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Relevance of Organic Farming and Effect of Climatological Conditions on the Formation of α-Acids, *β*-Acids, Desmethylxanthohumol, and Xanthohumol in Hop (*Humulus lupulus* L.)

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The concentrations of α-acids, *β*-acids, desmethylxanthohumol, and xanthohumol were monitored in the hop varieties Admiral (A), Wye Challenger (WC), and First Gold (FG) during the harvest seasons of 2003 through 2005. Hops grown under an organic regimen were compared to plants grown conventionally in hop fields in close vicinity. The concentrations of the key compounds depended very much on climatological conditions showing, in general, highest levels in poorest weather conditions (2004). Of the three varieties studied, FG was the only one showing a clear trend for higher concentrations of secondary metabolites under organic growing conditions than under conventional farming conditions. Cultivation of A and WC seems to be very sensitive to climatic conditions and environmental stresses caused by pests and diseases, thereby leading to various results. WC proved to be a rich source of bioactive chalcones, particularly desmethylxanthohumol.

KEYWORDS: Organic hop (*Humulus lupulus* L.); secondary metabolites; α-acids; *β*-acids; desmethylxanthohumol; xanthohumol

INTRODUCTION

For many centuries already, hop (*Humulus lupulus* L.) has been used for beer brewing, initially as a preservative to keep beer for more than a few days, but applications of hop in traditional medicine were also manifold. It is, therefore, not surprising that, already around the turn of the 19th century, botanical and morphological knowledge of the hop plant was extended to chemical investigations, and valuable reports date from that period continuing into the first half of the 20th century. The breakthrough in hop chemistry came during the 1960s and 1970s. Hop constituents of current high interest including α-acids and derivatives, *β*-acids, and xanthohumol (**Figure 1**) were isolated and identified, and stereochemistries were firmly established (*1*). During the past decade, research into the biological activities of hop constituents was active, and highly intriguing findings have been reported (reviewed in refs *2*−*4*). Furthermore, plant-breeding programs have led to the development of hop varieties that combine unusually high concentrations of α-acids (super-α-hops) with greatly improved resistance against the most relevant diseases. This latter aspect received particular attention after the outbreak of powdery mildew in the United States in 1997. Hop genetics began to be explored (*5*, *6*), and the first transgenic hop plants have been developed (*7*).

Over the past few years, hop research has been largely dedicated to prenylflavonoids in view of their extremely interesting bioactivities. Initially formed prenylflavonoids in hops are the prenylchalcones, xanthohumol (X) and desmethylxanthohumol (DMX) (**Figure 1**), which coexist in various ratios but with a predominance of X (up to 2% in dry hop cones; see ref *8*). DMX is considered to be a proestrogen, because spontaneous Michael-type cyclization renders the prenylflavanone, 8-prenylnaringenin (8-PN) (*9*), which is one of the most potent phytoestrogens currently known (*4*, *10*). Unlike other phytoestrogens (e.g., isoflavones), 8-PN is selective for the

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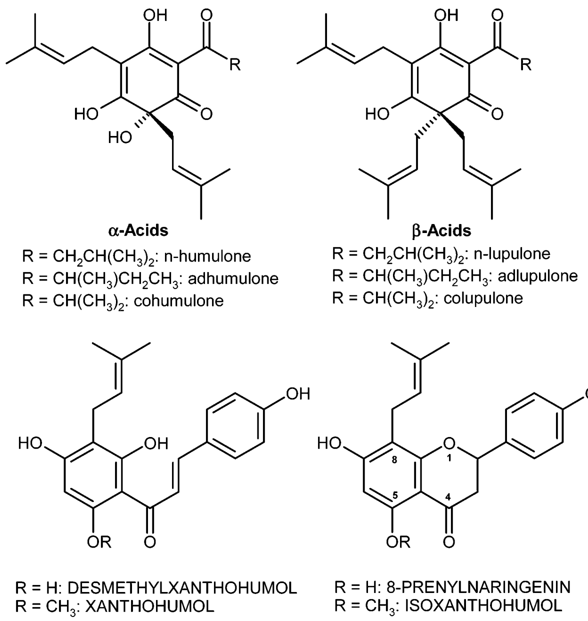

Figure 1. Structures of hop acids, prenylchalcones, and prenylflavanones.

estrogen receptor α (*11*, *12*), and it shows remarkable tissue specificity (*13*). X proved to possess a broad spectrum of cancer-inhibiting mechanisms at all stages of carcinogenesis (*14*) as well as a broad-spectrum antiinfective potential against microorganisms (*15*). In addition, X can be converted to the prenylflavanone, isoxanthohumol (IX, **Figure 1**), which is the main prenylflavonoid present in beer (thermal treatment during brewing stimulates the X-to-IX conversion; see ref *16*). We have recently discovered that particular intestinal microbiota are able to very efficiently transform IX into 8-PN; hence, the estrogenically inactive X (*17*) possesses an estrogenic potential through its isomeric IX (*18*).

In a previous study, we reported on the accumulation of α-acids, *β*-acids, desmethylxanthohumol, and xanthohumol during flowering of some selected hop varieties from small inflorescences to ripe cones during the harvest season of 2002 (Poperinge, Belgium) (*19*). In this study, we extended this investigation to the hop harvest seasons of 2003 through 2005 for three hop varieties, which were grown either conventionally or organically in nearby hop fields, thereby allowing comparisons to be made with respect to the formation of key secondary metabolites.

The development of organic hops is a response to the worldwide trend of reducing horticultural sprays and to restrict fertilizers to organic and composted manures. Organic hop farming is a positive management system, and organic is currently the only acceptable and legally recognized standard of chemical-free produce and products. Practices are designed to improve the richness and the stability of the soil by restoring its organic matter and avoiding synthetic fertilizers and pesticides. In Belgium, organic hop production is certified by Ecocert Belgium (www.ecocert.be, 23.09.2006). Farmland used to grow organic hops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years prior to harvest. Although organic produce remains a niche market, global sales have risen by about 20% per year for 5 years running (*20*). So-called "biological beers" ("biobeers" or "organic beers") have not had a real breakthrough yet, but demand for organically grown hops is increasing, whereas an apparent shortage is pushing prices upward.

Organic crops may contain higher levels of nutrients, but, in particular, increased concentrations of health-beneficial plant secondary metabolites in organic food could be important targets for organic farming. Abundant anecdotal evidence indicates that, on average, organic foods likely contain higher levels of phenolic metabolites than conventionally produced fruit and vegetables; however, few studies have directly addressed this issue (*21*). Up to 50% more phenolics have been found in organic samples of marionberries and corn with respect to conventionally grown crops (*22*). This may be due to the fact that organic plants need to have a much more intricate arsenal of compounds for defense against pests than plants that are protected by a variety of fertilizers and spraying agents. However, many environmental conditions influence the production of secondary metabolites, in particular phenolics, in plants. In this respect, we wish to present the results of a comprehensive study on the accumulation of bitter acids (α-acids and *β*-acids) and prenylchalcones [desmethylxanthohumol (DMX) and xanthohumol (X)] (**Figure 1**) in three hop varieties during the harvest seasons of 2003 through 2005 with a focus on organic farming versus conventional hop cultivation.

MATERIALS AND METHODS

Hop Sampling. Three hop varieties, Admiral (A), Wye Challenger (WC), and First Gold (FG), were investigated. Duplicates of all samples were carefully stored as voucher specimens in the facilities of the Botanical Garden of Ghent University. Conventionally grown hop samples were collected in the fields of the Free Technical Institute (VTI), Poperinge, Belgium, which are maintained by the Provincial Research and Advisory Centre for Agriculture and Horticulture (POVLT), Rumbeke, Belgium. Organic hop samples were collected at the hop farm of Joris Cambie, Poperinge, Belgium. These two sites are located only 300 m apart. Standard commercial chemical fertilizers were used for the conventional agricultural practice (ammonium nitrate once a month during April, May, and June, phosphate and sulfate during mid-April, in combination with cattle manure). As to hop protection, fungicides (active constituents: glyphosate, diquat, fosethyl-aluminum, metiram, dinocap, sulfur, copper oxychloride) were applied at regular time intervals (usually every 2 weeks), except for sulfur and copper oxychloride (weekly from early August until the end of August). Insecticides and acaricides (active constituents: tebufenpyrad, bifenthrin, imidacloprid, hexathiazox, amitraz, *λ*-cyhalothrin) were applied twice, one treatment being around mid-June and a second treatment taking place at the end of July. In the organic regimen, mainly decomposed cattle straw manure (30 tons per hectare) was used to fertilize the soil at the end of March, whereas only so-called *Bordeaux pap* (a mixture of 2 kg of copper sulfate and 1 kg of calcium carbonate in 100 L of water) mixed with soap served as a natural fungicide, which was applied every 2 weeks from the beginning of May until around August 20. Pyrethrum as bioinsecticide was used when necessary. Samples at three different developmental stages were collected each year. Stage 1 refers to the time period when inflorescences appeared (harvest dates: July 31, 2003, July 31, 2004, and August 9, 2005); stage 2 indicates small cones (length between 8 and 12 mm) (harvest dates: August 11, 2003, August 23, 2004, and August 22, 2005); and stage 3 points to full-grown hop cones (harvest dates: September 1, 2003, September 6, 2004, and September 5, 2005; i.e., just prior to the start of harvesting by the hop growers). Care was taken to sample morphologically homogeneous inflorescences and cones. Samples were immediately transferred to the laboratory and brought into 250-mL round-bottom flasks. Lyophilization was carried out for 36 h (Heto Lyolab 3000 from Heto-Holten, Allerod, Denmark, in combination with a DUO 5 Pfeiffer vacuum pump from Pfeiffer Vacuum, Asslar, Germany), and the dry materials were stored at −20 °C.

Extraction. Samples of hop inflorescences (250 mg) or cones (0.5−1 g) were ground, suspended in methanol containing 0.01% formic acid (10 mL for the inflorescences, 20 mL for cones), vortex-mixed (1 min), and stirred for 1 h. From the supernatant, ca. 1 mL was withdrawn and

Table 1. Climatological Conditions during the Months of July and August in 2003 through 2005 As Measured in Poperinge, Belgium [Source: Provincial Research and Advisory Center for Agriculture and Horticulture (POVLT), Rumbeke, Belgium]

	2003		2004		2005	
	July	Aug	July	Aug	July	Aug
temperature[a]	19.0 ± 2.3	20.1 ± 4.0	16.7 ± 2.2	18.5 ± 2.6	17.5 ± 2.4	16.2 ± 1.9
precipitation[b]	35.2	41.0	67.2	91.8	180.4	124.8
warm days[c]	8	14	3	8	4	1
dry days	20	27	13	9	14	20
wet days	11	4	18	22	17	11

[a] Mean temperature in °C ± standard deviation. [b] Millimeters (= L m^{-2}). [c] Mean temperature > 20 °C.

filtered over a 0.2-μm filter (regenerated cellulose, 13 mm; Alltech, Lokeren, Belgium).

Analytical Procedure. Quantitative analyses were done by high-performance liquid chromatography (HPLC) using a Waters 2695 Alliance Separations Module equipped with a C18 reversed-phase column (Varian Omnisphere, 250 × 4.6 mm, 5 μm). Water and methanol were used as solvents A and B, respectively, and both were acidified with 0.025% (v/v) formic acid. The gradient profile was as follows: 0−3 min, 45% B in A; 3−32 min, from 45% B in A to 95% B in A; 32−37 min, 95% B in A; 37−45 min, from 95% B in A to 45% B in A; 45−47 min, 45% B in A. The injection volume was 20 μL, the flow rate 1 mL/min, and the column temperature 35 °C. The UV-detection wavelengths were set at 314 nm (for α-acids and β-acids) and at 370 nm (for desmethylxanthohumol and xanthohumol), respectively, using diode array detection. Identification was routinely done by comparison of the retention times with those of authentic reference compounds, as well as by analysis of the respective UV spectra. A mixture of α-acids and β-acids (Versuchsstation Schweizerische Brauereien, Zurich, Switzerland) of well-known composition [ICE-1, International Calibration Extract-1; 17.69% cohumulone, 41.49% (humulone + adhumulone), 9.66% colupulone, 8.46% (lupulone + adlupulone)] served as external standard to quantify α-acids and β-acids. Desmethylxanthohumol and xanthohumol were isolated by semi-preparative HPLC according to published procedures (*9*, *23*). ^{1}H NMR and ^{13}C NMR data (Varian 300 MHz) were in accordance with reported data (*24*). Injections were done in twofold for five preparations of each sample, and standard deviations were calculated on the basis of peak area integration. Means were found statistically different when no overlap was found for the 95% confidence intervals.

RESULTS AND DISCUSSION

The choice of the three hop varieties, Admiral (A), Wye Challenger (WC), and First Gold (FG), was carefully made. First of all, A and WC had already been investigated in a previous study on the accumulation of α-acids, β-acids, desmethylxanthohumol (DMX), and xanthohumol (X) from the onset of flowering until the final formation of ripe cones (*19*). The selection of the hop varieties was motivated by their contrasting capacities for accumulation of the bioactive DMX and X, WC being characterized by high levels of DMX and low levels of X, whereas medium levels of DMX in connection to high and low levels of X were evident for A and FG, respectively. Furthermore, A, WC, and FG were available in two hop fields in each vicinity, where, in one field, hops were grown in a conventional way (further referred to as "conventional hops") and in the other field hops were grown organically (further referred to as "organic hops").

Weather Conditions during the Study Period. The concentrations of α-acids, β-acids, DMX, and X were monitored during the harvest seasons 2003, 2004, and 2005. The summer was hot and dry in 2003 and very rainy in 2004. The weather during the summer of 2005 was intermediate between these conditions. Climatological data collected in Poperinge for the months of July and August are given in **Table 1** (the climatic conditions of the first couple of days in September, when the mature cones were harvested, had very likely little influence on the results). The average temperatures and the numbers of warm and dry days were highest in 2003, whereas the precipitation was lowest compared to the other years. The precipitation was exceedingly high during July and August 2005, but the rain occurred in only 17 and 11 days, respectively, thereby indicating relatively few days with heavy rains intermingled with extended dry periods. The number of wet days (18 in July, 22 in August) characterized the miserable climatic conditions during the summer of 2004. Overall, the climatological conditions were substantially different during the harvest seasons chosen for this study.

General Patterns of Year-to-Year Variations. The analytical HPLC procedure used allowed us to determine all analytes in a single run, whereby the detection wavelengths were chosen according to the chromophores present, that is, 314 nm for the hop acids and 370 nm for the prenylchalcones. The hop acids represent each a mixture of two isomers, so-called n-humulone (invariably referred to as "humulone") and adhumulone for α-acids, n-lupulone (or "lupulone") and adlupulone for β-acids, and one homologue, cohumulone for α-acids and colupulone for β-acids. The HPLC analysis separates the isomers from the homologue in each series, and separation of the individual isomers was not necessary, because the aim was to evaluate the formation of α-acids and β-acids as separate groups over the study period. DMX and X were well separated, and they eluted prior to the hop acids (a representative chromatogram can be found in ref *19*).

Although priority is attached to the final concentrations at the onset of the harvest, we also monitored the accumulation of the key compounds throughout the flowering period. It is very important to stress that the two hop fields in Poperinge from which samples were withdrawn are only 300 m apart, thereby ensuring very similar environmental conditions with respect to soil composition and weather. Thus, differences in the contents of the target compounds of conventional hops (POVLT and Cambie) and organic hops (Cambie), for a given harvest year, should mainly depend on the different agricultural practices, rendering comparisons meaningful. Results of the quantitative analyses carried out on mature cones at the beginning of the harvest in 2003 through 2005 are given in **Table 2**, and a visual presentation of the results for α-acids and DMX is provided in **Figure 2**.

The most obvious general observations are (a) the concentrations of all compounds were highest for all hop varieties in 2004 and (b) they were, in general, lowest in 2003. It is remarkable that similar observations are true both for a so-called bitter hop such as Admiral, and for a so-called dual-purpose (relatively high levels of α-acids combined with a fine hoppy aroma) variety such as Wye Challenger. There are, obviously, some deviations from these general patterns that may be due partly

Table 2. Concentrations (%, w/w) of α-Acids, β-Acids, Desmethylxanthohumol (DMX), and Xanthohumol (X) in Full-Grown Cones of Several Hop Varieties during the Harvest Seasons of 2003, 2004, and 2005 (A, Admiral; WC, Wye Challenger; FG, First Gold; Org, Organic Hops; Conv, Conventionally Grown Hops) (for Experimental Conditions, See Materials and Methods)

	2003				2004				2005			
	α-acids	β-acids	DMX	X	α-acids	β-acids	DMX	X	α-acids	β-acids	DMX	X
A-Org	6.3 ± 0.56	2.6 ± 0.21	0.08 ± 0.003	0.54 ± 0.021	14.0 ± 1.48	6.5 ± 0.69	0.18 ± 0.006	1.17 ± 0.038	10.3 ± 1.59	4.9 ± 0.76	0.09 ± 0.007	0.87 ± 0.066
A-Conv	8.4 ± 0.56	3.3 ± 0.19	0.10 ± 0.002	0.73 ± 0.017	10.8 ± 0.74	4.6 ± 0.30	0.13 ± 0.007	0.95 ± 0.047	9.8 ± 1.50	5.7 ± 0.79	0.09 ± 0.007	0.90 ± 0.062
WC-Org	3.1 ± 0.53	3.9 ± 0.27	0.20 ± 0.008	0.19 ± 0.007	3.8 ± 0.13	5.2 ± 0.19	0.22 ± 0.011	0.29 ± 0.013	7.3 ± 0.95	4.3 ± 0.56	0.24 ± 0.018	0.47 ± 0.032
WC-Conv	1.7 ± 0.11	2.1 ± 0.07	0.13 ± 0.002	0.18 ± 0.004	6.5 ± 0.33	6.2 ± 0.27	0.30 ± 0.010	0.42 ± 0.013	5.2 ± 0.57	3.6 ± 0.36	0.18 ± 0.009	0.32 ± 0.017
FG-Org	2.6 ± 0.44	1.8 ± 0.29	0.09 ± 0.007	0.18 ± 0.014	7.9 ± 0.87	3.8 ± 0.41	0.14 ± 0.003	0.47 ± 0.015	6.5 ± 1.36	3.0 ± 0.56	0.10 ± 0.010	0.46 ± 0.043
FG-Conv	2.1 ± 0.34	1.1 ± 0.17	0.06 ± 0.005	0.15 ± 0.013	6.1 ± 0.39	2.4 ± 0.14	0.11 ± 0.003	0.39 ± 0.010	6.3 ± 0.83	3.1 ± 0.38	0.10 ± 0.006	0.44 ± 0.026

[a] Values are means of triplicate analyses (± standard deviation).

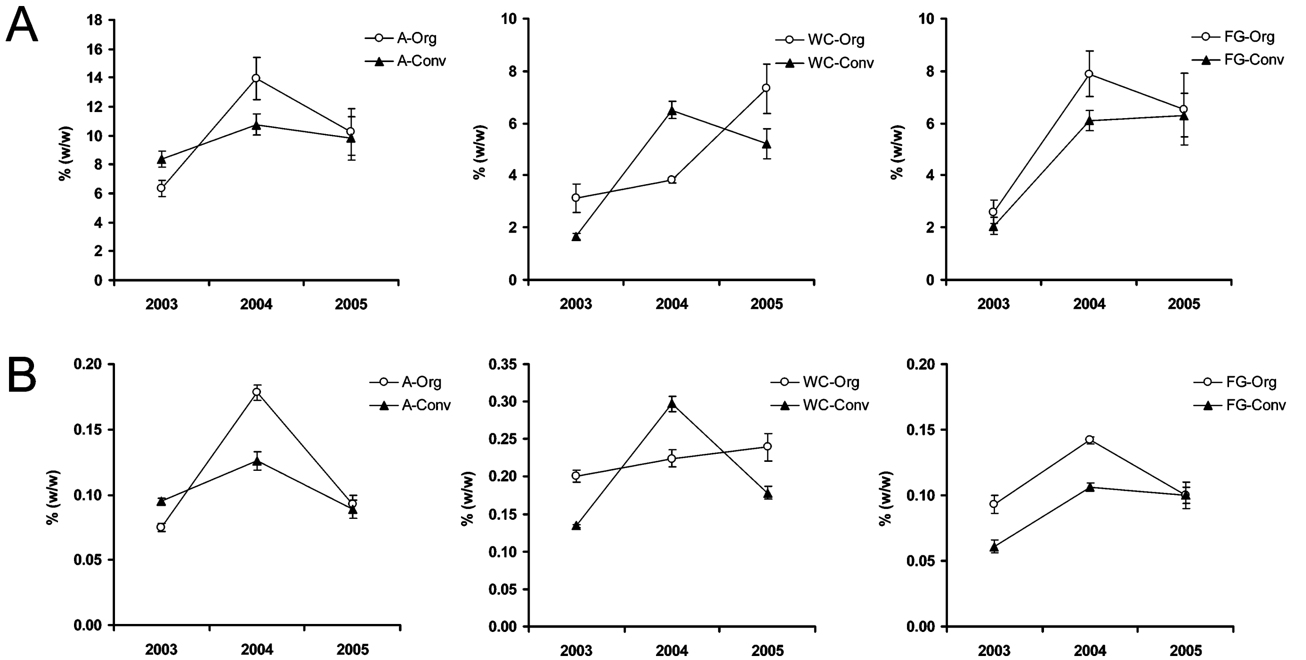

Figure 2. Concentrations (%, w/w) of α-acids (**A**) and desmethylxanthohumol (**B**) of several hop varieties at harvest in 2003, 2004, and 2005 (A, Admiral; WC, Wye Challenger; FG, First Gold; Org, organic hops; Conv, conventionally grown hops) (for experimental conditions, see Materials and Methods). Values are means of triplicate analyses, and error bars represent standard deviations.

to biological variability and partly due to specific factors in the field that are associated with the behavior of a particular hop variety in a particular season.

Interpretation of these results is not straightforward, as confounding factors related to the weather conditions undoubtedly intervene. On the one hand, the abnormally high temperatures already noted during the spring and early summer in 2003 led to unusually low final levels of hop secondary metabolites throughout Europe, in particular of α-acids and β-acids. On the other hand, the wet summer months of 2004 imposed increased stress to the plant, mainly due to hop aphids and downy mildew [observations in the Poperinge hop fields by the Provincial Research and Advisory Centre for Agriculture and Horticulture (POVLT), Rumbeke, Belgium; results not detailed]. Interestingly, hop aphids were a nuisance also in 2003, but, in view of the combination of high temperatures and low precipitation, downy mildew was suppressed. In contrast, long periods of increased susceptibility to infections due to downy mildew were evident throughout the summer months of 2005, but hop aphids could be readily controlled. Two-spotted spider mites were more active in 2003 than in the other two study years. In general, dry and warm weather favors the occurrence of spider mites, whereas warm and relatively humid weather favors hop aphids. Mild temperatures and changing weather conditions increase the risk of downy mildew. The correlation between weather conditions and powdery mildew is less clear.

Among-Variety Differences. Analysis of the profiles of individual hop varieties shows that only A is a high-α variety (14.0% in A-Org in 2004), and it is, at the same time, also clearly the highest in X (as high as 1.17% in A-Org in 2004), which confirms previous reports on a positive correlation between the concentrations of α-acids and X (*25*). The concentrations of β-acids are rather variable, although they more or less follow the patterns of the α-acids. WC proved to contain the highest levels of DMX over the three growing seasons, to a rough approximation twice as high as the other two hop varieties. Moreover, record concentrations of 0.24% DMX in organic WC in 2005 and even of 0.30% DMX in conventional WC were noted in 2004, whereas the concentration of DMX (0.201%) exceptionally superseded that of X (0.19%) in organic WC in 2003. Another odd feature associated with organic WC is the predominance of β-acids over α-acids in 2003 ($\beta/\alpha = 1.24$) and 2004 ($\beta/\alpha = 1.35$), but not in 2005 ($\beta/\alpha = 0.59$). Concentrations of α-acids and X in FG were more or less comparable to those in WC, whereas the concentrations of DMX were comparable to those in A. The levels of β-acids were always lowest in FG. These observations are in accordance with the rationale for selecting the three hop varieties.

Table 3. Concentrations (%, w/w) of α-Acids, β-Acids, Desmethylxanthohumol (DMX), and Xanthohumol (X) in Full-Grown Cones of Three Hop Varieties, Averaged over the Harvest Seasons of 2003, 2004, and 2005 (A, Admiral; WC, Wye Challenger; FG, First Gold; Org, Organic Hops; Conv, Conventionally Grown Hops) (for Experimental Conditions, See Materials and Methods)

	α-acids	β-acids	DMX	X
A-Org	10.18	4.68	0.115	0.524
A-Conv	9.66	4.54	0.103	0.857
WC-Org	4.76	4.67	0.221	0.315
WC-Conv	4.46	3.95	0.203	0.308
FG-Org	5.67	4.74	0.112	0.372
FG-Conv	4.82	2.20	0.089	0.327

Comparison of Conventional and Organic Hops. A previous study (*19*) has revealed that secondary hop metabolites accumulate from the onset of flowering, and these observations were fully confirmed by the present research. Thus, α-acids, β-acids, DMX, and X were present in samples taken at stage 1 (see Materials and Methods). The most significant increases in absolute quantities of the target compounds occurred obviously during the development from small cones (stage 2) to full-grown cones (stage 3). It seems clear that specific properties of a particular hop variety determine its potential for an increased production of secondary metabolites under organic farming conditions.

Comparisons of the contents of the target secondary metabolites of conventional (Conv) hops and organic (Org) hops over three harvest seasons are intriguing, as they may reveal sensitivities of particular hop varieties to an organic regime. A-Org (a high-α hop) performed much better than A-Conv only in 2004: 14.0% for α-acids in A-Org versus 10.8% in A-Conv; 6.5% for β-acids in A-Org versus 4.6% in A-Conv. A similar trend was noted for DMX and X: 0.18% for DMX in A-Org versus 0.13% in A-Conv; 1.17% for X in A-Org versus 0.95% for A-Conv. Interestingly, already from the appearance of inflorescences (data not shown), FG-Org possessed superior qualities with respect to FG-Conv, and this feature was maintained throughout the cultivation period, although the differences in concentrations of the relevant compounds derived from organic versus conventional growing decreased continuously, and at harvest most differences were no longer significant. Thus, FG gave a consistent profile, with FG-Org showing a trend for higher concentrations of all compounds in comparison to FG-Conv in all seasons. The sole exception was the concentration of β-acids for FG-Conv in 2005, which, however, was only marginally higher than that of FG-Org, 3.1% versus 3.0%, and this difference was not statistically significant. WC exhibited a much more peculiar behavior. WC-Conv had significantly higher contents of α-acids and β-acids, as well as of DMX and X, in 2004 than WC-Org. An outlier of 7.3% α-acids for WC-Org was found in 2005, and this same odd effect was apparent for X in WC-Org with a content of 0.47% in 2005. Although there is no obvious explanation for these unusually high levels, a parallelism was noted again between α-acids and X, and this observation lends support to the validity of the results. It should furthermore be noted that the ratios of DMX to X both in WC-Org and in WC-Conv are substantially higher than in the other hop varieties for all years. Overall, when the average concentrations of α-acids, β-acids, DMX, and X were examined over the harvest seasons 2003 through 2005 (**Table 3**), organic hops proved to be generally superior to conventional hops with some exceptions, for example, for X in A. This generalization should, however, be interpreted with great caution, as it covers only 3 years in a particular growing region. Moreover, the differences are not statistically significant, and many variables can influence the results.

The levels of the secondary metabolites as a function of the stresses caused by hop aphids and downy mildew are noteworthy. The increases of the concentrations of α-acids and X in A and FG occurred apparently in 2004 and 2005 when downy mildew was present, whereas, in WC (in particular WC-Org), the levels of α-acids were very low during 2003 and 2004, possibly under the influence of stress due to hop aphids. Remarkably, A and FG accumulated relatively high levels of DMX in the harvest season of 2004 when both hop aphids and downy mildew attacked the hop fields. These observations may hide some particular features, but, clearly, much more research is needed to gain insights into the sensitivities of individual hop varieties to various stress factors.

In summary, the results of the present study indicate that the three hop varieties investigated are very sensitive to various environmental factors that may either trigger enhanced biosynthesis of α-acids, β-acids, desmethylxanthohumol, and xanthohumol or inhibit the formation of these key compounds. Comparison of the varieties grown under an organic regimen to the same varieties grown under normal commercial production is confounded by weather variations and stresses caused by pests and diseases. It appears that only the hop variety First Gold shows a clear trend for enhanced production of secondary metabolites in organic production when compared to conventional production, and this behavior is consistent throughout the harvest seasons 2003, 2004, and 2005. The two other hop varieties did not show a uniform pattern. Wye Challenger, a hop variety known for its low pest resistance, performed much better under a conventional regime in the rainy season of 2004, whereas the highly resistant Admiral proved to be superior under an organic regimen in 2004.

LITERATURE CITED

(1) Verzele, M.; De Keukeleire, D. *Chemistry and Analysis of Hop and Beer Bitter Acids*; Elsevier: Amsterdam, The Netherlands, 1991.

(2) Stevens, J. F.; Page, J. E. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry* **2004**, *65*, 1317−1330.

(3) De Keukeleire, D.; Heyerick, A. Prenylflavonoids account for intriguing biological activities of hops. *Acta Hortic. (ISHS)* **2005**, *668*, 93−100.

(4) Chadwick, L. R.; Pauli, G. F.; Farnsworth, N. R. The pharmacognosy of *Humulus lupulus* L. (hops) with an emphasis on estrogenic properties. *Phytomedicine* **2006**, *13*, 119−131.

(5) De Keukeleire, J.; Roldán-Ruiz, I.; Van Bockstaele, E.; Heyerick, A.; De Keukeleire, D. Screening for genes involved in the biosynthesis of prenylated chalcones in hops (*Humulus lupulus* L.). *Acta Hortic. (ISHS)* **2005**, *668*, 175−190.

(6) Matoušek, J.; Vrba, L.; Novák, P.; Patzak, J.; De Keukeleire, J.; Škopek, J.; Heyerick, A.; Roldán-Ruiz, I.; De Keukeleire, D. Cloning and molecular analysis of the regulatory factor *HlMyb1* in hops (*Humulus lupulus* L.) and the potential of hops to produce bioactive prenylated flavonoids. *J. Agric. Food Chem.* **2005**, *53*, 4793−4798.

(7) Horlemann, C.; Schwekendiek, A.; Höhnle, M.; Weber, G. Regeneration and *Agrobacterium*-mediated transformation of hop (*Humulus lupulus* L.). *Plant Cell Rep.* **2003**, *22*, 210−217.

(8) Berwanger, S.; Frank, N.; Knauft, J.; Becker H. Biosynthetic ^{14}C-labelling of xanthohumol in hop cones. *Mol. Nutr. Food Res.* **2005**, *49*, 857−860.

(9) Hänsel, R.; Schulz, J. Desmethylxanthohumol: isolation from hops and cyclization to flavanones. *Arch. Pharm. (Weinheim)* **1988**, *321*, 37−40.

(10) Milligan, S. R.; Kalita, J. C.; Heyerick, A.; Rong, H.; De Cooman, L.; De Keukeleire, D. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 2249−2252.

(11) Schaefer, O.; Humpel, M.; Fritzemeier, K. H.; Bohlmann, R.; Schleuning, W. F. 8-Prenyl naringenin is a potent ER alpha selective phytoestrogen present in hops and beer. *J. Steroid Biochem. Mol. Biol*. **2003**, *84*, 359−360.

(12) Bovee, T. F. H.; Helsdingen, R. J. R.; Rietjens, I. M. C. M.; Keijer, J.; Hoogenboom, R. L. A. P. Rapid yeast estrogen bioassays stably expressing human estrogen receptors α and *β*, and green fluorescent protein: a comparison of different compounds with both receptor types. *J. Steroid Biochem. Mol. Biol.* **2004**, *91*, 99−109.

(13) Hümpel, M.; Isaksson, P.; Schaefer, O.; Kaufmann, U.; Ciana, P.; Maggi, A.; Schleuning, W.-D. Tissue specificity of 8-prenylnaringenin: protection from ovariectomy induced bone loss with minimal trophic effects on the uterus. *J. Steroid Biochem. Mol. Biol.* **2005**, *97*, 299−305.

(14) Gerhäuser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauft, J.; Neumann, I.; Scherf, H. R.; Frank, N.; Bartsch, H.; Becker, H. Cancer chemopreventive activity of xanthohumol, a natural product derived from hop. *Mol. Cancer Ther.* **2002**, *1*, 959−969.

(15) Gerhaüser, C. Broad spectrum antiinfective potential of xanthohumol from hop (*Humulus lupulus* L.) in comparison with activities of other hop constituents and xanthohumol metabolites. *Mol. Nutr. Food Res.* **2005**, *49*, 827−831.

(16) Stevens, J. F.; Taylor, A. W.; Clawson, J. E.; Deinzer, M. L. Fate of xanthohumol and related prenylflavonoids from hops to beer. *J. Agric. Food Chem.* **1999**, *47*, 2421−2428.

(17) De Keukeleire, D.; Milligan, S. R.; De Cooman, L.; Heyerick, A. The oestrogenic activity of hops (*Humulus lupulus* L.) revisited. *Pharm. Pharmacol. Lett.* **1997**, *7*, 83−86.

(18) Possemiers, S.; Heyerick, A.; Robbens, V.; De Keukeleire, D.; Verstraete, W. Activation of proestrogens from hops (*Humulus lupulus*) by intestinal microbiota; conversion of isoxanthohumol into 8-prenylnaringenin. *J. Agric. Food Chem*. **2005**, *53*, 6281−6288.

(19) De Keukeleire, J.; Ooms, G.; Heyerick, A.; Roldán-Ruiz, I.; Van Bockstaele, E.; De Keukeleire, D. Formation and accumulation of α-acids, *β*-acids, desmethylxanthohumol, and xanthohumol during flowering of hops (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2003**, *51*, 4436−4441.

(20) MacIlwain, C.; Nelson, L.; Giles, J.; Gewin, V. Organic: is it the future of farming? *Nature* **2004**, *428*, 792−798.

(21) Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventionally grown foods—results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281−293.

(22) Asami, D. K.; Hong, Y.-J.; Barrett, D. M.; Mitchell, A. E. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn growing using conventional, organic, and sustainable agricultural practices. *J. Agric. Food Chem.* **2003**, *51*, 1237−1241.

(23) Stevens, J. F.; Ivancic, M.; Hsu, V. L.; Deinzer, M. L. Prenylflavonoids from *Humulus lupulus*. *Phytochemistry* **1997**, *44*, 1575−1585.

(24) Stevens, J. F.; Taylor, A. W.; Deinzer, M. L. K. Quantitative analysis of xanthohumol and related prenylflavanones in hops and beer by liquid chromatography - tandem mass spectrometry. *J. Chromatogr. A* **1999**, *832*, 97−107.

(25) Krofta, K.; Poustka, J.; Nováková, K.; Hajšlová, J. Contents of prenylflavonoids in Czech hops and beers. *Acta Hortic. (ISHS)* **2005**, *668*, 201−206.

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