Journal of Applied Botany and Food Quality 88, 1 - 4 (2015), DOI:10.5073/JABFQ.2015.088.001

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Effects of growing system and season on the alkaloid content and yield of different sweet *L. angustifolius* genotypes

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(Received August 18, 2014)

Summary

Nine varieties and two breeding lines of sweet *Lupinus angustifolius* were cultivated under organic and conventional conditions in Northern Germany in growing seasons 2010, 2011 and 2012. The alkaloid content was significantly influenced by the growing system and year and also by genotype. The variety 'Vitabor' and the breeding line 'Bo 083521AR' revealed a very low alkaloid content in all years and cropping systems, while 'Sanabor', 'Borlu' and 'Boregine' had a higher content. In the years 2010-2012 significantly lower alkaloid contents (475 µg g^{-1}) were found under organic conditions than under conventional conditions (615 µg g^{-1}). The mean alkaloid level of all varieties and breeding lines in organic farming was highest in 2010 (640 µg g^{-1}) and lowest in 2012 (364 µg g^{-1}), depending on temperature during the seed filling period. Investigations on the yield of *L. angustifolius* revealed significant effects of the genotype, the year and the growing system.

Introduction

In the past the use of lupins for feed and food purposes was limited because of their high alkaloid content. Alkaloids are toxic and in particular unlimited feeding of bitter seeds resulted in a fatal disease called lupinosis (Hondelmann, 1984, 1996). Therefore, the interest in lupins free from alkaloids was steadily increasing (Hondelmann, 1984). On the one hand this can be achieved by debittering on a large scale and on the other hand by breeding (Hondelmann, 1996). At the beginning of the 20th century v. Sengbusch (v. Sengbusch, 1930) identified some sweet grains of lupins (*L. luteus* and *L. angustifolius*) which were the starting point for breeding sweet lupins. This opened new possibilities for using sweet lupins because of their high protein content (Jansen and Balko, 2012) without quantitative restrictions in feed and as food ingredients due to their functional properties (Wäsche et al., 2001). This largely broadened the original use of bitter genotypes of *Lupinus* species for soil improvement because of their long taproot (Clements et al., 1993) and ability to fix nitrogen due to symbiosis with the bacterium *Rhizobium* (Lindemann and Glover, 2003).
Due to this the so-called 'Old World' species *L. albus, L. luteus* and *L. angustifolius* as well the 'New World' type *L. mutabilis* achieved some agricultural importance (Hondelmann, 1984). Currently in Germany only *L. angustifolius* is cultivated, mainly due to its resistance to anthracnose (*Colletotrichum lupini*, Ruge-Wehling et al., 2008). Because of the advantageous features mentioned above, especially because of its ability to nitrogen fixation, *L. angustifolius* is of special importance in organic farming, in which the use of herbicides, pesticides, and mineral fertilizers is not allowed. Legume-based crop rotations are an option to supply nitrogen in organic farming systems to enhance soil fertility (Maeder et al., 2002). In this respect Temple et al. (1994) compared conventional, low input and organic farming concerning yield, weed biomass and percent N in leaves of different crops. For dry beans (legumes) no significant differences were detected between cropping systems in all years.

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For lupins it is known that the alkaloid content, which is of essential importance for food and feed is influenced by many environmental factors, i.e. differences in fertilizer application (Ciesolka et al., 2005 and Gremigni et al., 2003), soil pH (Jansen et al., 2012) and ambient temperatures during seed filling (Jansen et al., 2009).
The objective of this study was therefore to assess the effect of (i) the genotype, (ii) the growing system (organic versus conventional farming) and (iii) the year on the alkaloid content and yield of German *L. angustifolius* genotypes.

Material and methods

Plant material and cultivation

The seeds of all *L. angustifolius* cultivars and breeding lines were supplied by the seed company Saatzucht Steinach GmbH and Co. KG, Bocksee, Germany. Field experiments were carried out in four random replications with a plot size of 9.6 m² under organic and conventional farming conditions in Groß Lüsewitz (near Rostock, Mecklenburg-Western Pomerania, Germany, northern latitude: 54.071955, eastern longitude: 12.321031). Determinate types ('Boruta' and 'Haags Blaue') were sown with a sowing rate of 120 seeds m^{-2} and branched types ('Boregine', 'Borlu', 'Hagena', 'Probor', 'Sanabor', 'Sonate', 'Vitabor', 'Bo 083517AR'and 'Bo 083521AR') with 100 seeds m^{-2} in March/April in 2010, 2011, 2012 and were harvested at maturity in July/August of the respective season. The nine varieties and two breeding lines were registered or bred, respectively, between 2001 and 2008. Under organic conditions the following crop rotation was applied: potato, spring cereals, legumes, winter cereals, grass clover (twice). No manure, chemical seed treatment and chemical or mechanical weed controlwas conducted. Conventional cultivation was done according to good agricultural practice with the same crop rotation. The location Groß Lüsewitz is characterized by a loamy sand with a medium soil water holding capacity, pH value of 5.8, long-term mean (1972 - 2007) rainfall of 686 mm and mean temperature of 8.3 °C. Meteorological data for the years 2010 - 2012 were supplied by the University of Rostock, Faculty of Agriculture and Environmental Science, Institute for Environmental Engineering, Field of Hydrology.

Sample Collection

Yields were estimated at a moisture content of 11 % for each replication separately. In a next step seeds of each replication per genotype were mixed and pre-crushed using a Laboratory Break Mill SM 3 Brabender® (Brabender® GmbH & Co. KG, Duisburg, Germany) and after that ground to pass a 0.8 mm sieve with a Falling Number laboratory Mill 3100 (Perten Instruments, Hägersten, Sweden).
All whole meal samples were stored at 20 °C until analysis.

Analysis of alkaloids

The alkaloids in whole meal samples were analyzed by an Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) on Agilent J&W Ultra 1 column (25 m, 0.20 mm ID, 0.33 µm film thickness) and identified in the mass spectrometer Agilent 5975C according to WINK et al. (1995) and BERMUDES TORRES et al. (2002). This method was described in detail by JANSEN et al. (2009). Analyses were carried out in duplicate with variation coefficient lower than 4 %. The alkaloid content was calculated as the sum of the main alkaloids which are angustifoline, isolupanine, lupanine, and 13-hydroxylupanine in narrow leafed lupins.

Statistical Analysis

The statistical analyses were conducted using the software package SAS (version 9.3) for windows programs (SAS Institute, Inc., Cary, NC, USA). A generalized linear model for the analysis of variance (ANOVA) was applied, using the GLM procedure and a Tukey test ($\alpha = 0.05$) for comparing means (genotype, year and cultivation system).

Results

The field experiments with eleven sweet *L. angustifolius* over a period of three years showed significant differences in the alkaloid content between genotype and year. The influence of the growing system was significant, too (Tab. 1), but no significant interaction between genotype and growing system was found.

Tab. 1: Results of the ANOVA for alkaloid content.

Source	Degrees of freedom	p-value (2010-2012)
Genotype	10	<.0001
Year	2	<.0001
Growing system	1	0.0012
Genotype*Growing system	10	0.9185

The alkaloid content of different genotypes, years and cultivation systems is listed in Tab. 2.

The variety 'Vitabor' and the breeding line 'Bo 083521 AR' showed the lowest alkaloid content in all years and growing systems. 'Vitabor' varied between 69 µg g^{-1} and 158 µg g^{-1} in organic cultivation and between 127 µg g^{-1} and 341 µg g^{-1} in conventional farming. The breeding line 'Bo 083521 AR' varied between 71 µg g^{-1} and 151 µg g^{-1} and between 127 µg g^{-1} and 225 µg g^{-1}, respectively.

'Boregine', a well known variety with high yield showed high alkaloid contents between 308 µg g^{-1} and 1108 µg g^{-1} in organic systems and between 445 µg g^{-1} and 1312 µg g^{-1} under conventional conditions. The varieties 'Sanabor' and 'Borlu' were characterized by a very high alkaloid content in seeds, comparable to the content in seeds of 'Boregine'.

Fig. 1 and Tab. 2 show that higher alkaloid contents were detected in the conventional growing system.

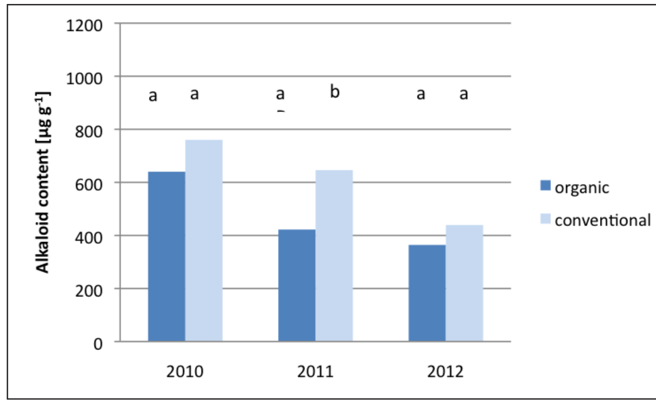

Fig. 1: Mean alkaloid content of eleven lupin genotypes cultivated under organic and conventional conditions (LS-means with the same letter are not significantly different).

The year and the associated different mean temperature during the growing season had an effect on the alkaloid content of the investigated sweet narrow-leaved lupins. The investigated eleven lupins accumulated a higher amount of alkaloids at higher temperatures (Fig. 2).

In the growing seasons 2010 - 2012 all genotypes, with the exception of 'Haagena', showed significant differences in yield between the growing systems, i.e. conventional and organic farming (Fig. 3).

The highest yield, both in organic and conventional farming was obtained by the cultivar 'Sonate'.

In general the yield of the lupins cultivated under organic conditions over the three years was significantly lower (2.96 to 4.02 t ha^{-1}) compared to the conventionally cultivated lupins (3.96 to 4.56 t ha^{-1}).

Tab. 2: Alkaloid content [µg g^{-1}] of nine varieties and two breeding lines of organic (O) and conventional (C) cultivated sweet narrow-leafed lupins in three years.

Genotype	Cultivation Year 2010		Cultivation Year 2011		Cultivation Year 2012		Mean value 2010-2012	
	O	C	O	C	O	C	O	C
Sanabor	942	1204	572	846	753	777	756	942
Borlu	1058	1273	489	751	508	667	685	897
Boregine	1108	1312	599	1027	308	445	672	928
Haagena	864	1075	421	796	653	557	646	809
Sonate	774	696	726	720	482	578	661	665
Bo 083517 AR	655	833	438	733	283	435	459	667
Probor	707	838	472	792	266	527	482	719
Haags Blaue	398	410	322	448	375	307	365	388
Boruta	220	189	455	594	209	286	295	356
Bo 083521 AR	151	187	71	225	94	127	105	180
Vitabor	158	341	82	170	69	127	103	213

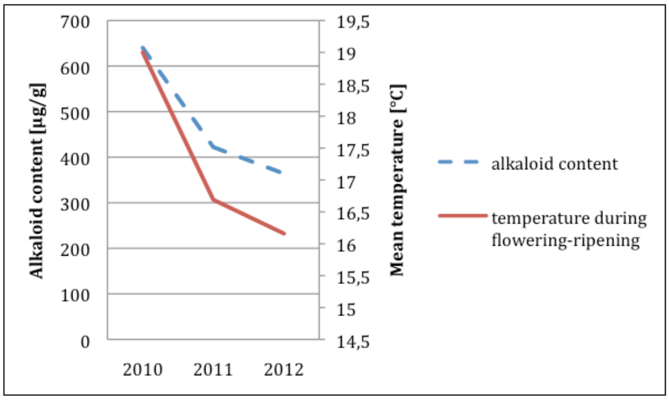

Fig. 2: Relationship between mean alkaloid content of eleven lupin genotypes cultivated under organic conditions and mean temperature from flowering to pod ripening (Pearson correlation coefficient R = 0.9997, p = ≤ 0.05).

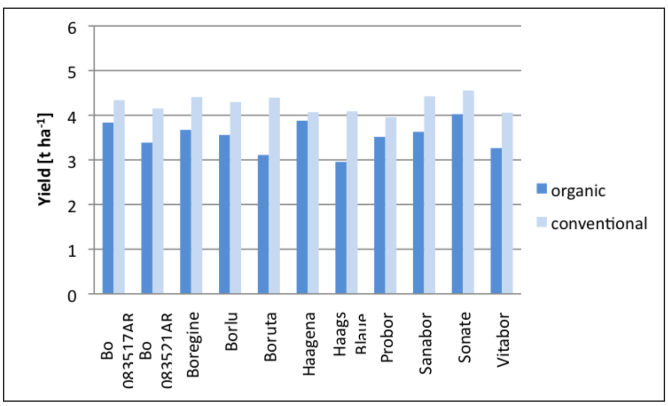

Fig. 3: Mean yield of *L. angustifolius* genotypes over a period of three years in organic and conventional growing systems cultivated lupins (all varieties had significantly different LS-means-values, with the exception of 'Haagena').

The results of ANOVA for yield of lupins cultivated under conventional and organic conditions are given in Table 3. Significant effects of the genotype, the year, and the cropping system were observed as well as respective significant interactions.

Tab. 3: Results of the ANOVA for yield.

Source	Degrees of freedom	F-Statistic	Pr > F
Growing system	1	244.65	<.0001
Genotype	10	8.47	<.0001
Year	2	230.47	<.0001
Genotype*Year	20	5.87	<.0001
Genotype*Growing system	10	4.06	<.0001
Genotype*Growing system*Year	20	4.13	<.0001

Discussion

Differences in the alkaloid content of sweet *L. angustifolius* are well known. In the descriptive variety list published by the Federal Plant Variety Office (Bundessortenamt, 2011) the variety 'Vitabor' is characterized by a very low alkaloid content, compared to the other tested varieties. This is also confirmed by the results of our work (Tab. 1), by the analyses of 11 genotypes of sweet narrow leaf lupins during 3 years.

Several studies compared quality parameters and the effects of organic and conventional growing systems, but in general only small and partially inconsistent differences were found. Possible effects on human health of organic and conventional food are examined in many studies, e.g. by Brand and Mølgaard (2001) as well Winter and Davis (2006). In general only small and partially inconsistent differences between organically and conventionally produced foods were detected. Only for nitrate and vitamin C a tendency to lower and higher contents, respectively was detected under organic conditions (Brand and Mølgaard, 2001).

Nevertheless, further investigations on nutritional important plant contents will be necessary to provide more answers to the question, whether organic or conventional cultivation can enhance the nutritional value.

Results of our study show that under organic conditions all *L. angustifolius* genotypes exhibited significantly lower alkaloid contents than under conventional conditions in all years. The reason for this may be that a higher N-content in the soil leads to higher concentrations of alkaloids. Gholamhosseinpour et al. (2011) stated that nitrogen is a constituent of the alkaloids which plays an important role in the synthesis of these ingredients. Therefore, increasing nitrogen supply leads to an increase in the alkaloid content. This has been shown for many species, e.g. lupins (*Lupinus succulentus*, Johnson et al., 1987), datura (*Datura innoxia Mill.*, Al-Humaid, 2004), periwinkle (*Catharanthus roseus L.*, Gholamhosseinpour et al., 2011) and for the total glycoalkaloid content in potatoes (Mondy and Munshi, 1990).

However, also contradictory results are reported e.g. for datura (Ruminska and Gamal, 1978) and potatoes (Hamouz et al., 2005). Ruminska and Gamal (1978) have shown that there is no remarkable influence of nitrogen fertilizer on the alkaloid content in *Datura innoxia Mill*. Hamouz et al. (2005) found, that growing under organic conditions did not influence the glycoalkaloid content of potatoes.

Due to these contradicting results new investigations with respect to the alkaloid content in relation to the growing system, i.e. organic versus conventional were conducted.

In 2012 the NH_4-N-level at Groß Lüsewitz was equal in soil samples of both cropping systems (0.1 mg $100g^{-1}$, 0-30 cm), but the NO_3-N-concentration in soil samples derived from conventional cultivation reached a level of 0.3 mg $100g^{-1}$ compared to organic soil samples with only 0.1 mg $100g^{-1}$.

It is known that the alkaloid content is influenced by a lot of environmental factors, such as temperature. Higher ambient temperatures during the seed filling led to a higher content of alkaloids (Jansen et al., 2009) in seeds of 6 older varieties of L. *angustifolius* registered between 1998 and 2004. As expected, in the present study the different temperatures during the growing period from 2010 to 2012 also had a significant influence on the alkaloid content (Fig. 3). Furthermore, genotypic differences were also observed in older studies conducted under organic conditions (Jansen et al., 2005) only.

As already mentioned the threshold for the alkaloid content for animal nutrition is 0.05 % and for human nutrition 0.02 % (Jansen et al., 2009) in Germany, while it is 200 mg kg^{-1} for contaminants and natural toxicants in Australia and New Zealand (Abbott et al., 2003). Alkaloid contents above this level were also detected by Muquiz et al. (1994) in genotypes of sweet *Lupinus albus* L. from different countries and locations. Reinhard et al. (2006) detected up to 2120 µg g^{-1} in seeds and flour of "sweet" L. *angustifolius* genotypes.

Small, but significant differences in yield between organic (3.53 t ha^{-1}) and conventional (4.25 t ha^{-1}) growing systems were found for 9 varieties and 2 new breeding lines. Similar results were obtained

in four years field trials for *Pisum sativum* and *Vicia faba* and in one year field trials of *L. angustifolius* (JANSEN and SEDDIG, 2007).
In conclusion the alkaloid content and yield of different narrow-leafed lupins is significantly influenced by the genotype, the growing season and the growing system (organic *vs*. conventional).
Significantly lower alkaloid contents and lower yields were found in organic farming compared to conventional farming. However, due to the lower alkaloid content and the only slightly reduced yield, which holds especially true for the cultivar 'Haagena', *L. angustifolius* is well suited for organic farming.

Acknowledgements
The Authors thank C. Leesch, M. Jugert and B. Lembke for excellent technical assistance.

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