Research Article

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A long-term comparison of the influence of organic and conventional crop management practices on the content of the glycoalkaloid α-tomatine in tomatoes

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Abstract

BACKGROUND: α-Tomatine, synthesized by *Lycopersicon* and some *Solanum* species, is a steroidal glycoalkaloid which functions to protect against pathogens and insects. Although glycoalkaloids are generally considered toxic, α-tomatine appears to be well tolerated in humans. α-Tomatine has numerous potential health benefits including the ability to inhibit cancer cell growth in *in vitro* studies. α-Tomatine is influenced by numerous agronomic factors including fertilization and nitrogen availability. Herein, the levels of α-tomatine were compared in dried tomato samples (*Lycopersicon esculentum* L. cv. *Halley 3155*) produced in organic and conventional cropping systems that had been archived over the period from 1994 to 2004 from the Long Term Research on Agricultural Systems project (LTRAS) at UC Davis.

RESULTS: The α-tomatine levels of tomatoes in both cropping systems ranged from 4.29 to 111.85 μg g^{-1} dry weight. Mean levels of α-tomatine were significantly higher in the organically grown tomatoes than conventional ones ($P < 0.001$). In the organic management system, α-tomatine content was also significantly ($P < 0.001$) different between cropping years, suggesting that other influencing factors such as environmental conditions also affect α-tomatine content in tomato.

CONCLUSIONS: The organically produced tomatoes had higher average α-tomatine content than their conventional counterpart over the 10-year study. Significant annual variability in the α-tomatine content in tomatoes was also observed and suggests that environmental factors, external to nitrogen fertilization, influence α-tomatine content in tomatoes.

Keywords: tomato; α-tomatine; organic; conventional

INTRODUCTION

The steroidal glycoalkaloid α-tomatine ($C_{50}H_{83}NO_{21}$) is found in *Lycopersicon* and *Solanum* species.[1,2] Glycoalkaloids are not required for plant growth and function. However, they function in plant defense mechanisms against fungi, bacteria, viruses and predatory insects.[3–5] The primary glycoalkaloids present in tomatoes are α-tomatine and dehydrotomatine (Fig. 1). These glycoalkaloids are ethers between a steroid alkaloid and a carbohydrate moiety (*Solanum*-type alkaloids). The aglycone portion is called tomatidine. These *Solanum* alkaloids have one nitrogen atom located in the F ring.[6,7] Production of *Solanum*-type glycoalkaloids is favored by the same conditions that promote the development of chlorophyll. The concentration of α-tomatine is highest in stems, leaves, and green tomatoes. It can reach high levels (up to 5% fresh weight) in leaves, flowers and green fruits of tomato.[8] Because of these high levels, synthesis is sensitive to nitrogen availability.[9] The levels of α-tomatine and dehydrotomatine in tomatoes ranged from 521 to 16 285 and 42 to 1498 mg kg^{-1} fresh weight, respectively.[2] Levels are highest in immature green fruit up to 500 mg kg^{-1} fresh weight. As the tomato ripens and changes color from green to red, the levels of the glycoalkaloids decrease.

In general, most glycoalkaloids are considered to be a negative attribute due to their inherent toxicity.[10] However, the levels of glycoalkaloids consumed from red–ripe tomatoes are not considered to be toxic to humans. In fact, in Peru an indigenous variant of *Lycopersicon esculentum* var. *cerasiforme* produces a red ripe tomato fruit with a very high α-tomatine content (in the range of 500–5000 mg kg^{-1} dry weight) and even at this level, human consumption of this fruit produces no ill effects.[11] It appears that α-tomatine is poorly absorbed in the gut and is hydrolyzed to the relatively non-toxic aglycone. More recent studies of glycoalkaloids over the past decade suggest that they possess beneficial effects depending on dose and conditions of their use. α-Tomatine has been reported to have numerous health

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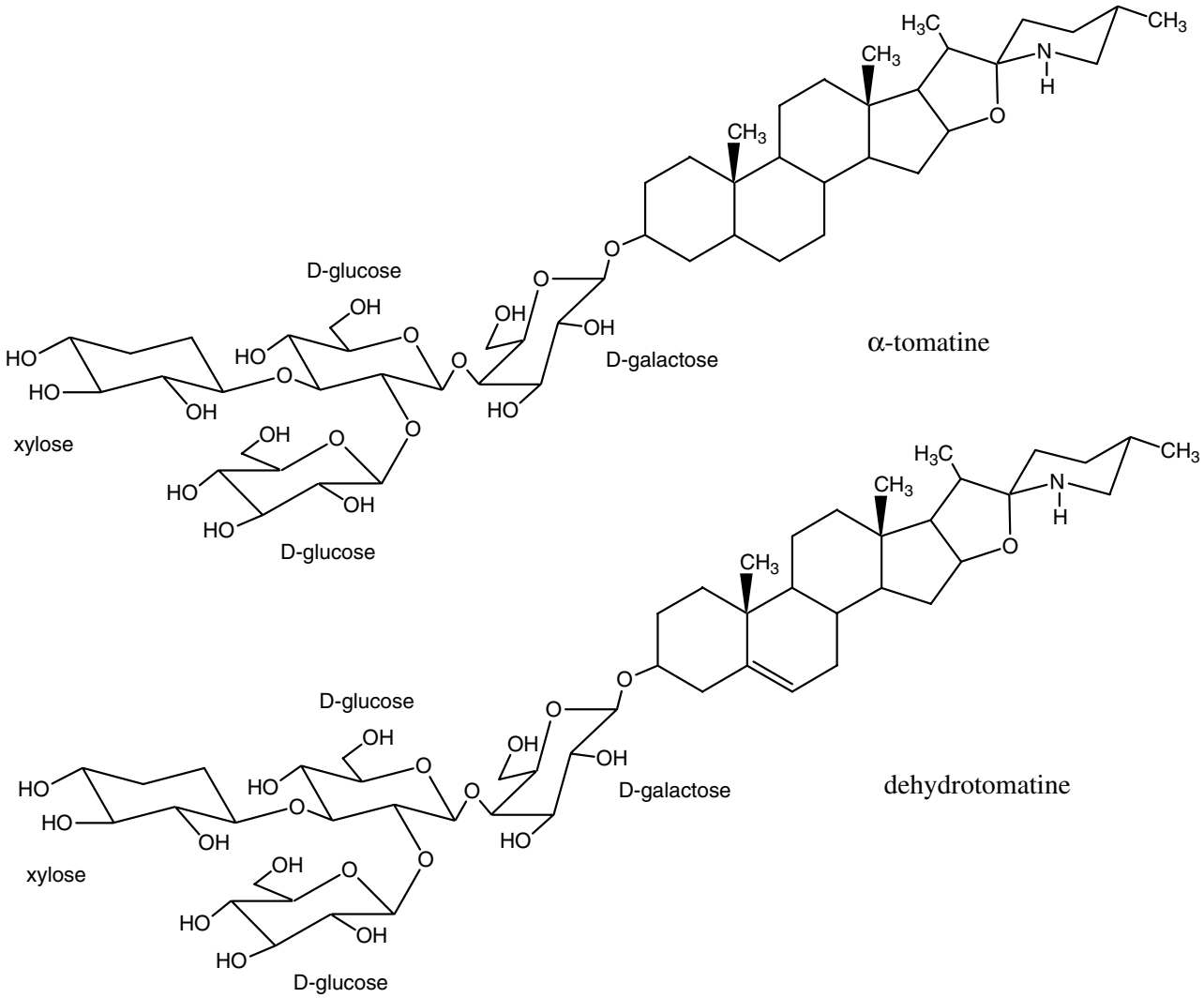

Figure 1. Structure of α-tomatine and dehydrotomatine.

promoting properties including: anti-cancer activity against tumor cell lines,[12] lowering plasma low-density lipoprotein cholesterol and triglyceride levels,[13] and enhancing the immune system.[14] In a recent study, Choi *et al*.[15] showed a negative correlation between α-tomatine and macrophage expression of tumor necrosis factor-α *in vitro* and that α-tomatine has high activity against prostate cancer cells. Given the data in support of a role for α-tomatine in the prevention of these chronic diseases, interest in understanding the levels in tomatoes has increased. As α-tomatine is a secondary plant metabolite, similar to other secondary plant metabolites, levels may be influenced by the agronomic environment in which they are grown.[16,17]

Multiple factors including genetics, soil type, fertilization practices, ripening stage, and abiotic and/or biotic stress can influence glycoalkaloid levels in plants.[9,18–20] Studies also demonstrate that the α-tomatine content of tomatoes is dependent on the tomato variety and anatomical part of the tomato plant.[2,21] The influence of different types of fertilizers and cropping system on the production of bioactives in tomatoes has been investigated.[16,22,23]

Fundamental differences between organic and conventional production systems, particularly in soil fertility management, may affect the nutritive composition of plants, including secondary plant metabolites. Organic systems emphasize the accumulation of soil organic matter and fertility over time through the use of cover crops, manures and composts and rely on the activity of a diverse soil ecosystem to make nitrogen (N) and other nutrients available to plants. Conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants. The availability of inorganic nitrogen, in particular, has the potential to influence the synthesis of secondary plant metabolites, proteins and soluble solids. Mitchell *et al*.[23] proposed that increased tomato crop growth and development rates, and greater biomass accumulation in well-fertilized crops would also correlate with decreased allocation of resources towards the production of starch, cellulose and non-nitrogen containing secondary metabolites. Given that many secondary plant metabolites are produced for defense against herbivores and are inducible by pathogens or wounding, possible differences in pest pressure between conventional and organic systems might also influence levels in food crops.

Both conventional and organic agricultural practices include combinations of farming practices that vary greatly depending upon region, climate, soils, pests and diseases, and economic factors guiding the particular management practices used on

the farm. Many of these influences change continuously, so a steady state condition may never be achieved on most farms. The dynamic nature of agricultural systems also makes adequately controlled comparisons of produce quality, free from confounding influences, experimentally challenging. Reviews of studies comparing the nutritional quality of conventionally and organically produced vegetables demonstrate inconsistent differences with the exception of higher levels of ascorbic acid (vitamin C) and less nitrate in organic products.[22–26] However, these data are difficult to interpret since cultivar selection and agronomic conditions varied widely and different methods of sampling and analysis were used in the investigations cited. In contrast, comparisons of cropping systems, using long-term research plots that have been managed consistently over time, provide a means to overcome many of the confounding factors associated with farm-based sampling. Additionally, the effects of changes over time in cropping system behavior can be evaluated using archived soil and plant samples and a reasonable estimate of the causes of those changes can be made.

The present study is an example of the use of long-term research to address complex processes operating in cropping systems. The specific objective was to compare the levels of α-tomatine in tomato samples (*Lycopersicon esculentum* L. cv. *Halley 3155*) produced in conventional and organic cropping systems that had been archived over the period from 1994–2004 from the Long Term Research on Agricultural Systems project (LTRAS) at UC Davis which began in 1993 (http://ltras.ucdavis.edu). LTRAS was designed to detect and estimate changes in crop productivity trends and other factors correlated with sustainability, which result from differing irrigation and fertilization practices. It includes an organic cropping system in which maize and tomatoes are grown in rotation and compared to the same crops produced conventionally. This archive of tomato samples is unique in California and perhaps the world.

MATERIALS AND METHODS

Chemicals and samples

α-Tomatine, (3β,5α,25*S*)-spirosolan-3-yl β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, was purchased from MP Biomedicals Inc. (Solon, OH, USA). α-Solanine (99%) was obtained from Sigma (St Louis, MO, USA). The C_{18} cartridge (1 g, 6 mL), acetic acid (glacial), and all solvents were from Fisher Scientific (Fair Lawn, NJ, USA).

Tomato cultivation in the LTRAS cropping systems

Following uniform cropping with Sudan grass (*Sorghum vulgare* L.) in 1992 and 1993, ten different cropping systems were established in 1993 using 0.4-ha plots. Each cropping system was replicated three times and both crops of the 2-year rotations were present each year. Plots were large enough to allow for the use of commercial scale farm equipment. Irrigation amounts were measured using flow meters located at each irrigated plot. Systems differ in the amount of irrigation received (rain fed or irrigated), in the amounts of nutrients applied as fertilizers and in organic matter applied to the soil as crop residues, winter legume cover crops, and/or composted manure (http://asi.ucdavis.edu/rr). Conventional plots received herbicides and other pesticides as needed while organic crops received only organically approved pesticides such as sulfur and Bt compounds (a natural pesticide made by the bacterium Bacillus thuringiensis). Crop yields and total biomass were measured every year and analyzed for total N and C. Sample archiving included yearly plant and fruit samples from all cropping systems, and time zero and subsequent soil samples collected every few years. Systems rather than single inputs were compared, so a valid comparison required that each system was managed carefully to achieve its potential yields. For example, both the conventional and organic maize/tomato systems had the same tomato cultivar (Halley 3155) over the period of interest and were irrigated similarly as needed but the short-term availability of organic N sources can require more total N input to meet crop N needs. The systems compared are model systems, chosen to include representative crops rather than more complex, changeable crop rotations. California farmers rarely follow fixed crop rotations as markets change, and organic farmers, especially, tend to have more complex rotations than the one studied at LTRAS.

Crop management practices follow best management practice guidelines in the region. Conventional tomatoes received 50 kg ha^{-1} of an N-P-K starter fertilizer and 118 kg ha^{-1} of ammonium nitrate at side-dress. A combination of tillage and herbicides were used for weed control. Aphids, mites and stinkbugs occur periodically and were controlled as necessary, similar to practices in commercial fields. In both treatments, processing tomatoes were transplanted at the rate of 22 500 plants ha^{-1} (10 000 per acre). Transplanting in the organic treatment followed incorporation of a winter legume cover crop consisting of hairy vetch (*Vicia villosa* Roth) and field peas (*Pisum sativum* L.). Transplanting of all plots occurred within a period of 2–4 days in spring, commonly around the middle of April. Prior to incorporation of the cover crop, 9 Mg ha^{-1} of composted poultry manure currently is top dressed and incorporated with the cover crop. The amount of N present in cover crops varies from year to year, but typically, organic plots currently receive between 240 and 260 kg N ha^{-1} year in addition to the N fixed by the legume cover crop. During the first three cropping cycles, to more rapidly increase soil organic matter levels and soil fertility, 19 Mg ha^{-1} of composted manure was added to tomato crops. This was reduced after organic matter levels had increased to a near constant level. Tomatoes were harvested in August each year when the field as a whole reached 90 % ripe fruit. A commercial tomato harvester was used for main plot harvests.

Sampling and preparation of plant material

Immediately prior to harvest of the main plot, samples for the archive were collected from four 3.1 m^2 sub-sample areas (sub-plots) within the larger main plots. Total plant biomass and the yield of the red ripe fruit were determined for each sub-plot. A random sample of 20 ripe fruit from the four sub-plots was washed and oven dried at 60 °C, ground, and stored in glass containers in the dark at 20 °C until analyzed. Samples from conventional and organic plots from even-numbered years (1994–2004) were chosen for α-tomatine analysis. Three of the sub-plots ($n = 3$) for each treatment (three organic; three conventional) were analyzed in each of the even years (1994–2004). These sub-plot samples were analyzed in triplicate.

Analyais of α-tomatine

α-Tomatine analysis followed the method of Cataldi *et al.*[10] A 250 mg of pestle-pulverized, air-dried tomato sample was combined with 5 mL of 1% acetic acid in water. To this mixture, 25 μL of a 1 mg mL^{-1} standard solution of α-solanine was added as an internal standard. Internal standard recovery was 92–93% for α-solanine. The mixture was extracted at room temperature for 1 h by

using a Lab-Line Orbit Environ-Shaker (Lab-Line Instruments Inc, Melrose Park, IL, USA). The homogenate was centrifuged for 15 min. The pellet was suspended in 5 mL of 1% acetic acid in water, shaken and centrifuged. Supernatant combined was applied to a HyperSep C_{18} SPE cartridge (1 g, 6 mL, Thermoscientific, Bellefonte, PA, USA), which was preconditioned with 6 mL of methanol and 6 mL of water, passed through C_{18} used for the solid-phase extraction). The sample was washed through using 5% methanol in water (v/v) and eluted with methanol. Eluent was filtered through a 0.45 µm PTFE filter (Millipore, Bedford, MA, USA).

Tomatine was resolved by reversed-phase high-performance liquid chromatography (HPLC; Shimadzu Scientific, Columbia, MD, USA) using a Prodigy ODS (5 µ), 150 mm × 2.00 mm (Phenomenex, Torrance, CA, USA). The HPLC system was equipped with a SIL-10A injector, binary 10 AD pumps, and SPD-10A UV detector (Shimadzu Scientific). The mobile phase gradient was 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B): 35–38% B in 5 min, 38% B from 5 to 15 min, 38–65% B in 5 min. The column was re-equilibrated for 10 min between runs. The HPLC was interfaced to a Quattro LC triple-quadrupole mass spectrometer (Micromass, Altrincham, UK) equipped with a dual orthogonal (*ZSPRAY*) ion source. Samples were run in positive ion mode using a capillary voltage of 3.0 kV. The source temperature and a desolvation gas temperature were 150 °C and 400 °C, respectively. Peaks corresponding to α-tomatine and solanine were identified by full scanning liquid chromatograph–electrospray ionization/mass spectrometry (LC-ESI/MS). Structural verification of peaks having ions with molecular ions corresponding to α-tomatine and solanine was achieved by product ion scanning LC-ESI/MS/MS. α-Tomatine was quantified through multiple reaction monitoring mode with the limit of detection of 5 ng mL^{-1}. The cone voltage (20–89 V) and collision energy (55–80 V) were optimized separately for each compound for accurate quantitative data. The ratio of peak area of α-tomatine to an internal standard, α-solanine, was determined and then plotted as a function of the concentration of α-tomatine.

Statistical analysis

The data were analyzed statistically by ANOVA and Duncan's multiple range tests using the SAS 9.2 version (SAS Institute Inc., Cary, NC, USA). A P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The α-tomatine levels of tomatoes ranged from 4.29 to 111.85 µg g^{-1} on a dry weight basis. This is similar to results reported earlier by Friedman and Levin,[20] who found that the α-tomatine content of ripe red tomatoes ranged from 0.3 to 6 µg g^{-1} fresh weight when considering that the moisture content of fresh tomato is around 95%.[21] Tomato samples in this study were harvested in the red–ripe stage. The mean levels of α-tomatine (Fig. 2) were significantly ($P < 0.001$) higher in the organically grown tomatoes (42.25 µg g^{-1} dry weight) as compared to the conventionally grown ones (23.16 µg g^{-1} dry weight) most years. During this study, *Verticillium* wilt broke out in 2004 in both systems. The conventional system received treatment for the wilt, whereas the organic system did not. No significant difference in α-tomatine levels was observed between two different cropping systems in 2004. This suggests that pathogen stress may not have a critical role of determining α-tomatine content in ripe tomatoes.

According to the C/N balance hypothesis,[27] plants will emphasize the synthesis of growth-related compounds with high N

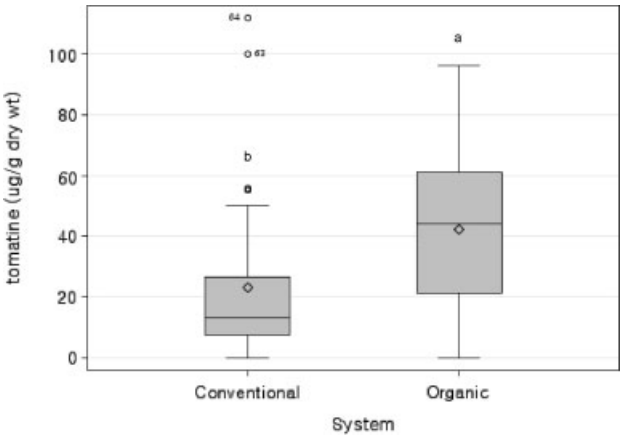

Figure 2. Comparison of α-tomatine content (µg g^{-1} dry weight) in organically and conventionally grown tomatoes from 1994 to 2004. Means with same letters are not significantly different.

content (e.g. proteins, DNA, peptides, etc.) when soil N is readily available.[27,28] α-Tomatine, however, is primarily a carbon-based compound (Fig. 1). Hoffland *et al*.[9] demonstrated that the α-tomatine content of tomato leaves correlates with the ratio of C/N and proposed that the carbohydrate moiety, rather than the N concentration, limits the biosynthetic rate of α-tomatine. In the present study, a higher average α-tomatine content was observed in the organic tomatoes as compared to the conventional tomatoes (Fig. 2). This would suggest that the readily available nitrogen in the conventional plots is not a primary factor in the biosynthesis of α-tomatine and supports the observations by Hoffland *et al*.[9] To date, studies comparing the phytochemicals content of conventional and organic crops demonstrate inconsistent differences. This, in part, can be explained by the relative rates of release of nutrients from various fertilizers, which can result in various C/N ratios in plants, and influence the production of secondary metabolites.[27,29] In organic plots, the soil microbes must first release the nitrogen before it can be assimilated. This can result in a more balanced production of primary and secondary plant metabolites and may help explain why α-tomatine levels were higher in the organic plots.

Figure 3 demonstrates the changes in the tomatine content (µg g^{-1} dry weight) in the organic (A) and conventional tomatoes (B) collected over 10 years of the LTRAS trials (1994–2004). In the organic cropping system, the α-tomatine levels are significantly ($P < 0.001$) different among cropping years (Fig. 3A), whereas in the conventional system the α-tomatine content is only statistically different in 1996 and 2002 (Fig. 3B). Total N application rate continuously decreased from 1994 to 1998 in the organic system and then increased almost up to the starting level in 2000, while the conventional system received the same total N input during 1994–2004. In both cropping systems, α-tomatine levels in the tomatoes produced in 1996 were highest, although not statistically higher in the conventional system (Fig. 3A and B). The soil organic matter in the organic plots at LTRAS reached a quantitative limit of accumulation in 1998 and the amount of composted manure applied to organic plots was reduced.[29,30] It appears that this influenced the levels of α-tomatine in these tomatoes; the 1998 tomatoes receiving the lowest nutrient application had significantly lower levels of α-tomatine. Mondy and Munshi[31] reported that total glycoalkaloid content of potatoes increased significantly with increasing levels of N fertilization when ammonium nitrate was applied to the soil. Additionally, Cronk *et al*.[32] found that increases in the glycoalkaloid

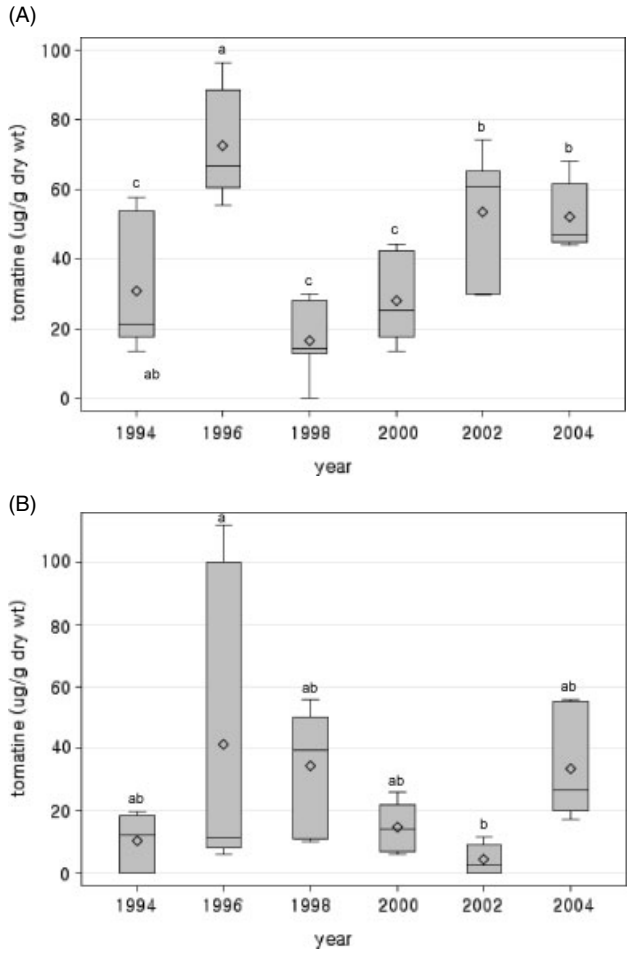

Figure 3. Changes of α-tomatine content (μg g^{-1} dry weight) in organic (A) and conventional tomato (B) over 10 years of the LTRAS trial (1994–2004). Within the cropping system, tomatine content annotated by the same letter are not significantly different at $\alpha = 0.05$.

content due to N fertilization varied with potato cultivar. Similar variety comparisons have not been made in tomatoes. Increases in N fertilization have been shown to increase the chloroplast content of the plant through increased.[33] The enzymes that are required for glycoalkaloid synthesis are located in chloroplasts and therefore could be influenced by leaf growth.

In conclusion, the organically produced tomatoes had higher average α-tomatine content than their conventional counterpart when using the same tomato cultivar. Significant variability in the α-tomatine content in tomatoes treated with the same N application over 10 years (conventional plots) was also observed. This suggests that environmental factors, external to nitrogen fertilization, influence affect α-tomatine content in tomatoes.

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