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*Research Article*

# Effect of the Soil pH on the Alkaloid Content of *Lupinus angustifolius*

**Gisela Jansen, Hans-Ulrich Jürgens, Edgar Schliephake, and Frank Ordon**

*Institute for Resistance Research and Stress Tolerance, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, 18190 Sanitz, Germany*

Correspondence should be addressed to Gisela Jansen, gisela.jansen@jki.bund.de

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Field studies were conducted in growing seasons 2004, 2005, and 2010 to investigate the effect of different soil pH values on the alkaloid content in seeds of *Lupinus angustifolius*. Two-year experiments with eleven cultivars were carried out in acid soils with an average of pH = 5.8 (Mecklenburg-Western Pomerania) and on calcareous soils with an average pH of 7.1 (Bavaria), respectively. In addition, in 2010, eight cultivars were grown in field experiments in soils with pH values varying between pH = 5.3 and pH = 6.7. In all experiments conducted on soils with a higher pH (pH = 6.7 and pH = 7.1), a significantly lower alkaloid content was detected in all *Lupinus angustifolius* cultivars than on soils with a lower pH (pH = 5.3 and pH = 5.8). Results clearly show that the alkaloid content is significantly influenced by the soil pH but genotypic differences regarding the reaction to different pH values in the soil were observed.

## 1. Introduction

Lupins as protein crops can be used in many ways. They are grown for green manure, for animal feed, or for human nutrition. Unlimited feeding of bitter seeds led to the disease "lupinose" in former times. It was caused by the alkaloids contained in bitter lupins [1]. Only breeding of the so-called sweet lupins [2] facilitates the use of lupins to a greater extent in animal feed and for human nutrition. But besides genetics the alkaloid content in sweet lupins, for example, *Lupinus angustifolius,* is influenced by different environmental factors. Apart from drought, heat, and nutrient deficiencies, plants are largely affected by the soil pH [3]. Lupin species are differing concerning their demands for optimal growth, but in general commercial cultivars of lupins grow poorly on alkaline or neutral soils [4]. In general blue lupins (*Lupinus angustifolius*) and especially yellow lupins (*Lupinus luteus*) are less sensitive to calcareous soils than white lupins (*Lupinus albus*) [5, 6]. The soil pH considerably influences yield and protein content of *Lupinus angustifolius* [7, 8], that is, the higher the soil pH the lower the kernel yield and protein content. Besides this, the pH value has an impact on the production of secondary metabolites [9–11], for example, out of many soil parameters analysed, the highest correlation of the production of the glycoside salidroside in *Rhodiola sachalinensis* was observed to the soil pH [9]. Similar results were obtained for the alkaloid production, for example, when the pH value in cell culture media of *Lupinus polyphyllus* decreased from pH = 5.5 to pH = 3.5, the alkaloid production increased [12].

The objective of our field studies was to assess the influence of the soil pH on the alkaloid content of narrow-leafed lupin cultivars (*Lupinus angustifolius*).

## 2. Materials and Methods

*2.1. Field Trials.* Seeds of *Lupinus angustifolius* cultivars Borlana, Borweta, Bordako, Boruta, Borlu, Bora, Boregine, Boltensia, Bolivio, Vitabor, and Haags Blaue were supplied by the seed company Saatzucht Steinach (Bornhof, Germany). The variety Sonet was provided by Kruse Saatzucht (Münster, Germany).

In 2004 and in 2005, field experiments with 11 cultivars of *Lupinus angustifolius* (except Haags Blaue) were carried out under organic farming conditions in Bogen (Bavaria, northern latitude: 48.912925, eastern longitude: 12.692792) and in Gross Luesewitz (Mecklenburg-Western Pomerania,

Table 1: Characteristic of locations.

| | Mecklenburg-Western Pomerania | | Bavaria | |
|---|---|---|---|---|
| | Gross Luesewitz | Bornhof | Bogen | Gründl |
| Soil type | Loamy sand | Sand | Sandy clay loam | Sandy loam |
| pH value | 5.8 | 5.3 | 7.2 | 6.7 |
| Mean annual rainfall (mm) | 620 | 558 | 803 | 822 |
| Mean annual temperature (°C) | 8.2 | 8.2 | 7.7 | 8.6 |

Source: [8, 13].

Table 2: Content of alkaloids in different cultivars of *Lupinus angustifolius* at different locations (2004 and 2005).

| Genotype | Year | 13-Hydroxylupanine | | Angustifoline | | Isolupanine | | Lupanine | |
|---|---|---|---|---|---|---|---|---|---|
| | | Bo | GL | Bo | GL | Bo | GL | Bo | GL |
| | 2004 | 45.5 | 215.5 | 20.8 | 102.8 | 7.0 | 23.7 | 16.8 | 179.2 |
| Bolivio | 2005 | 52.2 | 157.8 | 41.8 | 87.3 | 10.2 | 26.6 | 6.6 | 204.7 |
| | **Mean** | **48.9** | **186.7** | **31.3** | **95.1** | **8.6** | **25.2** | **11.7** | **192.0** |
| | 2004 | 2.4 | 73 | 2.3 | 52.1 | 2.3 | 11.3 | 7.5 | 136.5 |
| Boltensia | 2005 | 1.8 | 58.1 | 1.5 | 50.5 | 0.0 | 16.8 | 1.2 | 216.8 |
| | **Mean** | **2.1** | **65.6** | **1.9** | **51.3** | **1.2** | **14.1** | **4.4** | **176.7** |
| | 2004 | 15.5 | 119.9 | 13.3 | 78.3 | 6.8 | 24.2 | 27.7 | 199.1 |
| Bora | 2005 | 2.1 | 94.3 | 0.6 | 72.1 | 1.0 | 34.4 | 4.5 | 293.8 |
| | **Mean** | **8.8** | **107.1** | **7.0** | **75.2** | **3.9** | **29.3** | **16.1** | **246.5** |
| | 2004 | 13.8 | 135.2 | 9.3 | 98.7 | 3.6 | 17.5 | 10.5 | 231.9 |
| Bordako | 2005 | 4.9 | 80.1 | 4.6 | 80.7 | 1.4 | 26.8 | 2.6 | 340.8 |
| | **Mean** | **9.4** | **107.7** | **7.0** | **89.7** | **2.5** | **22.2** | **6.6** | **286.4** |
| | 2004 | 12.7 | 67.6 | 7.4 | 46.3 | 4.6 | 18.8 | 10.5 | 113.6 |
| Boregine | 2005 | 1.8 | 75.5 | 0.8 | 66.7 | 0.5 | 33.6 | 0.3 | 245.7 |
| | **Mean** | **7.3** | **71.6** | **4.1** | **56.5** | **2.6** | **26.2** | **5.4** | **179.7** |
| | 2004 | 5.0 | 67.4 | 2.8 | 49.6 | 1.9 | 11.1 | 4.1 | 103.9 |
| Borlana | 2005 | 5.1 | 43.7 | 5.5 | 41.1 | 1.1 | 14.1 | 0.9 | 171.9 |
| | **Mean** | **5.1** | **55.6** | **4.2** | **45.4** | **1.5** | **12.6** | **2.5** | **137.9** |
| | 2004 | 12.6 | 118.6 | 5.7 | 79.5 | 3.3 | 18.8 | 9.6 | 151.2 |
| Borlu | 2005 | 3.8 | 36.4 | 5.3 | 31.6 | 1.5 | 11.2 | 5.5 | 106.3 |
| | **Mean** | **8.2** | **77.5** | **5.5** | **55.6** | **2.4** | **15.0** | **7.6** | **128.8** |
| | 2004 | 18.7 | 53.3 | 8.3 | 31.0 | 3.3 | 6.9 | 14.8 | 82.0 |
| Boruta | 2005 | 2.7 | 34.7 | 0.5 | 32.0 | 0.0 | 7.2 | 2.4 | 115.0 |
| | **Mean** | **10.7** | **44.0** | **4.4** | **31.5** | **1.7** | **7.1** | **8.6** | **98.5** |
| | 2004 | 11.2 | 119.2 | 6.4 | 87.0 | 3.0 | 12.4 | 17.3 | 167.4 |
| Borweta | 2005 | 5.0 | 19.5 | 2.9 | 23.9 | 0.0 | 8.2 | 4.2 | 136.7 |
| | **Mean** | **8.1** | **69.4** | **4.7** | **55.5** | **1.5** | **10.3** | **10.8** | **152.1** |
| | 2004 | 32.7 | 135.6 | 22.7 | 96.1 | 4.9 | 14.8 | 32.2 | 215.3 |
| Sonet | 2005 | 35.7 | 89.8 | 36.8 | 98.1 | 3.7 | 15.1 | 55.4 | 364.0 |
| | **Mean** | **34.2** | **112.7** | **29.8** | **97.1** | **4.3** | **15.0** | **43.8** | **289.7** |
| | 2004 | 4.5 | 19.9 | 2.0 | 11.3 | 1.7 | 3.4 | 3.0 | 25.7 |
| Vitabor | 2005 | 2.2 | 14.7 | 0.7 | 10.1 | 0.0 | 3.1 | 0.6 | 36.9 |
| | **Mean** | **3.4** | **17.3** | **1.4** | **10.7** | **0.9** | **3.3** | **1.8** | **31.3** |

BO: Bogen, GL: Gross Luesewitz.

Table 3: Mean content (±SD) of alkaloids in different cultivars of *Lupinus angustifolius* at different locations (2004 and 2005).

| Alkaloid | Bogen | | Gross Luesewitz | |
|---|---|---|---|---|
| 13-Hydroxylupanine | 13.3 ± 14.9 | a | 83.2 ± 50.5 | b |
| Angustifoline | 9.2 ± 11.5 | a | 60.3 ± 29.5 | b |
| Isolupanine | 2.8 ± 2.6 | a | 16.4 ± 8.9 | b |
| Lupanine | 10.8 ± 13.1 | a | 174.5 ± 87.6 | b |

Means for the same alkaloid between the locations with different letters are significantly different from each other (alpha = 0.05).

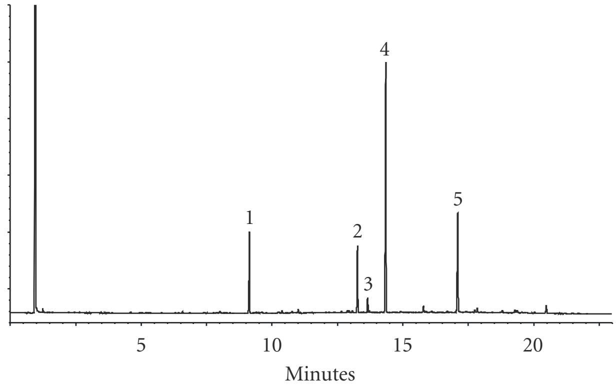


Figure 1: Gas chromatogram for different alkaloids (1 ISTD-Caffeine, 2 Angustifoline, 3 Iso-Lupanine, 4 Lupanine, 5 13-Hydroxy-Lupanine) present in cultivar Haags Blaue grown at Bornhof.

northern latitude: 54.071955, eastern longitude: 12.321031). Field experiments were conducted in a randomized block design with four replications with a plot size of 9.6 $m^2$. The alkaloid content in these trials was analysed on a mixed sample of each cultivar. In 2010 the variety Boruta and the variety Haags Blaue as well as six newly developed breeding lines (51–56) of the Saatzucht Steinach were grown in field experiments in fourfold replications in 4.2 $m^2$ plots at different soils at Gründl (Bavaria, northern latitude: 48.519305, eastern longitude: 11.816175) and Bornhof (Mecklenburg-Western Pomerania, northern latitude: 53.477371, eastern longitude: 12.911754) under conventional growing conditions. In this experiment the alkaloid content was measured separately for each plot. In Table 1 the characteristics of the locations are given.

*2.2. Determination of the Alkaloid Content.* Grain samples of about 250 g were randomly taken and grounded as described by [14]. All wholemeal samples revealed a dry matter content of about 90% and were stored at 20°C until analysis. The subsequent alkaloid analysis in lupin whole meal flour was carried out according to [14–16]. Main alkaloids were calculated as the sum of alkaloids shown in Figure 1. The main alkaloids in narrow-leafed lupins are angustifoline, isolupanine, lupanine, and 13-hydroxylupanine.

The determination of the alkaloid content was performed twice per sample with a coefficient of variation lower than 4%.

*2.3. Statistical Analysis.* To assess the effects of the location on the alkaloid content, a generalized linear model for the analysis of variance (ANOVA) was applied, using the GLM procedure of the software package SAS (version 9.3) followed by a Tukey test ($\alpha = 0.05$) for comparing the means. The two datasets were analysed separately.

## 3. Results and Discussion

The alkaloid content in sweet narrow-leaf lupin cultivars is in general very low. Already Sengbusch [17] suggested to call lupins alkaloid-poor (0.05% alkaloids in seeds) or alkaloid-free (0.025% alkaloids in seeds). Nevertheless, the seed alkaloid content of sweet lupins is influenced by different environmental factors such as fertilizers [18–22], ambient temperature during initiation of flowering up to pod ripening [14], and drought stress [23]. In 2004 and 2005, the mean daily temperature at the beginning of flowering until harvest in August was very similar [14]. The experiments were also carried out under uniform agronomic management (fertilizer, herbicides, etc.), so that the abiotic stress factors temperature, drought, and nutrient deficiency as well as mechanical damage can be neglected. In 2004 at Bogen the main alkaloid content of the cultivars tested ranged between 11.2 $\mu$g/g for the cultivar Vitabor and 92.5 $\mu$g/g for the cultivar Sonet and 90.1 $\mu$g/g for Bolivio. At Gross Luesewitz the alkaloid content ranged between 60.3 $\mu$g/g for cultivar Vitabor and 521.2 $\mu$g/g for cultivar Bolivio. The alkaloid content of all varieties tested is shown in Table 2. In 2005, as expected, the lowest alkaloid content was also found in the cultivar Vitabor (Bogen 3.5 $\mu$g/g and Gross Luesewitz 64.8 $\mu$g/g) and the highest content in Sonet (Bogen 131.6 $\mu$g/g and Gross Luesewitz 567.0 $\mu$g/g).

Out of all alkaloids analyzed the lupanine content shows the largest increase with decreasing soil pH. Christiansen et al. [23] reported that drought stress during the vegetative phase reduces mostly the concentration of lupanine, 13-hydroxylupanine, and angustifoline, whereas isolupanine is affected to a much smaller extent.

The differences in the alkaloid content between the locations in Mecklenburg-Western Pomerania and Bavaria are significant (Table 3).

Figure 2 clearly demonstrates that the alkaloid production is significantly higher when lupins are cultivated at a lower pH (mean value of two years is shown). However, also clear differences are observed between cultivars opening the opportunity to breed cultivars with low alkaloid content under low pH conditions, for example, Vitabor.

In 2010 also two cultivars and six newly developed breeding lines of *Lupinus angustifolius* were analyzed concerning the alkaloid content at locations with different soil pH. At Gründl (pH = 6.7) the total alkaloid content ranged between 0.0166% and 0.1293% while at Bornhof (pH = 5.3) the alkaloid content was in general higher and ranged between 0.029% and 0.1810% (Figure 3). The variety Boruta and

Table 4: Mean alkaloid content estimated on soil with pH = 5.8 and pH = 7.2 observed in cultivars of *Lupinus angustifolius* at different locations (2004 and 2005, $n = 22$).

| Location | Main alkaloid content [%] | |
|---|---|---|
| | 2004 | 2005 |
| Mecklenburg-Western Pomerania (pH = 5.8) | 0.0330 | 0.0339 |
| Bavaria (pH = 7.2) | 0.0043 | 0.0029 |
| Least significant difference (Tukey, $\alpha = 0.05$) | 0.0084 | 0.01 |

Table 5: Mean alkaloid content estimated on soil with pH = 5.3 and pH = 6.7 observed in actual varieties and breeding lines of *Lupinus angustifolius L.* at different locations (2010, $n = 32$).

| Location | Alkaloid content [%] |
|---|---|
| | 2010 |
| Mecklenburg-Western Pomerania (pH = 5.3) | 0.0687 |
| Bavaria (pH = 6.7) | 0.0355 |
| Least significant difference (Tukey, $\alpha = 0.05$) | 0.0081 |

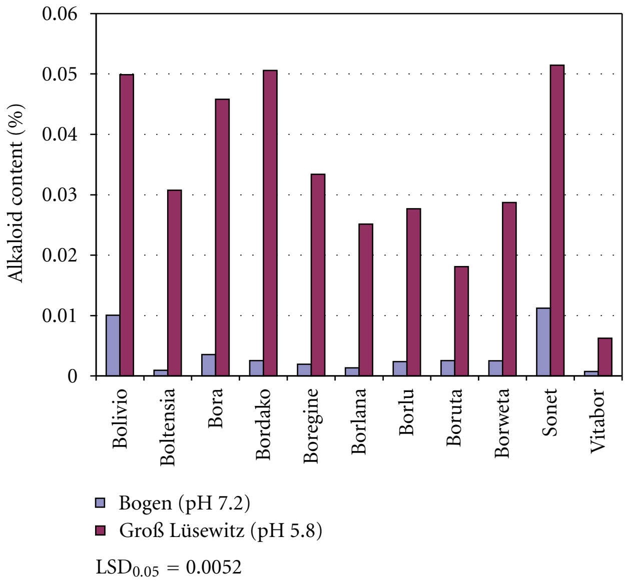


Figure 2: Influence of the soil pH on the main alkaloid content of *Lupinus angustifolius* (mean of 2004 and 2005).

two new breeding lines revealed the lowest alkaloid content (Figure 3).

Concerning the average main alkaloid content in cultivars and breeding lines significant differences between locations were observed in 2010 (Table 5) as it was previously observed in 2004 and 2005 (Table 4). In Germany, there is no law concerning the upper threshold of alkaloids in lupins for animal feed and human nutrition, but in general the upper threshold for use in animal and human nutrition is 0.05% and 0.02%, respectively. In 2010 this threshold has been exceeded in general at the soil with the lower pH (Mecklenburg-Western Pomerania) and also by some cultivars in 2004/2005.

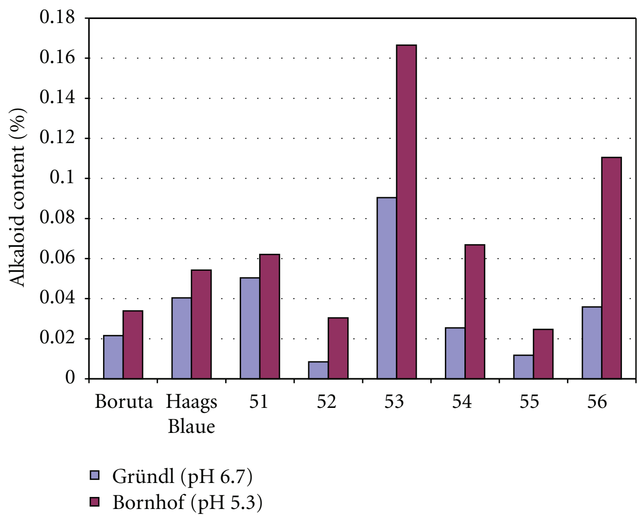


Figure 3: Influence of the soil pH on the main alkaloid content of *Lupinus angustifolius* cultivars and breeding lines (2010).

For lupin cultivation a rather low soil pH (pH = 5.0–6.8) is recommended [5]. Jansen et al. [16] reported that the grain yield at pH = 7.2 is lower than at pH = 5.8. On the other hand the alkaloid content of *Lupinus angustifolius* decreases significantly at a higher pH (Figures 2 and 3). Therefore, for a high yield combined with low alkaloid content, the soil pH is of prime importance, although it has to be taken into account that also additional environmental factors, for example, drought stress, fertilization, and temperature, can have an adverse effect on the alkaloid content in lupin seeds. Yaber Grass and Leicach [24] noted that a significant increase in the total alkaloid content was observed from samples of *Senecio grisebachii* growing in highly deteriorated soil compared to those from samples grown in less deteriorated ones.

The results presented show that additional breeding efforts are needed to achieve low alkaloid content also under low soil pH. As shown in Figures 2 and 3 genetic variation concerning this trait is present in cultivars and new breeding lines which could be exploited in the future.

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