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Soybean Tocopherol Concentrations Are Affected by Crop Management

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Soybeans are an important source of tocopherols, which have health-beneficial properties. Previous studies have demonstrated that environmental factors may affect soybean tocopherol concentrations; the impact of specific crop management strategies, however, remains poorly understood. Experiments were conducted for 2 years at three sites in Quebec to determine the impact on soybean tocopherol concentrations of seeding rate, row spacing, seeding date, cultivar, and P and K fertilization. Total and α-, γ-, and δ-tocopherol concentrations were determined by high-performance liquid chromatography. Overall, α-tocopherol was the most responsive to the factors evaluated; the response of other tocopherols was often lower or inconsistent across environments. The seeding rate affected α-tocopherol concentrations in three out of five environments; seeding at a rate of 40 seeds m^{-2} resulted in 4% higher concentrations than seeding at a higher rate. Wide row spacing (more than 36 cm) resulted in two out of five environments in 6% higher α-tocopherol concentrations as compared to narrower row spacing. The seeding date had a greater impact; mid- to late-May seeding across four environments resulted in 45% greater α-tocopherol concentrations than seeding at later dates. Phosphorus and K fertilization had a negligible impact on tocopherol concentrations. Across experiments, large differences were observed between environments; plants grown in northern environments consistently had lower concentrations of α- and γ-tocopherols but higher concentrations of δ-tocopherol. Differences between cultivars were also consistent, ranging between 10 and 30%, depending on the tocopherol. Results demonstrate that soybean tocopherol concentrations are affected by crop management and thus suggest that specific recommended agronomic practices may need to be established for the production of soybeans for the functional food market.

KEYWORDS: Soybean; *Glycine max* [L.] Merr.; tocopherols; health-beneficial compounds; agronomic practices

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) contains high levels of compounds with health-beneficial properties, which are used by the nutraceutical and functional food industries, including tocopherols. Tocopherols could play a role in cardiovascular diseases and cancer prevention and may impact the immunological system (*1*, *2*). Tocopherols exist in four forms (i.e., α, β, γ, and δ); α-tocopherol has the greatest antioxidant activity. In addition, the dietary reference intake for vitamin E is currently based solely on α-tocopherol (*3*). Thus, most interest for tocopherols resides in α-tocopherol; however, as certain health properties have also been attributed to other tocopherol forms, interest for these remains (*2*).

Factors affecting tocopherol concentrations of field-grown soybeans remain poorly researched; however, it appears that they are both genetically and environmentally determined. Large variations have been reported among soybean genotypes, but concentrations in specific cultivars have also been reported to vary greatly when grown in different environments (*4*−*7*). Despite the fact that environmental and genotype × environment effects have been reported for tocopherols, information on the impact of specific environmental factors remains limited to few studies (*8*−*10*). These investigated, under controlled conditions, the impact of temperature and/or drought during seed filing stages. Results varied depending on the studies, which differed in intensity of the stress imposed. Britz and Kremer (*9*) reported that total tocopherol concentrations in mature soybean seeds are only slightly affected by temperature and moisture stress. However, increased temperature and drought cause an increase in the proportion of α-tocopherol due to a large increase in α-tocopherol concentration (2−3-fold) combined with a reduction in γ- and δ-tocopherol concentrations. This is in accordance with reports of a greater variation in the α-tocopherol than total tocopherol concentration across environments (*4*, *6*). In contrast, Dolde et al. (*10*) reported an opposite trend with a very strong negative relation between temperature during seed development and total tocopherol concentrations; the total tocopherol content of soybean oil was reduced by as much as 95% at high

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temperatures. It is not known how environmental factors other than temperature and moisture may impact soybean tocopherol concentrations.

To our knowledge, the impact of crop management and specific agronomic practices on soybean tocopherol concentrations has never been investigated. Agronomic practices contribute in changing microenvironmental and edaphic conditions to which plants are exposed and thus may impact plant growth and composition. Agronomic practices have specifically been demonstrated to affect concentrations in crops of a range of secondary metabolites with health-beneficial properties, including isoflavones in soybean and lycopene in tomatoes (*11*, *12*). It is thus important to understand factors affecting soybean tocopherol concentrations, especially if specific target concentrations may be required by the nutraceutical and functional food industry, which is often the case in specialty value-added productions. The objectives of this study were thus to determine the impact of specific crop management practices on tocopherol concentrations, with an emphasis on α-tocopherol. Experiments were conducted in multiple environments in Quebec, Canada, to determine the impact of (i) seeding rate and row spacing, (ii) seeding date, (iii) cultivar, and (iv) P and K fertilization.

MATERIALS AND METHODS

Experiment and Treatment Descriptions. A series of three field experiments were conducted in 2007 and 2008 at a minimum, each year, of two out of three sites located in Sainte-Anne-de-Bellevue, QC (45°24′ N, 73°57′ W; environments later referred to as MTL07 and MTL08), Saint-Mathieu-de-Beloeil, QC (45°36′ N 73°14′ W, later referred to as SMB07 and SMB08), and Normandin, QC (48°51′ N, 72°32′ W, later referred to as NOR07 and NOR08), for a total of 4−6 environments.

The first experiment investigated the impact of row spacing and seeding rate in five environments (MTL07, MTL08, SMB07, SMB08, and NOR07). Three row spacings (18, 36, and 90 cm; 71 cm was used instead of 90 cm in Saint-Mathieu-de-Beloeil) and three seeding rates (40, 50, and 60 seeds m^{-2}) were evaluated for two early maturing cultivars [AC Proteina (MG 0) and OAC Vision (MG00); AC Proteina was replaced by Gaillard (MG00) in Normandin, the northernmost site].

The second experiment investigated the impact of seeding dates in six environments (MTL07, MTL08, SMB07, SMB08, NOR07, and NOR08). Four seeding dates were evaluated (mid-May, early June, mid-June, and late June) for two cultivars (AC Proteina and OAC Vision, with Gaillard replacing AC Proteina in Normandin).

Finally, the third experiment investigated the impact of K and P fertilization levels in four (MTL07, MTL08, SMB07, and NOR07) and five environments (MTL07, MTL08, SMB07, NOR07, and NOR08), respectively. Five fertilization rates were evaluated (0, 50, 100, 150, and 200 kg K ha^{-1} and 0, 25, 50, 75, and 100 kg P ha^{-1}) for two cultivars (AC Proteina and OAC Vision, with Gaillard replacing AC Proteina in Normandin). Phosphorus and K were evaluated separately.

In all cases, except for the variable under investigation, field management practices were done following local recommendations for conventional soybean production (*13*). Seeding was done in a prepared seedbed the second or third week of May at an 18 cm row spacing and a seeding rate of 50 plants m^{-2}. Seeds in all environments were inoculated at seeding with a commercial rhizobial inoculant (Liphatech, Milwaukee, WI). Plots were fertilized with sufficient P and K during field preparation to support maximal seed yield as recommended by soil tests. In addition, at Normandin, 25 kg N ha^{-1} was added along with the P and K fertilizers. The soil of most fields had an optimal pH for soybean (i.e., between 6.2 and 7.0), and initial P and K levels were classified as average or good (i.e., 60−150 kg P ha^{-1}) for P and good or high (i.e., 150−500 kg K ha^{-1}) for K, based on local soil analysis standards (*14*). Finally, weeds were controlled using appropriate herbicides based on local recommendations. The plot size varied depending on the site but was on average approximately 1.25 m × 5 m. Plots in all environments were harvested with a self-propelled combine when plants reached physiological maturity. Harvesting occurred between late September and mid-October. The seed moisture content was determined, and yields were expressed on a dry matter (DM) basis. Subsamples of approximately 50 g were then randomly selected for each plot in each environment and stored at 4 °C until grinding prior laboratory analyses.

Tocopherols Extraction and Quantification. Finely ground seeds were supersonicated in 80% aqueous ethanol (with 5 μg mL^{-1} of tocol as internal standard) for 15 min at room temperature. Hexane saturated with pyrogallol was then added and allowed to stand for 30 min. This was followed by centrifugation for 10 min at 10000*g*, with the hexane layer then isolated and evaporated overnight. Finally, 80% aqueous ethanol was added, followed by high-speed vortexing for a few seconds.

δ-, γ-, and α-Tocopherol were separated by high-performance liquid chromatography (HPLC) using a Varian system (Walnut Creek, CA) equipped with a ProStar 210 solvent delivery system, a model 410 autosampler, and a ProStar 325 UV detector. Separation was carried out on an Inertsil ODS-3 reverse phase column (5 μm, 3.0 mm × 250 mm; GL Sciences, Japan). The column was maintained at 40 °C with a 0.5 mL min^{-1} flow rate using CH_3CN/CH_3OH (75:25 v/v) for 25 min. Detection was made at 295 nm. δ-, γ-, and α-Tocopherol standards (Sigma Aldrich, St. Louis, MO) were used to prepare calibration curves. Individual tocopherols were each quantified based on the resulting curves. The total tocopherol content was calculated by summing up the content of the individual tocopherols.

β-Tocopherol is another tocopherol found in soybean seeds but only in very small concentrations [i.e., <2% of total tocopherol (*4*)]. It is a structural isomer of γ-tocopherol that comigrates with γ-tocopherol on reverse phase columns (*9*). As β- and γ-tocopherol have similar calibration curves using standards (*5*), β-tocopherol was thus measured as γ-tocopherol if present; therefore, the total tocopherol was accurately quantified.

Statistical Analyses. Plots in each of the experiments were arranged in a randomized complete block design with four replicates, with split-plot or split−split-plot restriction as required, with multiple factors being evaluated. The row spacing and seeding rate experiment had a split−split-plot restriction, and the others had a split-plot restriction. In the row spacing and seeding rate experiment, cultivars were assigned to main plots, row spacing was assigned to subplots, and seeding rate was assigned to subsubplots. In the seeding date experiment, cultivars were assigned to main plots, and seeding dates were assigned to subplots. Finally, in the fertilization experiments, cultivars were assigned to main plots, and fertilization rates were assigned to subplots.

All data were subjected to an analysis of variance (ANOVA) using the General Linear Model procedure in SAS (*15*) to identify significant treatment effects and interactions. Replicates were considered random effects, and all other factors were fixed. Combined analyses across environments could not be performed due to differences in treatments used in the different environments. In all experiments, comparisons between means were made using least significant differences at a 0.05 probability when ANOVA indicated model and treatment significances. For the fertilization experiments, the significance of trends (linear, quadratic, or cubic) was assessed using a single degree of freedom orthogonal comparisons (*16*). Only significant effects ($P < 0.05$) are presented and discussed.

RESULTS

Row Spacing and Seeding Rate Experiment. The seeding rate and row spacing affected the α-tocopherol concentration mainly through main effects; no interaction between these two factors was observed in any of the five environments. Seeding at a rate of 40 seeds m^{-2} resulted in a 4% higher α-tocopherol concentration than seeding at a rate of 50 or 60 seeds m^{-2}, in three out of five environments (**Figure 1**). No differences between seeding rates were observed in either year at Saint-Mathieu-de-Beloeil. Row spacing of 36 cm or wider resulted in 6% higher α-tocopherol concentrations as compared to narrower row spacing in Sainte-Anne-de-Bellevue in both years (data not presented). Interactions between cultivar and row spacing, however, indicated that the response depended on the cultivar, with only one responding in both years, OAC Vision in MTL07 and AC Proteina in MTL08.

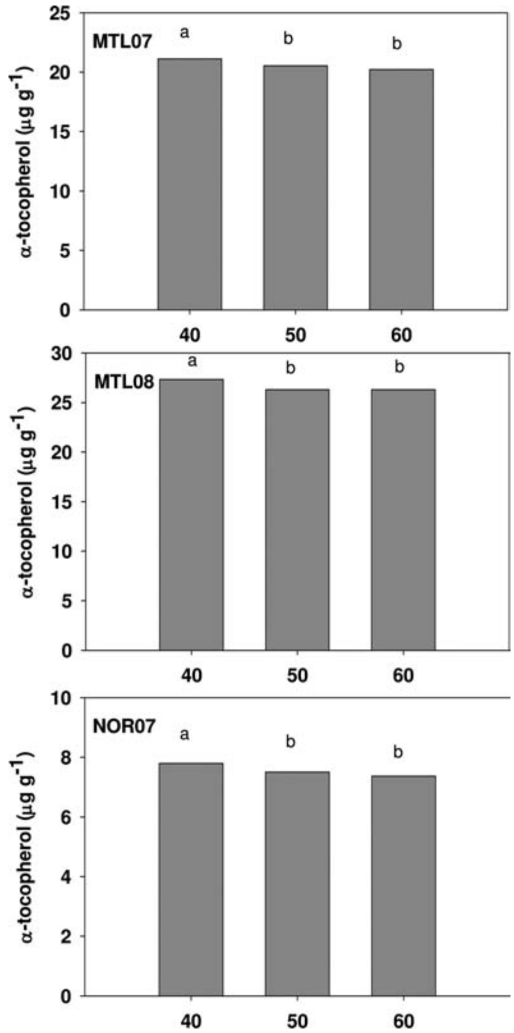

Figure 1. α-Tocopherol concentrations in soybean seeded at three seeding rates (40, 50, and 60 seeds per m^2) and grown in three environments in Quebec (MTL07, Ste.-Anne-de-Bellevue, 2007; MTL08, Ste.-Anne-de-Bellevue, 2008; and NOR07, Normandin, 2007). Results are the averages for two cultivars and three row spacings at each site and represent seeding rate main effects. Means for a given site followed by different letters are significantly different ($P < 0.05$). Note the differences in scales of the *y*-axes.

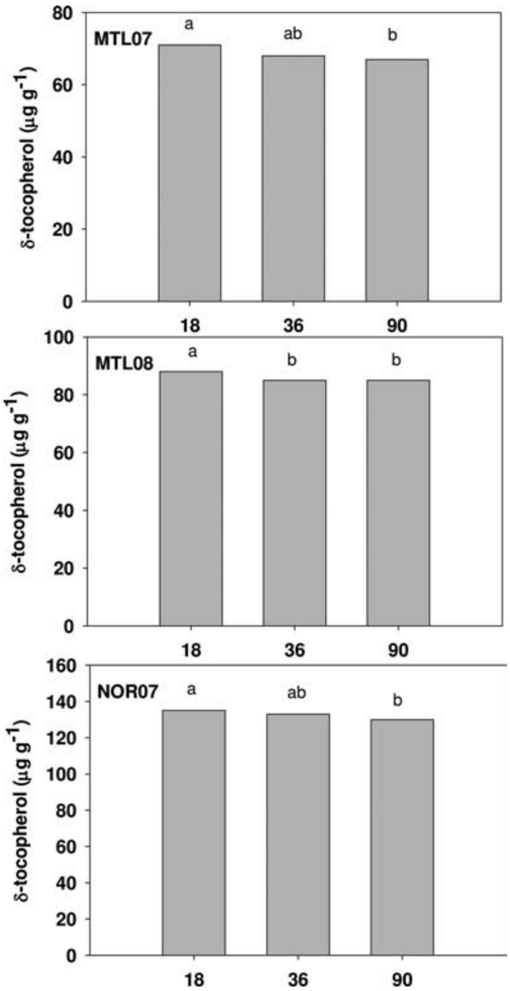

Figure 2. δ-Tocopherol concentrations in soybean seeded at three row spacings (18, 36, and 90 cm) and grown in three environments in Quebec (MTL07, Sainte-Anne-de-Bellevue, 2007; MTL08, Sainte-Anne-de-Bellevue, 2008; and NOR07, Normandin, 2007). Results are the averages for two cultivars and three seeding rates at each site and represent the row spacing main effects. Means for a given site followed by different letters are significantly different ($P < 0.05$). Note the differences in scales of the *y*-axes.

Finally, cultivar differences were observed in three out of five environments; AC Proteina and Gaillard had on average 30% greater α-tocopherol than OAC Vision (data not presented).

The impact of seeding rate and row spacing on concentrations of other tocopherols varied depending on the environment but was generally minimal. No interactions between factors were observed for any tocopherol. Total and γ-tocopherol were not affected by either seeding rate or row spacing. δ-Tocopherol was affected by row spacing only, in three out of five environments; concentrations were 4% greater at a 18 cm row spacing than wider row spacing (**Figure 2**). Differences between cultivars were consistent across environments; concentrations of δ-, γ-, and total tocopherol were on average 10, 13, and 12% greater, respectively, in AC Proteina and Gaillard than OAC Vision (data not presented).

Although direct comparisons between sites could not be made because of differences in some of the cultivars used and in row spacing treatments, the contribution of individual tocopherols to total tocopherol overall varied considerably between northern and southern environments. While the total tocopherol concentration was relatively stable across sites (average of 278 μg g^{-1}), the contribution of specific tocopherols varied greatly. Indeed, in Normandin (North), γ-, δ-, and α-tocopherol represented 52, 45, and 3% of the total tocopherol, as compared to proportions of 64, 30, and 6% in Sainte-Anne-de-Bellevue and Saint-Mathieu-de-Beloeil (South). The concentration of α-tocopherol was 2.4-fold greater in southern sites; concentrations increased along a North−South axis, averaging 8, 15, and 23 μg g^{-1} in Normandin (48°51′ N), Saint-Mathieu-de-Beloeil (45°36′ N), and Sainte-Anne-de-Bellevue (45°24′ N), respectively.

Finally, the seed yield response to both seeding rate and row spacing was highly consistent across environments (data not presented). Few interactions, namely, between row spacing and cultivar, reflected differences in the magnitude of cultivar response, although trends were comparable. Overall, there was a positive relation between seeding rate and seed yield, with yields maximized by a seeding rate of 60 seeds m^{-2}. In the case of row spacing, seed yields were greatest with row spacings of 18 or 36 cm.

Seeding Date Experiment. The seeding date affected α-tocopherol in five out of six environments (**Figure 3**). In four environments, concentrations were maximized by the earliest seeding date (i.e., mid-May). In two of these four environments

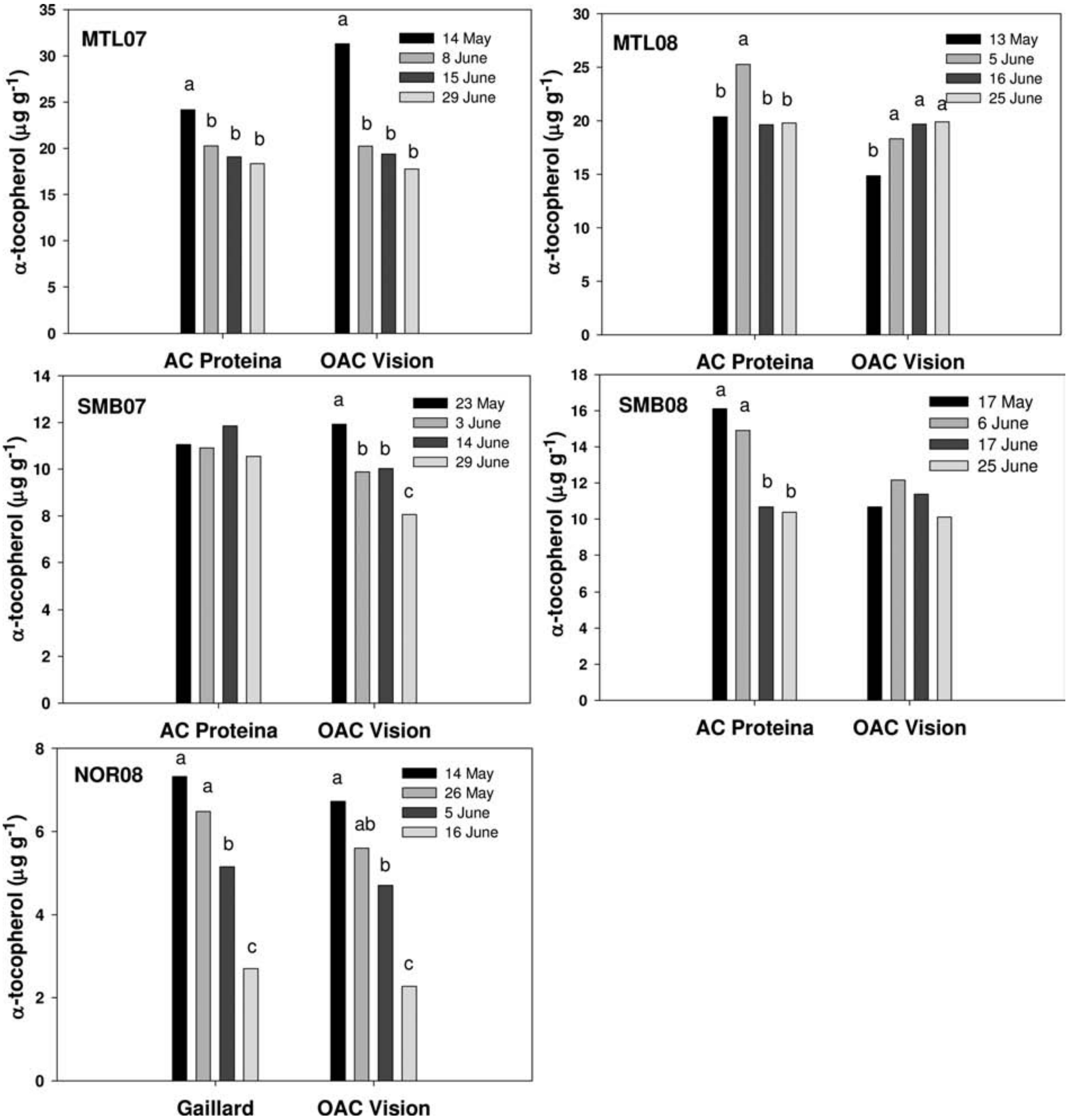

Figure 3. α-Tocopherol concentrations in two soybean cultivars grown in five environments in Quebec (MTL07, Sainte-Anne-de-Bellevue, 2007; MTL08, Sainte-Anne-de-Bellevue, 2008; SMB07, Saint-Mathieu-de-Beloeil, 2007; SMB08, Saint-Mathieu-de-Beloeil, 2008; and NOR08, Normandin, 2008). Means for a given cultivar at a given site followed by different letters are significantly different ($P < 0.05$). Note the differences in scales of the *y*-axes.

(SMB07 and SMB08), significant interactions between seeding date and cultivar, however, reflected that this response was observed for only one of the two cultivars. Overall, seeding in mid-May resulted in 45% greater α-tocopherol than seeding at a later date. The response to seeding date was the greatest in NOR08, where there was a 183% difference in α-tocopherol concentration between seeding in mid-May and mid-June. The response observed in MTL08 differed from that observed in the four other environments; the α-tocopherol concentration was maximized with the early June seeding for AC Proteina and seeding anytime in June for OAC Vision. As reported for the seeding rate and row spacing experiment, differences between cultivars were consistent across environments with α-tocopherol concentrations, and those of all other tocopherols were greater in AC Proteina and Gaillard than OAC Vision. Also, large differences were again observed between the three sites irrespective of the year; α-tocopherol concentrations increased along a North−South axis and were 4-fold greater in Sainte-Anne-de-Bellevue than Normandin.

Seeding date effects were observed for δ-tocopherol in all six environments (**Figure 4**). The response to seeding date varied depending on the site and also cultivar, as shown by significant interactions between seeding date and cultivar in five environments. In most cases, however, the interaction reflected differences in the magnitude of the response of cultivars, and only in one case, it reflected a cross-over interaction (i.e., SMB08). In three of the six environments (i.e., MTL07, SMB07, and NOR08), the response was consistent for both cultivars; concentrations were 24% greater with the later seeding date (i.e., mid- to late June). In the other three environments, the response differed. In MTL08, the trend was the opposite the earlier seeding date maximizing concentrations; in NOR07, the second seeding date (i.e., May 24) maximized concentrations. Finally, in SMB08, the date maximizing concentration differed depending on the

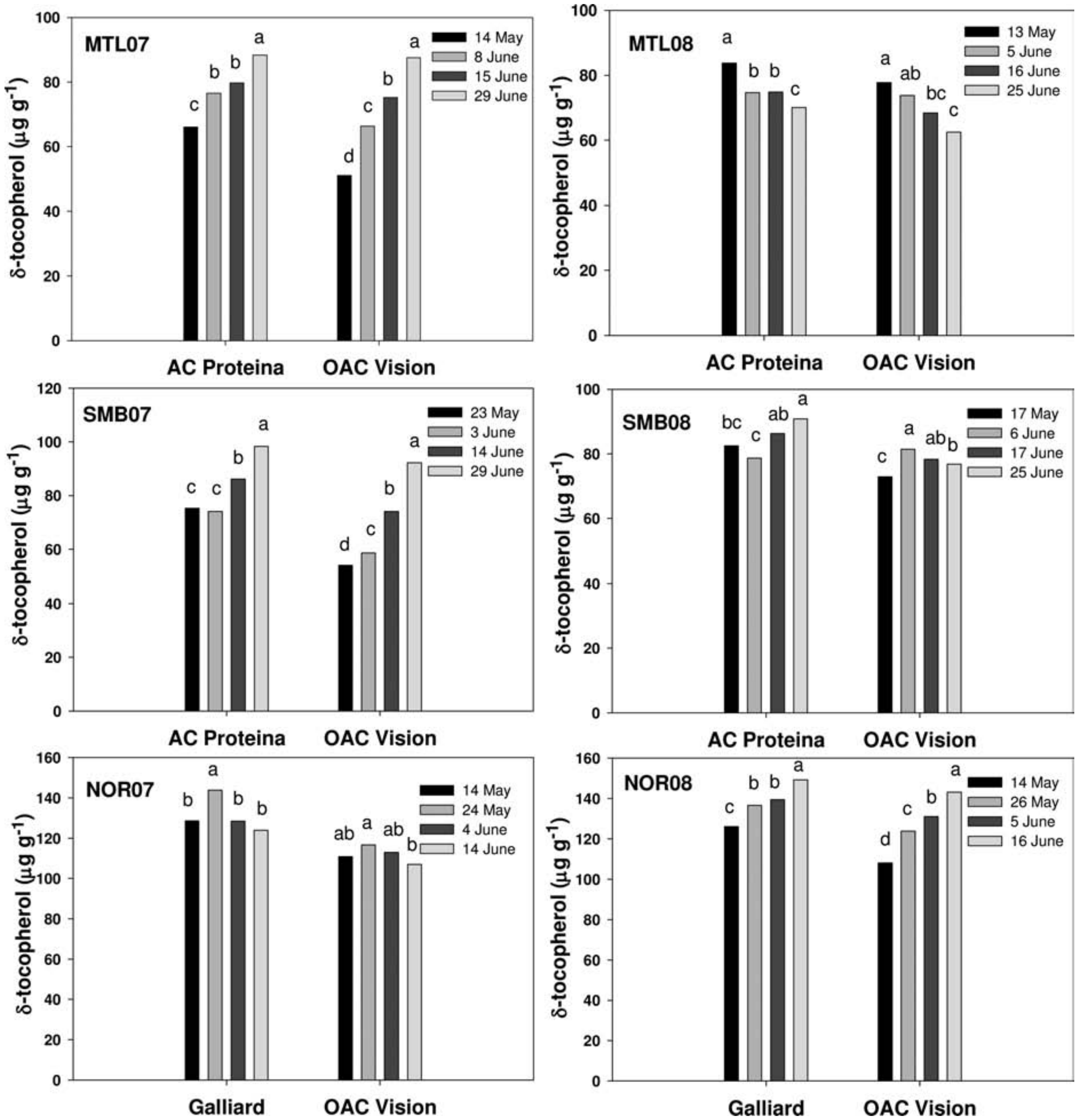

Figure 4. δ-Tocopherol concentrations in two soybean cultivars grown in six environments in Quebec (MTL07, Sainte-Anne-de-Bellevue, 2007; MTL08, Sainte-Anne-de-Bellevue, 2008; SMB07, Saint-Mathieu-de-Beloeil, 2007; SMB08, Saint-Mathieu-de-Beloeil, 2008; NOR07, Normandin, 2007; and NOR08, Normandin, 2008). Means for a given cultivar at a given site followed by different letters are significantly different ($P < 0.05$). Note the differences in scales of the *y*-axes.

cultivar and was early to mid-June for OAC Vision and mid- to late June for AC Proteina. As reported for α-tocopherol, large differences between sites were observed for δ-tocopherol; concentrations were 67% greater (127 vs 76 $\mu g\ g^{-1}$) in Normandin than at southern sites (i.e., Saint-Mathieu-de-Beloeil and Sainte-Anne-de-Bellevue).

Although seeding date effects were observed for γ-tocopherol and total tocopherol in four out of six environments, the response varied significantly depending on the site; the date maximizing concentration was highly inconsistent (data not presented). Finally, for seed yields, although the exact seeding date maximizing yields varied depending on the environment, seeding in mid- to late June consistently led to large yield reductions;yields were often half of those resulting from earlier seeding dates (data not presented).

Fertilization Experiments. Phosphorus and K fertilization had very limited effects on concentrations of all tocopherols. In addition, no interactions between fertilizer treatments and cultivars were observed. The only fertilizer effect observed was that of P on α-tocopherol; concentrations were reduced linearly by P in one out of five environments (**Figure 5**). The reduction in α-tocopherol caused by P fertilization was 7% between 0 and 100 kg P ha^{-1}. The initial soil P level in this particular environment was 102 kg P ha^{-1}; according to local classification, this soil is considered to have "average" soil P levels (*14*). The soil of most fields in the these experiments was classified as "average" or "good" for P and "good" or "high" for K, based on local soil analysis standards (*14*). On the basis of local recommendations for seed yield, fertilization for such fields should be between 20 and 60 kg P ha^{-1} and 0 and 40 kg K ha^{-1}. However, the seed yield response was overall very limited; a linear increase was observed only in one environment for P (data not presented). Fertilization (P and K) thus seems to have minimal effects on the concentrations of all tocopherols. Similar differences between cultivars and

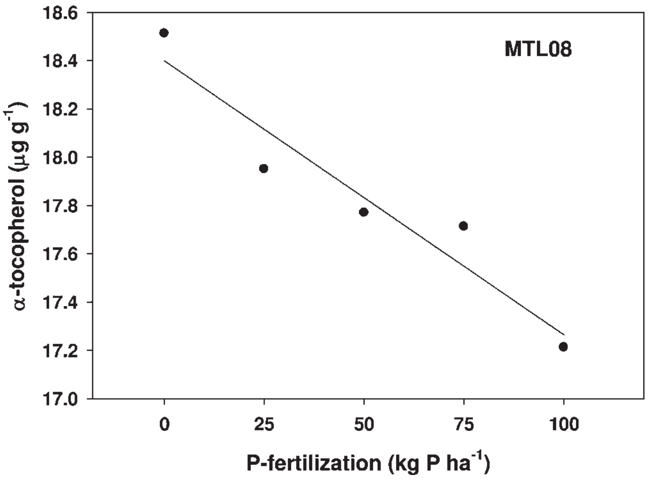

Figure 5. α-Tocopherol concentrations in soybean fertilized at different P rates. Results are the averages for two cultivars grown in Sainte-Anne-de-Bellevue, Quebec, in 2008 (MTL08). Linear regression was significant ($P < 0.05$).

sites as observed in previous experiments were also noted in this experiment (data not presented).

DISCUSSION

All crop management factors evaluated affected tocopherol concentrations to some degree. The response level depended on the factor evaluated and the tocopherol form. To our knowledge, this is the first study demonstrating an impact of crop management and agronomic practices on soybean tocopherol concentrations. Location, seeding date, and cultivars were the factors affecting tocopherol concentrations the most and P and K fertilization the least. Among all tocopherols, α-tocopherol was overall the most responsive. This is in accordance with previous studies, which also reported a greater variation of α-tocopherol than other tocopherols across environments (*4*, *6*).

The seeding date affected the concentration of all tocopherols. α-Tocopherol concentrations were maximized by the earliest seeding date (i.e., mid-May) in four out of six environments. Overall, seeding in mid-May resulted in 45% greater α-tocopherol than seeding at a later date. In the case of δ-tocopherol, the trend was the opposite, as concentrations were maximized by mid- to late June seeding in three out of six environments, while the response was inconsistent in the other two environments. Finally, in the case of total and γ-tocopherol, while a response was observed in four out of six environments, trends were highly inconsistent across environments. Such results suggest that specific tocopherols may be differently affected by the environmental factors, which are modified by a change in seeding date. Further studies in controlled environments should be conducted to identify which particular environmental factors are responsible for these differential responses to seeding date. Possible factors could include soil temperature, air temperature, and irradiance level and quality at specific crop developmental stages, factors that were all demonstrated to affect the concentration of other health-beneficial compounds in soybean (*12*). The seeding date was previously reported by several to affect soybean isoflavones; later seeding resulted in higher concentrations (*17*−*19*). Such a response is attributable in part to the fact that late seeding resulted in the exposition of developing seeds to lower temperatures, which has been hypothesized to lead to increased isoflavone synthesis in seeds. This opposite response to the seeding date of isoflavone and α-tocopherol suggests that it might not be possible to concurrently maximize the concentrations of these compounds, which are both currently of great interest to the nutraceutical and functional food industries.

Cultivar differences were consistent across environments and experiments, ranging between 10 and 30% depending on the tocopherol. In most cases, cultivar differences were greater than differences caused by other treatments evaluated, except for seeding date. While tocopherol concentrations for a given cultivar varied depending on the environment, concentrations of all tocopherols were generally greater in AC Proteina and Gaillard than OAC Vision. Such results are in accordance with other studies, which also reported that although actual tocopherol concentrations are affected by environmental conditions, performance and ranking of cultivars are relatively stable (*5*, *6*).

The other factors evaluated, seeding rate, row spacing, and P and K fertilization levels, had a much lower impact on tocopherol concentrations. The maximum effect of seeding rate was 4% for α-tocopherol, and that of row spacing was 6% for α-tocopherol and 4% for δ-tocopherol. The reason for such smaller impact as compared to other factors evaluated may stem from the fact that the different treatments only produced subtle differences in microclimatic conditions experienced by the crop. A study looking at the impact of row spacing on soybean isoflavones concentration reported a lack of response in two environments (*18*). In the case of P and K fertilization, a response to P was only observed in one out of five environments, while none was observed for K. Our results are in accordance with those of Seguin and Zheng (*20*) who reported for soybean isoflavones a lack of response to P, K, S, and B fertilization in soils with "average" to "high" initial soil fertility.

While the total tocopherol concentration was relatively stable across sites, large differences were observed in the concentrations of specific tocopherols and their contributions to total tocopherol. Differences were clearly site-specific and were consistent in both years of experimentation. Large and consistent differences were observed between Normandin (48°51′ N) and the two southern sites [Sainte-Anne-de-Bellevue (45°24′ N) and Saint-Mathieu-de-Beloeil (45°36′ N)]; Normandin had much greater concentrations and proportions of δ-tocopherol and lower α- and γ-tocopherol. Across experiments and years, the α-tocopherol concentration in Normandin was less than half of that observed in the more southern sites. Large differences in soybean tocopherol concentrations and proportions were also reported in Brazil between different sites located between latitude 25° South and 7° North, although no clear geographical pattern could be established (*4*). The observation of large differences between sites is important, as it suggests that depending on desired traits some regions could be better suited than others for the production of soybean for value-added markets. In the present case in eastern Canada, for example, if the demand is for soybeans with greater α-tocopherol contents, southern sites would be at an advantage over northern ones.

Tocopherol differences between specific seeding dates, sites, row spacing, and seeding rate could be due to a range of factors including among others air and soil temperature at specific crop developmental stages, daylength, and irradiance levels. Such environmental factors might impact the expression of genes involved in the synthesis of enzymes implicated in the last steps of tocopherol transformation or the activity of these enzymes. Indeed, δ- and γ-tocopherol share a common precursor, MPBQ (2 methyl-6-phytyl benzoquinol), from which they are synthesized. While δ-tocopherol is directly synthesized from MPBQ by 2-methyl-6-phytylbenzoquinol, γ-tocopherol requires an additional step and thus the action of two enzymes: MPBQ-specific methyl transferase and 2,3-dimethyl-5-phytylbenzoquinol-specific translocase (*1*). This later enzyme is also responsible for the synthesis of β-tocopherol from δ-tocopherol, while α-tocopherol is synthesized from γ-tocopherol through its methylation by the enzyme γ-tocopherol methyl transferase. Differences observed between treatments and sites could thus be attributable to an effect on one or several of the following enzymes: MPBQ-specific methyl transferase, 2-methyl-6-phytylbenzoquinol, and/or 2,3-dimethyl-5-phytylbenzoquinol-specific translocase. In *Arabidopsis* leaves, abiotic stresses were reported to reduce the α-tocopherol proportion (*21*). This response was associated with a reduction in γ-tocopherol methyltransferase expression.

While monitoring the seed yield response to factors evaluated was not a key objective of the current project, it is essential that any improvement in seed quality (e.g., increased tocopherol concentrations) does not come at the expense of seed yields. In the present study, the response trend of seed yield and α-tocopherol to factors evaluated was generally comparable, except for row spacing. The responses of seed yield and δ-tocopherol to seeding date were, however, opposite, meaning that delaying seeding to maximize δ-tocopherol concentration would most likely be uneconomical. Previous work with 20 soybean genotypes has reported negative correlations between seed yield and total and γ-tocopherol; no correlation was reported between seed yield and α- or δ-tocopherol (*6*).

In conclusion, results demonstrate that several crop management strategies may impact soybean nutraceutical value by affecting the concentrations of tocopherols that have health-beneficial properties. Of the factors studied, seeding date, cultivars, and site had the largest impact. The impact that crop management has on soybean tocopherol concentrations suggests that specific recommended agronomic practices may need to be established for the production of soybeans for the functional food and nutraceutical markets. Our results suggest that these may be different than those currently existing for conventional commodity soybeans, depending on the compound of interest. Finally, results demonstrate the importance of including agronomic studies in the development of functional food and nutraceutical products, given the impact that agronomic practices may have on raw material composition.

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