

Shade effects on chlorophyll content, gas exchange and nutrient content of cranberry vines exhibiting yellow vine symptoms

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Abstract

Yellow vine (YV) on cranberry (*Vaccinium macrocarpon* Ait.) is a symptom of stress that might reduce upright net photosynthetic carbon assimilation (A) through both, stomatal effects, which reduce the internal CO₂ concentration (C_i), and nonstomatal factors. This study evaluated the shade effects on reversing the effects of YV symptoms in chlorophyll and nutrient content, and uprights gas exchange. Shaded vines were affected in the same way as unshaded yellow vines and their net photosynthetic carbon assimilation was not affected by stomatal activity in contrast with the normal green vines. However, A was not limited by stomatal activity ≥ 250 mmol m⁻² s⁻¹. Chlorophyll a concentration was positively correlated with A ($r = 0.53$ $P \leq 0.05$), shaded and YV had significantly lower total chlorophyll concentration relative to normal vines. Chlorophyll b was less affected by YV symptoms. Plant tissue were collected in autumn and analyzed for individual nutrient composition. Manganese levels were excessive in all samples; this was especially true for yellow vines, suggesting that the yellow vines may be under more water stress. Shading yellow vines did not change their nutrient composition relative to unshaded yellow vines. It is plausible that excess water on the bog is the major cause of the yellow vine as growers have a cultural practice of applying 25 mm of irrigation water a week regardless of the evaporative demand or field capacity.

Key words: Yellow vine, cranberry, *Vaccinium macrocarpon*, stress, chlorophyll, gas exchange, nutrient

Introduction

Shading effects could have negative impact on a plant, but impact will be reduced if the plant is shade acclimated. Acclimation is a growth process in which each newly produced leaf has a set of biochemical and morphological characteristics suited to the particular environment (Taiz and Zeiger, 2010). The ability to acclimate under different conditions is very important, for instance in some shady habitats, plants can receive less than 20% of their photosynthetically active radiation (PAR). In most circumstances, shaded leaves have more total chlorophyll per reaction center and a higher ratio of chlorophyll b to chlorophyll a. On the other hand, the leaves exposed to the sun tend to have more rubisco and a larger pool of xanthophyll cycle components than shaded leaves (Taiz and Zeiger, 2010).

Reduced photosynthetic carbon assimilation and therefore reduced crop dry matter accumulation is a principal effect of environmental stresses in plants of agricultural importance. Photosynthesis is an important parameter used to monitor plant response to abiotic stress. There are several indications that cranberry (*Vaccinium macrocarpon* Ait.) vines are periodically under environmental stress such as during a frost night when cranberry buds or berries are exposed to temperatures below what they can actually tolerate (Workmaster and Palta, 2006). Stress can influence various plant processes and attributes including leaf water potential, stomatal conductance, transpiration, net photosynthesis, canopy-air temperature difference, crop water stress index, and leaf wilting (O'Toole *et al.*, 1984).

Yellow vine (YV) symptoms on cranberry are caused by an interaction of environmental stresses on the plant that are not well

understood (Wei *et al.*, 2010). Symptoms appear as yellowing along leaf margins with areas along leaf veins remaining green. Symptoms usually appear acropetally, affecting older leaves first. Growers often note severe YV symptoms during fruit set and early fruit growth when demand for resources is high (in late July and early August). Anecdotal evidence suggests that YV symptoms are most likely due to nutritional imbalances that are probably secondary to root problems caused by water stress (DeMoranville, 2010, pers. comm.). Too much or too little water can lead to poor root development and plants are unable to move enough nutrients and water to meet the demands of both shoots and fruits (DeMoranville *et al.*, 2009). This creates a competition for resources in which the developing fruit and the youngest leaves are the best competitors, leaving the older leaves showing YV symptoms.

We have previously reported that PS II is the main target of photoinhibition and speculated a possible role of photoinhibition associated with the YV syndrome in cranberry plants (Wei *et al.*, 2010). In a subsequent study, we used spectrometric and HPLC analyses and observed that YV leaves contained 26 to 28 % less chlorophyll than normal cranberry leaves. This demonstrates that YV syndrome is associated with a poor photosynthetic activity and is problematic for the long-term growth and crop production (Zhang *et al.*, 2011). While long term solutions for bogs consistently exhibiting YV symptoms may require changes in water management practices, some short term fixes may reduce symptoms within a growing season. It has been reported anecdotally that shaded areas on cranberry bogs tend to recover from YV symptoms quicker than unshaded areas (pers. comm. Monika Shuler). Shade treatment may provide a short term solution for YV symptoms.

The extent to which photosynthetic activity is altered in cranberry vines exhibiting YV is not well understood. Therefore, the objective of this study was to evaluate the effect of shading on chlorophyll concentrations, nutrient content, and gas exchange of cranberry vines exhibiting YV symptoms.

Materials and methods

Four shade structures were installed at a commercial cranberry bed in Carver, MA, in June 2009 and 2010. This peat-based bog has been in production for over 12 years. The shade structures were erected in a section of the bog that is generally very wet and where yellow vine symptoms are common. Structures were constructed from 5 cm diameter PVC tubes and consisted of a 4 by 2 m frame on 2 m legs; legs were buried 1 m deep on the bog. The top and three sides of each structure were covered with shade cloth (allowing 65% light penetration (Home Depot)). The four shade structures were installed over patches of yellow vines; four areas outside the shades with yellow vines, and another four areas of similar size to the shade structures with normal (green) vines were also marked for data collection.

Cranberry uprights were collected for both chlorophyll and nutrient analysis. Vines were collected from mid July to the end of August for chlorophyll analysis and vines were collected for nutrient analysis at the end of August. Chlorophyll was extracted from fresh leaves and the absorbance of each extraction was measured at 663 nm and 645 nm using a Hitachi U-1100 spectrophotometer (Hitachi High-Technologies Co.) (Fu and Huang, 2001). Absorbance measurements were used to calculate the concentrations of chlorophyll a (using absorbance at 663 nm) and chlorophyll b (using absorbance at 645 nm) according to the methods of Arnon (1949). Total chlorophyll concentration and the ratio of chlorophyll a to chlorophyll b were also calculated. Chlorophyll concentrations are reported as milligrams of chlorophyll per gram of fresh leaves. Samples were dried and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2-mm screen and analyzed for nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, sodium, iron, manganese, copper, zinc, and boron (Mid-West Labs, Omaha, NE).

Gas exchange: Gas exchange measurements were taken on uprights using an infrared gas analyzer (CIRAS-2, PP systems Inc., Amesbury, MA) with an automatic broadleaf or conifer cuvette [PLC5(C), PP Systems Inc.], between 1000HR and 1400HR. Gaseous exchange measurements were taken on uprights of (i) vines showing yellow vine syndrome, (ii) vines grown under shade and (iii) normal green vines in July and August, 2010. The average irradiance ranged between 700 to 1400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf temperature was maintained at ambient air temperature and water vapor pressure of air entering the cuvette was set at 50 to 70% of outside air, and this varied by day. Air flow was 400 mL min^{-1} with CO_2 maintained at 360 ± 10 ppm. The leaf cuvette was clamped around 10 cm of the youngest growth of an upright for approximately 5 min, until a steady rate ($\pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$) was reached, and then measurements of net carbon assimilation were taken. Upright-level gas exchange parameters used to quantify the response to YV were: carbon assimilation [A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)], sub stomatal CO_2 concentration [Ci ($\mu\text{mol mol}^{-1}$)] and stomatal conductance [gs ($\text{mmol m}^{-2} \text{s}^{-1}$)]. Leaf area index

(LAI) was obtained by destructively sampling measured uprights using an LI-3100 Leaf Area Meter (Li-COR, Lincoln, NE).

Data analysis: Trends in gas exchange measurements were determined by non-linear regression modeling (A vs. gs; Ci vs. gs) using Proc NLIN, while the relationship between A vs. Ci was determined by linear regression techniques in Proc REG (SAS Institute, 2008). Chlorophyll and nutrient data were subjected to an analysis of variance in Proc GLM (SAS Institute, 2008). Single degree of freedom comparisons were used for mean separation among treatments for chlorophyll concentrations while Fisher's protected LSD (5%) was used to separate means of the nutrient data. Chlorophyll components were also correlated with carbon assimilation in Proc COR (SAS Institute, 2008).

Results and discussion

Gas exchange: The relationship between net carbon assimilation with stomatal conductance is represented in Fig. 1. Yellow vines occur in a region where changes in stomatal conductance results in increased net CO_2 assimilation up to 200 $\text{mmol m}^{-2} \text{s}^{-1}$ (Fig. 1). In this region, A is limited by rate of increase in gs. The shaded vines seem to be affected in the same way as the unshaded yellow vines while the normal vines (green) exhibited the highest A but occurred in a region where large changes in gs did not result in increase in A (Fig. 1). The region where green vines occur indicates that stomatal conductance greater than 200 $\text{mmol m}^{-2} \text{s}^{-1}$ did not

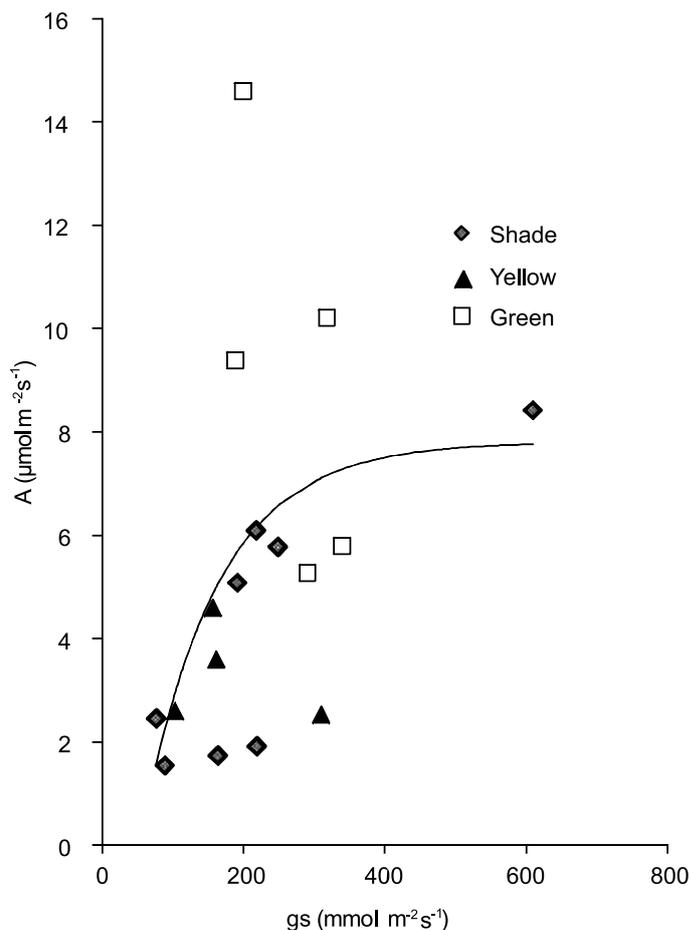


Fig. 1. The relationship between carbon assimilation (A) and stomatal conductance (gs) of cranberry vines showing yellow vine symptoms, under limited shade and normal green vines. The curve represents the best-fit relationship obtained by nonlinear regression analysis of pooled data ($r^2=0.52$).

affect A and thus other physiological processes were limiting rate of net CO_2 assimilation.

Carbon assimilation was practically independent of stomatal behavior at g_s higher than $250 \text{ mmol m}^{-2} \text{ s}^{-1}$ but g_s limited A below $6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$, a range where the A - g_s relationship became almost linear (Fig. 1) and similar results were reported with celery grown under varying NaCl concentrations (Everard *et al.*, 1994). Stomatal movements can be affected by various environmental factors, including plant water status, CO_2 concentration and light (Raschke, 1975). Light has often been suggested to exert an indirect effect on stomata via lowering of the CO_2 concentration by photosynthesis (Kim *et al.*, 2004).

The stomatal conductance in relationship with carbon assimilation in our study concurs with the findings of Sawyer (1932) on several cultivars of cranberry that are still in production (*e.g.*, var Howes). Sawyer (1932) noted that stomata in cranberry leaves never open widely, as they do in potato and other plants. The behavior of the guard cells in regulating the stomatal openings is very erratic and in general it would appear as though stomatal control in cranberry adjusts poorly to changing conditions of light, temperature, and moisture. Our results on stomatal conductance for normal vines are similar to those reported by Kumudini (2004) and Hattendorf and Davenport (1996). However, stomatal conductance for the yellow vines were very low but were slightly greater than those reported by Croft *et al.* (1993).

Yellow vine stomatal conductance resembles those of a cranberry vine experiencing water stress under field conditions (Croft *et al.*, 1993). It must be noted that plants in this study were irrigated

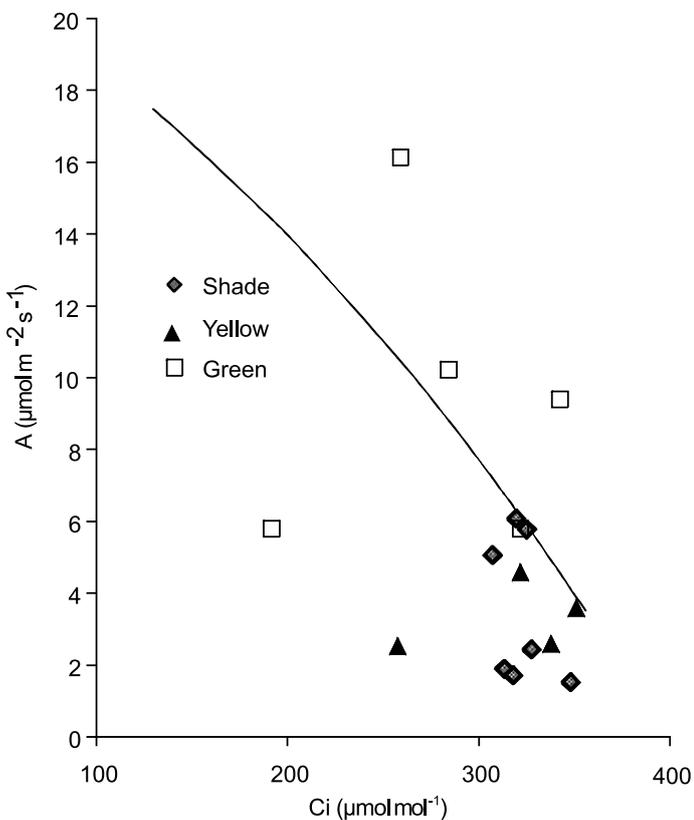


Fig. 2. The relationship between carbon assimilation (A) and intercellular CO_2 concentration (C_i) of cranberry vines showing yellow vine symptoms, under limited shade and normal green vines. The curve represents the best-fit relationship obtained by nonlinear regression analysis of pooled data ($r^2=0.72$).

regularly, indicating that some other secondary mechanism might have been responsible for this behavior. Cranberry stomatal conductance has been characterized as similar to xeromorphic plants due to their sunken stoma and because they are covered with a thick layer of epicuticular waxed stomata (Croft *et al.*, 1993).

Carbon assimilation decreased with an increase in internal CO_2 concentration for all cranberry vines (Fig. 2). Green vines (normal) were associated with a high carbon assimilation rate compared with yellow vines. In fact, the yellow vines defined the tail-end of the linear relationship while the green vines defined the top of the graph, thus at low internal CO_2 , green vines resulted in higher carbon fixation. Usually, photosynthesis rates of C_3 plants are enhanced under elevated CO_2 (Acock and Allen, 1985; Cure and Acock, 1986) unlike C_4 plants that have shown little increase in photosynthetic capacity (Kramer, 1981; Mooney *et al.*, 1991). However, there are some exceptions to this rule, for instance, a strawberry crop grown with little or no applied fertilizer in a greenhouse experiment exhibited such severe photosynthetic acclimation to elevated CO_2 and photosynthesis rate decreased at CO_2 above $600 \text{ } \mu\text{mol mol}^{-1}$ (Keutgen *et al.*, 1997). The fundamental cause of acclimation of photosynthesis to elevated CO_2 is thought to be inability of plants to fully utilize the extra photosynthates produced (Stitt and Krapp, 1999). Lack of sink capacity may result from nutrient deficiency, low temperature, restricted rooting volume, or lack of a reproductive sink. Cranberry with YV symptoms in our study typified this characterization of photosynthetic acclimation and sink limitation. Cranberry, being a C_3 plant, is prone to reach CO_2 saturation such that the most critical influence to photosynthesis becomes the enzyme rubisco's ability for CO_2 carboxylation (Taiz and Zeiger, 2010).

The low carbon assimilation could be indicating fewer PSII fixation sites in YV vines compared with normal vines since the first phase in photosynthesis is light dependent and thus is limited by the ability of light harvesting sites. Yellow vines have a lower chlorophyll concentration compared with normal vines (Table 1). The lower chlorophyll content in YV might be due to the direct photodamage of the chlorophyll molecule by excess light energy, since it is the main target in photoinhibition within photosystem II (Aro *et al.*, 1993). There is good evidence that photoinhibition occurs because of the damage to the oxygen-evolving complex of photosystem II (PSII) (Hakala *et al.*, 2005). Photoinhibition occurs when light harvested by PSII exceeds the capacity for electron transport (Taiz and Zeiger, 2010), it leads to the closure of the PSII and thus increases the chlorophyll fluorescence. Photoinhibition damages the photosynthetic systems, thereby decreasing photosynthetic activity. In addition there could have been a reduction in carbon assimilation enzymes' activity or carbohydrate accumulation.

Intercellular CO_2 concentration decreased with increase in stomatal conductance (Fig. 3). Stomatal conductance became less responsive to changes in C_i after a g_s of $200 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ was obtained. Leaf gas exchange often involves partitioning the effects of an abiotic stress into either stomatal or nonstomatal effects. Stomatal effects involve gas diffusion processes through the stomata, while nonstomatal effects are biochemical in nature and often analyzed in terms of ribulose biphosphate carboxylase oxygenase (RuBP) activity and its regeneration. In this study,

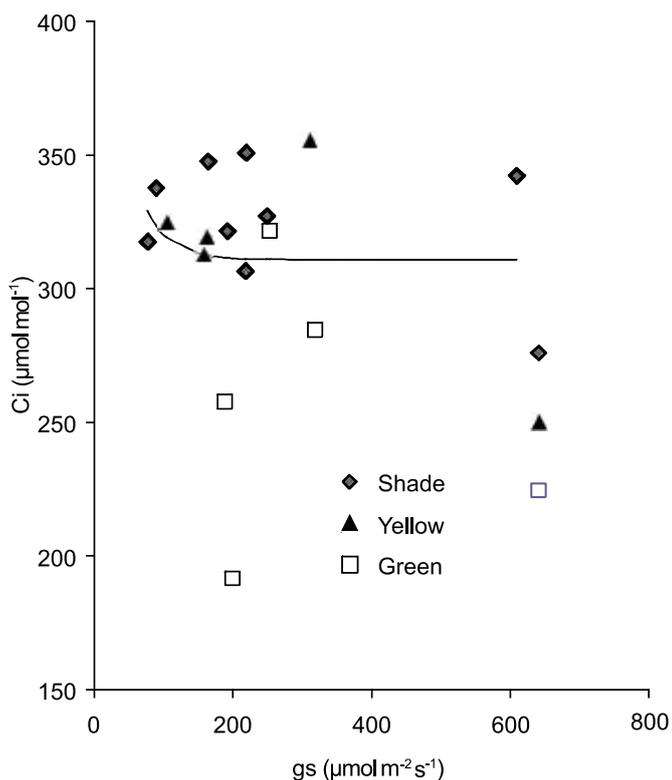


Fig. 3. The relationship between intercellular CO₂ concentration (C_i) and stomatal conductance (g_s) of cranberry vines showing yellow vine symptoms, under limited shade and normal green vines. The curve represents the best-fit relationship obtained by nonlinear regression analysis of pooled data ($r^2=0.65$).

stomatal closure appears to have influenced YV's response to A, but green vines were limited by the inhibition of metabolic processes or nonstomatal factors that led to reduced RuBP regeneration and inhibition of A. Similar results were obtained in cotton in a drought stress experiment where they found that stomatal closure was the initial plant response to a mild drought and increasing drought resulted in progressive down regulation of the metabolic processes (Flexas and Medrano, 2000).

Chlorophyll content: There was a significant contrast between unshaded yellow vines and unshaded green vines for all parameters. As would be expected, yellow vines had significantly lower concentrations of chlorophyll than green vines. Shading increased the concentration of chlorophyll a by 12% although this was not statistically significant (Table 1).

Chlorophyll b concentrations in yellow vines were not significantly affected by shading (Table 1). The concentration of chlorophyll b in the normal green vines was 60% greater than yellow vines under different treatments (Table 1). Shading does not seem to influence changes in chlorophyll b concentration. The ratio of chlorophyll a to chlorophyll b was similar across treatments and much higher for unshaded yellow vines than for normal green vines. Although concentrations of both chlorophyll a and chlorophyll b were significantly reduced in yellow vines, it appears that chlorophyll b was less affected by factors that cause YV symptoms. Green vines had a 75% greater total chlorophyll concentration compared with yellow vines.

Chlorophyll a concentration was positively correlated with carbon assimilation ($r = 0.53^*$) while total chlorophyll concentration was also positively correlated with carbon assimilation ($r =$

Table 1. Chlorophyll a, b, Chl a:b ratio and total chlorophyll concentrations of normal and yellow cranberry vines under a shade treatment

Treatment	Chl a	Chl b	Total Chl	Chl a:b
	mg g ⁻¹			
Green (normal)	1.54	0.60	2.14	2.56
Shade	1.01	0.38	1.39	2.66
Yellow	0.84	0.38	1.22	2.22
Contrasts				
Green vs. Shade	*	*	*	NS
Green vs. Yellow	**	*	**	NS
Shade vs. Yellow	NS	NS	NS	NS

*Significant at $P \leq 0.05$ or ** $P \leq 0.01$, and NS is non-significant at $P \leq 0.05$.

0.5*). There was no significant relationship with chlorophyll b ($r = 0.35$, $P=0.18$). The higher chlorophyll b may indicate that yellow vines do not affect the light harvesting system of cranberry leaves. The ratio of chlorophyll a to chlorophyll b is at variance with previously recorded ratios using an HPLC and spectrometric analysis (Wei *et al.*, 2010). The differences in these ratios could be due to sampling, growth stage of cranberry uprights and the analytical methods.

Nutrient content: Analysis of leaf nutrient concentration data did not reveal any relationship between shading and nutrient levels nor did it show that there was a significant difference between nutrient levels of green and yellow vines for macronutrients. It is however, interesting to note that, iron and manganese concentrations were significantly different between yellow vines and normal green vines. Iron levels were 175 mg L⁻¹ for shaded yellow vines, 140 mg L⁻¹ for unshaded yellow vines and 286 mg L⁻¹ for normal green vines. Levels of manganese were higher in yellow vines (1161 mg L⁻¹ shaded; 1008 mg L⁻¹ unshaded) than in normal green vines (884 mg L⁻¹). Excessive levels of manganese are an indication of overly wet soils. While manganese levels were excessive in all samples, this was especially the case for yellow vines, suggesting that the yellow vines may be under more water stress (due to excess moisture in the bog-personal observation) than green vines. It is very plausible that excess water on the bog is the major cause of the yellow vine as growers have a cultural practice of putting 25 mm of irrigation water a week regardless of the evaporative demand or field capacity (Hattendorf and Davenport, 1996).

We did not have normal green vines under shade since our hypothesis was that shading cranberry with yellow vines will change the color back to green and improve photosynthesis function. We were mostly interested in comparing yellow vines under shade and unshaded with the normal green vines as control. Our results seem to suggest that shading yellow vines does not necessarily shift their photosynthesis function or chlorophyll content as had been postulated from anecdotal observations.

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