 and –7.5 kPa (Caron et al., 2016; Laurent, 2015; Pelletier et al., 2013), and growers can use overhead irrigation to avoid water stress when the lower threshold is reached. Most of the cranberry beds are equipped with drain tiles for removing excess water after rainfall or sprinkler frost protection, but drainage systems can also be used for subirrigation where the water table depth is controlled by adding water in drain tiles. Historically, cranberries were grown under wet conditions with shallow water tables around 23–38 cm below the ground surface (Eck, 1990), but there is evidence that higher yields can be attained when the water table is ≈60 cm deep (Pelletier et al., 2015).

Although drainage problems could induce significant yield limitation (Baumann et al., 2005), the time to remove the excess of water greatly depends on the water table depth before rainfall (Pelletier et al., 2015). Recent improvements in water management and the outcome of efforts at promoting the benefits of drier soil conditions could explain in part the yield increases observed in recent years in the province of Québec (Canada). Indeed, the average yield in conventional production systems increased by 38% over the last decade (from 24,000 kg·ha⁻¹ in 2004 to 33,000 kg·ha⁻¹ in 2014) (APCQ, 2015).

A decline in photosynthesis is one of the first physiological responses to soil waterlogging (Liao and Lin, 2001). Poor aeration in the root zone generally leads to a reduction in root cellular respiration and permeability, followed by a decline in water absorption (Bhattarai et al., 2005). Stomata then close gradually, transpiration is reduced, and carbohydrate translocation from leaves to roots is inhibited (Liao and Lin, 2001). The extension of root axes can be severely reduced as a result of ethylene production under anaerobic conditions resulting in injury to the plant (Smith and Restall, 1971). It has been shown in many crops, such as blueberries (Davies and Flore, 1986a), cherries (Beckman et al., 1992), and sunflowers (Grassini et al., 2007), that leaf gas exchange is affected by the lack of oxygen in the rhizosphere. Depending on the growth stage at which soil waterlogging occurs, such reduction in CO_2 assimilation may reduce the number of fruiting uprights, number of flowers, fruit set, and fruit size (Kozlowski, 1997). Hence, lower carbon assimilation under saturated soil conditions could

reduce plant growth and lead to significant yield loss. The magnitude of these impacts will depend on the time required to recover and return to preflooding values when waterlogging ends. However, although the reduction in plant gas exchange under soil waterlogging and the recovery time are species dependent (Kozlowski, 1997), little is known about these relationships in cranberry production—yet essential to improve the design of drainage systems and avoid yield limitations caused by inadequate drainage. Therefore, the objectives of the present study were to determine the effect of soil waterlogging duration on cranberry gas exchange and to investigate the recovery time after removing the excess water.

Materials and Methods

The experiments were carried out in an 18-m³ controlled growth chamber (BDW80, Conviron, Winnipeg, Manitoba, Canada) located at Université Laval in Québec City, Canada (lat. 46°46′ N, long. 71°16′ W). Mature 'Stevens' cranberry plants were collected as 0.06-m² square mats of vines from a fine sand field in October and grown in 0.27-m³ containers filled with fine to medium sand. Particular attention was paid to avoid damaging the roots and shoots when removing each mat from the soil. Plants were overwintered in the dark at an air temperature of 4 °C and relative humidity (RH) of 80% for 2880 h, satisfying the recommended time of 2500 h (Eady and Eaton, 1972). For the first week of breaking dormancy (d = 1 to d = 7), temperature was set to 15 °C from 1300 to 1800 HR and to 6 °C from 0100 to 0500 HR with constant hourly steps between these periods. RH was controlled at 50% during the day and 80% during the night, following a time course similar to temperature. From d = 8 to d = 14, the temperature was set to 21 °C day/11 °C night and from d = 15 to d = 147, to 25 °C day/16 °C night. Plants were exposed to a 13-h photoperiod during breaking dormancy and a 15-h photoperiod during the growing season. Metal halide high-intensity discharge lamps (n = 33, 400 W each) were used to provide a photosynthetic photon flux density (PPFD) of 600 µmol·m⁻²·s⁻¹ at plant level, corresponding to light-saturated conditions for cranberry (Kumudini, 2004). Each experimental unit (EU) of 0.27 m³ was seated in a 0.76-m³ container. Water was added in the large container and small holes were drilled in the bottom of the small container to ensure water entering into the soil by upward flux. A Mariotte bottle was connected to the large container to replace the water lost by evapotranspiration (Soppe and Ayars, 2003). Mariotte bottles were filled each week for maintaining a constant water table depth. Except for fertilization, no water was added to the soil surface. Fertilizers were applied at 10%, 50%, and 100% of flowering for a total dose of 40 kg·ha⁻¹ N, 60 kg·ha⁻¹ P, and 125 kg·ha⁻¹ K. Nutrients were mixed with 250 mL of water per EU and manually applied with a commercial watering can. Flowers were hand pollinated each day with a small paintbrush

from d = 44 to d = 65. This resulted in fruit set (≈40%) similar to that observed in the field (Pelletier et al., 2015), with no significant difference among EUs.

Treatments consisted of soil waterlogging time varying from 0 (control) to 5 d (1, 2, 3, and 5 d) and were applied at three different growth stages: bud elongation (d = 24 after dormancy), flowering (d = 51), and fruit development (d = 77; i.e., ≈2 weeks after fruit set). Soil in the control treatment was never waterlogged. To apply the saturation treatments, the water level in the 0.76-m³ containers was increased until the soil was completely saturated by capillary rise, as evidenced by a thin layer of water at the soil surface. At the end of each saturation treatment, the water in the large containers was removed. Because of the high saturated hydraulic conductivity of the soil and the absence of restrictive constraints, water drained away in less than 30 min after the treatment period.

Measurements of photosynthesis (P_n), g_S, and transpiration (E) were performed each day using the same uprights between 1300 and 1600 HR on the day before treatment application and for the next 12 d (except for the 6th, 8th, 10th, and 11th d) using a portable photosynthesis system (LI-6400XT; LI-COR, Lincoln, NE) equipped with a 6-cm² chamber and a red/blue light-emitting diode light source (6400-02B, LI-COR). Leaf gas exchange was measured up to 7 d after the termination of the longest saturation treatment to allow estimation of the recovery time. A different set of uprights was selected at each development stage. One-year-old leaves were used at the bud elongation stage, whereas current year foliage was used at the flowering and fruit development stages. Leaves were first acclimated under 800 μmol·m⁻²·s⁻¹ PPFD, 25 ± 1 °C leaf temperature, 400 μmol·mol⁻¹ leaf chamber air CO_2, 400 μmol·s⁻¹ air flow rate, and gas exchange variables (P_n, g_S, and E) were recorded once they had reached steady state. The leaf-to-air vapor pressure deficit (VPD_l) was maintained at 1.2 ± 0.1 kPa during measurements. Because of the difficulty to ensure a good contact between the foliage and the leaf thermocouple, the latter was positioned to measure air temperature instead of leaf temperature and the energy balance method was used for estimating leaf temperature and calculating related gas exchange parameters. After measurements, leaves were collected and digitized with a flatbed scanner to determine leaf area using the ImageJ software (W.S. Rasband, U.S. National Institutes of Health, Bethesda, MD) and measured gas exchange values were adjusted accordingly.

The experimental setup was a randomized complete block design with four replicates by treatment for a total of 20 EUs. Differences between the saturated and control treatments were analyzed using the contrast statement of the PROC MIXED of SAS 9.3 (SAS Institute, Cary, NC).

Results

The sensitivity of cranberry plants to hypoxic soil conditions depended on crop stage. At the bud elongation stage, there was a rapid decline in P_n when vines were exposed to soil saturation. Indeed, P_n was significantly decreased by 28% after the first day of soil saturation compared with the control treatment, and the overall difference was 46% after 5 d of treatment (Fig. 1A). In contrast, the responses of leaf photosynthesis to saturated soil conditions were slower during the flowering stage. There was a gradual decline in P_n as the number of days with saturated soil conditions increased but P_n started to be significantly lower than the control only on the fifth day of saturation (–34%). Cranberry vines were not sensitive to short-term soil saturation during the fruit development stage, as no significant difference was observed between treatments, even 5 d after saturating the soil. The reduction of P_n under hypoxic soil conditions could be partly due to stomatal limitation. Indeed, mean intercellular CO_2 concentration (C_i) declined from 320 μmol CO_2/mol in control plants to 283 μmol CO_2/mol in treated vines after 5 d of saturation at the bud elongation stage. Moreover, g_S declined significantly after 3 d of exposure to soil saturation during bud elongation and flowering, but remained similar to control values during fruit development (Fig. 1B). The overall decrease in g_S after 5 d of saturation was 68% during bud elongation and 45% during flowering. As VPD_l was controlled at a constant value throughout measurements, the changes in leaf transpiration attributable to saturated soil conditions were similar to those in g_S (data not shown).

The P_n and g_S values measured after drainage were normalized relative to those observed on the first day of measurement (d = 0) to compare the time required for cranberry uprights exposed to 1 to 5 d of soil saturation to return to P_n and g_S values of the control treatment. During bud elongation, P_n of vines that had been waterlogged for 1 d was similar to that of control vines on the second day after drainage occurred (Fig. 2A). In contrast, it took between 4 to more than 10 d for cranberry plants exposed to longer saturation times to reach P_n values of the controls (Fig. 2B). Indeed, P_n in the 2-d saturation treatment was still 27% lower than in the control treatment after 10 d of recovery (Fig. 2B) and ≈40% lower in the 5-d treatment after 7 d of recovery (Fig. 2D), thereby suggesting that the full recovery time of CO_2 assimilation is longer than 7–10 d in severely waterlogged cranberry vines.

Interestingly, at the flowering stage, P_n in treatments that were saturated between 1 and 3 d (Fig. 2A–C) returned to values similar to those of the control treatment on the first day after drainage. However, there was a trend in P_n values of control plants to increase faster over time, resulting in a significant difference between treated and control vines on day 6 to 8 after drainage, depending on the duration of the saturation treatment (Fig. 2A–C). In the 5-d treatment, P_n remained lower than the control treatment at least 7 d after drainage (Fig. 2D). At the fruit development stage, although the differences in P_n between treated and control vines were significant on a few sampling days, they were relatively small, which is consistent with soil saturation having no effect on P_n during fruit development (Fig. 1A). Overall, there were no significant differences between g_S of the control and saturation treatments, except at the bud elongation stage for the 2- and 5-d treatment only (data not shown).

Discussion

Photosynthetic capacity is often inhibited in flooding-intolerant plants exposed to saturated soil conditions (Liao and Lin, 2001). Our results demonstrate that cranberry is sensitive to waterlogging. On the basis of long-term weekly measurements, Laurent (2015) also observed a significant reduction in P_n and g_S of cranberry after the first week of waterlogging and suggested that photosynthesis inhibition could occur sooner. In this study, gas exchange measurements were performed on a daily basis and a significant decline in P_n was found after the first day of waterlogging (Fig. 1A). Of course, such reduction of photosynthesis may have occurred earlier than we observed, i.e., within hours of saturating the soil. Because the decrease in g_S occurred 2 d after the reductions in P_n (Fig. 1B), our results suggest that soil saturation induced nonstomatal limitations of photosynthesis. It has indeed been shown that lower P_n in the first days after waterlogging could be associated with a reduction of ribulose 1,5 bisphosphate regeneration (Bradford, 1983), a decrease in carboxylation efficiency (Fernandez, 2006), or a decrease in the chlorophyll content of leaves (Insausti and Gorjón, 2013). For poorly adapted species, a major constraint due to the excess of water is an inadequate supply of oxygen to root tissues, the diffusion of oxygen through water being 10⁴ times slower than in the air (Jackson and Colmer, 2005). The lack of aeration in the rhizosphere may induce different physiological responses that lower plant CO_2 assimilation and could impact crop yield.

This study is the first to examine the short-term effects of soil waterlogging in cranberries; however, similar results have been reported for other *Vaccinium* species. For instance, photosynthetic carbon assimilation of rabbiteye blueberry (*Vaccinium ashei* Reade) declined by 36% after 1 d of soil saturation and by 62% after the fourth consecutive day of saturation and was associated in part with stomatal limitations (Davies and Flore, 1986a). In highbush blueberries (*Vaccinium corymbosum* L.), a decrease in g_S brought about by a 56% reduction in root hydraulic conductivity resulted in lower carbon assimilation within 1–2 d of flooding (Davies and Flore, 1986b). Similar results were also reported for other crops, such as in sour cherries (*Prunus cerasus* L.), where carbon assimilation was affected less than 12 h after flooding and declined by 68% over 5 d (Beckman et al., 1992).
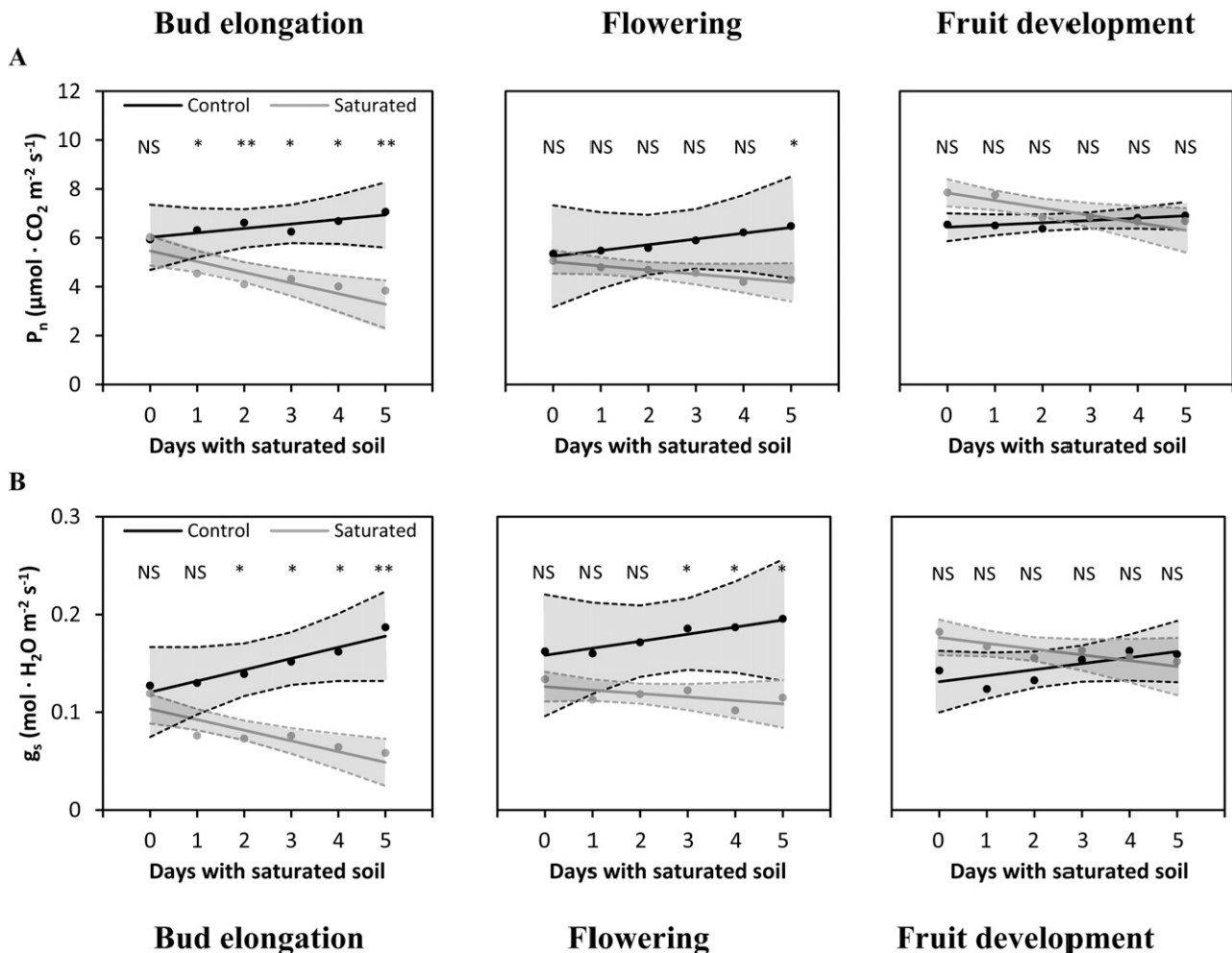
Fig. 1. Changes in (**A**) photosynthesis (P_n) and (**B**) stomatal conductance (g_S) as a function of the number of days with saturated soil conditions at the stage of bud elongation, flowering, and fruit development. Sixteen experimental units were saturated for 1 to 5 d (1, 2, 3, 5 d; n = 4 per treatment), and four control units were sampled each day. Day 0 corresponded to the day before the saturation treatments, and four experimental units were drained after 1, 2, 3, and 5 d. Significance levels are: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), nonsignificant (NS). Means and 95% confidence intervals are indicated.

Gas exchange was negatively affected by soil waterlogging during bud elongation and flowering, but not during fruit development (Fig. 1). The effects of waterlogging varying with plant ontogeny are species specific (Kozlowski, 1997). For example, the early stages of maize were also found to be the most susceptible to an excess of water (Zaidi et al., 2004). In contrast, four flooding days occurring 8 d before snap beans (*Phaseolus vulgaris* L.) flowering resulted in 96% of plant survival and a similar harvest index than the control treatment. However, when flowering occurred 8 d after flooding, only 4% of the plants survived and there were no pods to harvest (Lakitan et al., 1992). Because the same plants were used for all waterlogging treatment periods in our experiment, a carry-over effect could have masked the growth stage effect. This has been observed in other crops such as in wheat where waterlogging before anthesis was found to indeed enhance tolerance after anthesis by increasing P_n, g_S, and E (Li et al., 2011). In any case, waterlogging affected cranberry gas exchange at least in the early development stage.

Flooding is commonly used as a pest management tool in cranberry production. It has been demonstrated that a fall flood is efficient to limit cranberry fruitworm emergence and reduce dewberry weed (DeMoranville et al., 2005), whereas a 10-d flood during fruit set reduced the overall weed coverage (Sandler and Mason, 2010). Lower carbohydrate accumulation in cranberry uprights is a direct consequence of spring flood (Vanden Heuvel and Goffinet, 2008). Although vines were not covered by water in our experiment, the reduction in P_n observed here suggests that soil saturation is sufficient to impact plant growth and fruit development. It is important to note that flooding used as an integrated pest management tool may be economically beneficial in comparison with pesticide applications; however, there is a need for better tools that avoid the detrimental consequences of flooding on final crop yield. Frost protection by overhead irrigation can also lead to soil saturation (Perry, 1998). Consecutive nights of frost protection may result in several days of soil waterlogging when the water table is too shallow. Although frost protection by irrigation is essential to prevent damage to

the crop, it is recommended to lower the water table before irrigation to avoid saturation of the soil for extended periods and thereby limit crop loss (Pelletier et al., 2015). Automated intermittent irrigation during frost protection (i.e., the cycling of irrigation) is another method to limit soil waterlogging and the concomitant impairment of cranberry yield (P. Jeranyama, unpublished data). In general, P_n in the saturated treatments did not return to control values immediately after drainage. It thus appears that nonstomatal limitations of photosynthesis were more important than stomatal limitations and have prevailed through recovery. Indeed, C_i values were only 11% lower after 5 d of saturation, and were similar with control for all treatments after drainage at bud elongation, whereas no differences were observed during flowering and fruit development. In some cases, it took more than 10 d to recover the gas exchange rates of controls, in line with results for other *Vaccinium* species. In highbush blueberries (*V. corymbosum* L.), the recovery of g_S to preflood values required 18 d after 24 d of flooding, whereas more than 18 d were necessary in rabbit-eye blueberries (*V. ashei* Reade) (Davies and Flore, 1986a).
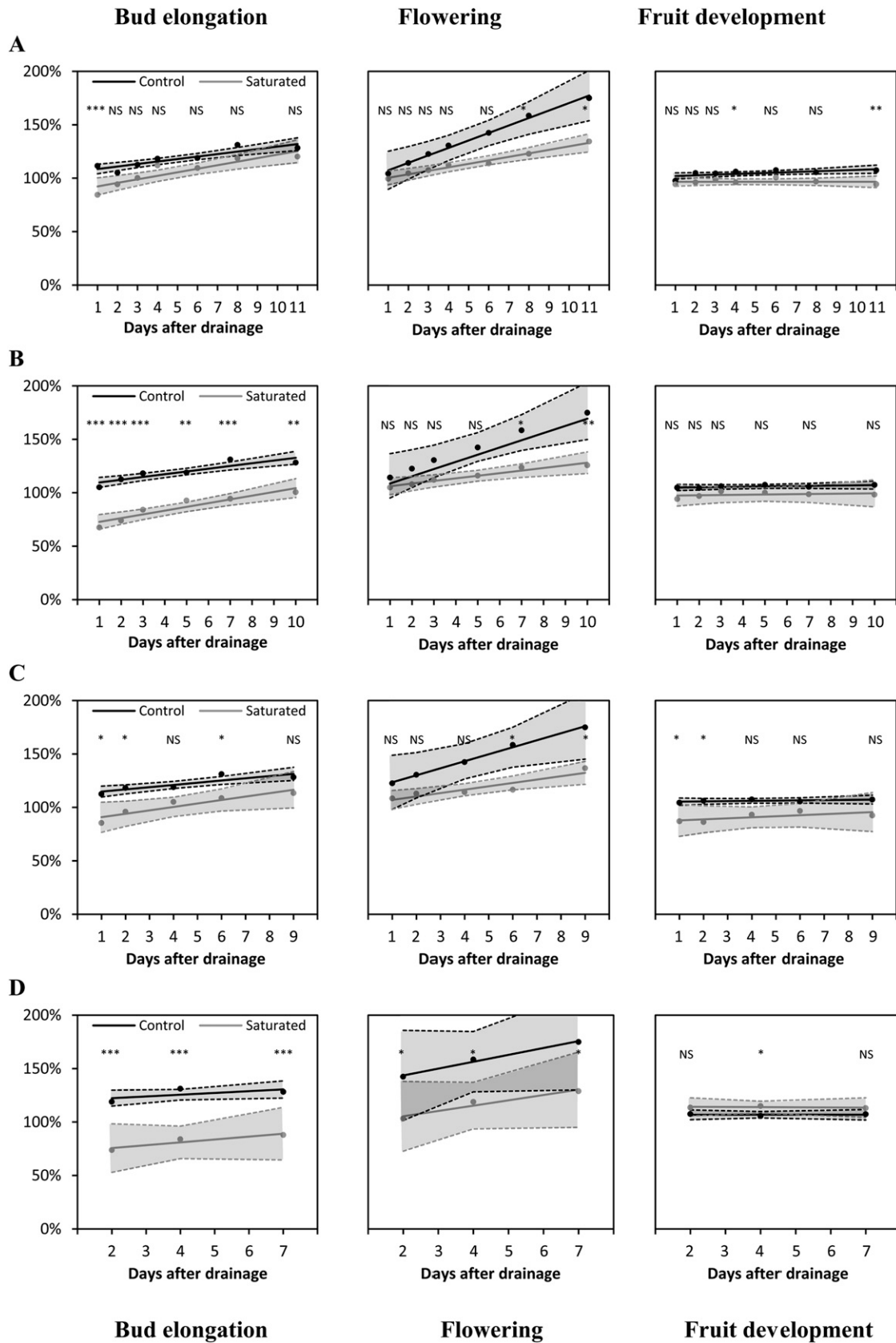
Fig. 2. Photosynthesis as a function of the number of days after drainage at the stage of bud elongation, flowering, and fruit development for treatment with (**A**) 1, (**B**) 2, (**C**) 3, and (**D**) 5 consecutive days of soil saturation. Values were standardized according to the first day of measurement. Significance levels are: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), nonsignificant (ns). Means and 95% confidence intervals are indicated.

According to our data, waterlogging should be avoided in cranberry production especially during early development. Efficient subsurface drainage is an excellent approach to controlling soil saturation. With predicted increases in rainfall intensity and frequency due to climate change and since actual drainage systems allow water to be removed in more than 24 h after precipitation in some cases (Pelletier et al.,

2015), further work should focus on implementation of the drainage system design.

In summary, P_n was significantly reduced after 1 d of soil waterlogging at the bud elongation stage and after 5 d at the flowering stage. After water drained away, the recovery time was dependent of the waterlogging duration, but varied from 1 d to more than 10 d. Drainage is therefore an important management practice to avoid the negative effects of soil waterlogging on cranberry gas exchange.

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