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RESEARCH ON LOW-ALKALOID CONCENTRATION IN VARIETIES OF LUPIN (*LUPINUS SP.*) IN LITHUANIA

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Summary. Testing for alkaloids in lupine varieties was conducted at the Voke Branch of the Lithuanian Institute of Agriculture during a competitive trial of feeding lupine (*Lupinus* sp.) in 2006-2008. The samples were taken from feeding yellow lupine (*Lupinus luteus* L), 'Trakiai' and 'Vilčiai' varieties, as well as narrow-leaved lupine (*Lupinus angustifolius* L.), 'Vilniai' variety, and selection line N1702. Alkaloid concentration was estimated in the periods of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development of fruits (BBCH 71-75) and seed ripening (BBCH 85-88). The test material included exsiccates of leaves, stems, flowers, pods and seeds. According to the results, the average alkaloid levels were similar in yellow forage lupine (*Lupinus luteus* L.) varieties 'Trakiai' and 'Vilčiai'. The 'Vilniai' variety on average contained more alkaloids than the narrow-leaved selection line N1702. Analysis of the average alkaloid levels revealed that in the yellow lupine (*Lupinus luteus* L.) varieties 'Trakiai' and 'Vilčiai' alkaloid levels in the stems were lower than in the narrow-leaved (*Lupinus angustifolius* L.) variety 'Vilniai' and N1702 in all phenological stages. The average alkaloid level in leaves was lower than in stems at the stage of flowering (BBCH 64-67). In our study, the highest average alkaloid levels were found in pods (0.114 ± 0.007) and flowers (0.114 ± 0.006) in narrow-leaved lupines, while the lowest level was found in seeds (0.022 ± 0.003) of yellow lupines.

Key words: forage lupins; alkaloids; vegetation periods; vegetative and generative organs.

INTRODUCTION

Before 1926, lupines had been used as siderates only. E. Bauer and A. Pryanishnikov were the first to speak about the natural existence of low-alkaloid lupines, however, research into this field was hampered by the absence of reliable and rapid methods of determining alkaloid plants (Gataulina, 2002). In 1928, R. Sengbush from the Central German Institute of Genetics proposed a method which was applied to analyze 1.5 million of alkaloid plants, and three non-alkaloid mutants of yellow lupine and two such mutants of narrow-leaved lupine were established (Kurlovich, 2002). The absence of alkaloids was determined to be an inherited trait, and the obtained individuals by their yields equalled alkaloid plants.

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These individuals were used in selection work which resulted in the first famous varieties of Munchenberg sweet lupines. Low alkaloid concentration in lupines is derived from biochemical mutation. The first forage varieties of lupines were developed by the method of individual selection from alkaloid populations in which low-alkaloid mutants, though rarely, still did occur (Kurlovich, 2002). Alkaloid content is a dominant trait which in yellow lupine is determined by four, in narrow-leaved by five and in white lupine by eight genes (Phan et al., 2007). Cross-pollination of low-alkaloid and alkaloid lupine varieties was found to produce the alkaloid in F_2 and splitting occurs into alkaloid and non-alkaloid generations at a 3:1 ratio. The role of alkaloids in plants is not yet fully clear. Alkaloids are supposed to protect plants from pests which are put off grazing by the acidic taste (Wink and Hartmann, 1982). Another theory proclaims alkaloids to be useless products of protein metabolism (Clements et al., 1996). Yet another opinion is that alkaloids accumulated in the underground parts of a plant, participate in metabolic processes, induce root growth and, on leaching into soil, make a barrier to microorganisms (Peneva, 2006). However, none of the above theories gives an exhaustive explanation of the significance of alkaloids to plants because some plants accumulate them while others do not. Alkaloids show an uneven distribution in plant organs: some plants accumulate them mostly in seeds and others in leaves, roots or cortex, in parenchymal tissue or in cells. The same plant may accumulate both similar and different alkaloids. During the vegetation period, alkaloid concentration undergoes changes, the peak coinciding with the flowering. At the end of vegetation, alkaloids accumulate in seeds and roots (Hondelmann, 1984). Alkaloid concentration in a plant depends on numerous factors such as age, environmental impacts and geography, also on how the soil is fertilized (Breitmaier, 2002). Lupine (*Lupinus* sp.) is a universal plant with numerous useful properties. It may be used both as fodder and for soil fertilization. As fodder, low-alkaloid lupine species such as yellow fodder lupine (*Lupinus luteus* L.) and narrow-leaved forage lupine (*Lupinus angustifolius* L.) are used. Of course, lupines produce alkaloids not in order to supply them to man or animals. Various alkaloids function in plants as insecticides, herbicides, fungicides or pest deterrents (Lee et al., 2008; Gataulina, 2002). There is also an opinion that lupine alkaloids may destroy toxic fungi in forage and thus favour forage assimilation (Hondelmann, 1984). There are studies to show that low levels of alkaloids exert no effect on human and animal organism, while in larger quantities they may cause acute ailments or even death. Lupine alkaloids exhibit not only toxic but also pharmacological properties. In yellow fodder lupine, alkaloid concentration may vary from 0.005% to 1.7% while in narrow-leaved lupine from 0.005% to 3.0%. Low alkaloid levels in lupines are considered to vary from 0.025% to 0.099%. Lupine varieties breeding with low alkaloids amount or without alkaloids might give new perspectives for lupine use not only in feed production but also in the food industry. The aim of the present study was to determine alkaloid concentration variations in different lupine varieties of the species *Lupinus*

luteus L. and *Lupinus angustifolius* L. in the vegetative and generative organs at different developmental phases.

MATERIALS AND METHODS

The study was carried out in 2006-2008 at the Voke Branch of the Lithuanian Institute of Agriculture. The experimental plots were established on sandy loam on carbonaceous fluvial-glacial gravel eluviated soil (IDp), according to FAO-UNESCO classification *Haplic Luvisols* (*LVh*) with the following agrochemical indices: pH – 5.6-6.2, humus 1.37-2.5%, mobile P_2O_5 and K_2O 130-250 mg kg^{-1} and 146-254 mg kg^{-1}, respectively. Competitive trials of the varieties were carried out according to a selection scheme (Maknickiene, 2007).

The samples were taken from feeding yellow lupine (*Lupinus luteus* L), 'Trakiai' and 'Vilčiai' varieties, as well as narrow-leaved lupine (*Lupinus angustifolius* L.), 'Vilniai' variety, and cropper of No. 1702. Selection line No. 1702 was selected by an individual selection method from collection sample No. 3186. Selected genotype had low alkaloids amount and intensive pink flower color. Alkaloid concentration was estimated in the periods of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development of fruit (BBCH 71-75) and seed ripening (BBCH 85-88). The test material included exsiccates of leaves, stems, flowers, pods and seeds.

Freeze-dried plant material was finely ground at room temperature and 15 ml 5% (w/v) trichloroacetic acid was added to 200 mg plant material. The suspension was kept at room temperature for 2 h followed by centrifugation at 3000 rpm for 15 min. An aliquot of 12 ml of the supernatant was subsequently alkalinized with 25% (v/v) ammonia to pH ~11 and extracted twice with 25 ml dichloromethane. The pH was then raised to 14 by the addition of 10 M sodium hydroxide and again the solution was extracted twice with 25 ml dichloromethane. The organic extracts were dried over anhydrous sodium sulphate, collected in a flask containing 100 µg of internal standard (*n*-eicosane) and concentrated *in vacuo*. The residues were reconstituted in *c*. 1 ml ethyl acetate. Usually 50 µl phloem sap or 300 µl xylem sap were made up to 1 ml with 25% (v/v) ammonia. The aqueous solution was extracted four times with dichloromethane (2 ml) and the combined organic extracts were dried over anhydrous sodium sulphate and concentrated upon heating (40°C) under a continuous stream of nitrogen. The residue was reconstituted in methanol (100 µl) containing caffeine (10 µg) as internal standard.

The method of Lee et al. (2007) with modifications was used for chromatographic analysis. The alkaloid quantities were recalculated as a percentage of the dry matter concentration.

A statview ANOVA program was used for statistical analysis of the data. The obtained data were assessed by the method of dispersion analysis, employing the ANOVA ($LSD_{0.1}$) statistical data processing software (Tarakanovas, 2002).

RESULTS AND DISCUSSION

In 2006-2008, two forage lupine species (each with two genotypes) were analysed for alkaloid levels at the stages of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development

of fruit (BBCH 71-75) and seed ripening (BBCH 85-88), separately in vegetative and generative parts of the plants.

The results showed different alkaloid content in different lupine genotypes. The highest alkaloid content was found in leaves of 'Vilniai' (0.108 %) at the phase of fruit development (BBCH 71-75) (Table 1). Analysis of the average alkaloid levels revealed that in the yellow lupine varieties 'Trakiai' and 'Vilčiai' alkaloid levels in the stems were lower than in the narrow-leaved varieties 'Vilniai' and N1702 in all phenological stages. The average alkaloid level in leaves was lower than in stems at the stage of flowering (BBCH 64-67). The average alkaloid content in leaves during inflorescence emergence (BBCH 51-55) was higher than in the stems except variety 'Trakiai'. Also, at the stage of flowering (BBCH 64-67) in all genotypes alkaloids in stems were higher than in leaves. At fruit development stage (BBCH 71-75) alkaloids in leaves were higher than in stems except for genotype 'Vilčiai'.

Alkaloid levels in lupines undergo distinct periodical changes. In plants, they have been found to be the intermediate forms of nitrogen metabolism, in which these compounds are rendered harmless and accumulate (Kurlovich, 2002; Barbachi, 2000). There has been data on the possible role of alkaloids in the processes of respiration, oxidation of various compounds such as ascorbic and citric acids, hydroquinone, pyrogallol, enzyme synthesis (Lee et al., 2007). The quantitative distribution of alkaloids in different stages of lupine development is shown in Fig. 1. The average alkaloid level in cv. Vilniai was highest at the stages of flowering (BBCH 64-67) (0.089 ± 0.003) and development of fruit (BBCH 71-75) (0.088 ± 0.002). Alkaloid levels were influenced also by the species and genotype. The average alkaloid levels in

Table 1. Alkaloid concentration in leaves and stems of some lupine species (T. Vokė, 2006-2008, average data).

Genotype (A)	Vegetative organs (B)	Phenological growth stages (C), dry matter %		
		Inflorescence emergence (BBCH 51-55)	Flowering (BBCH 64-67)	Development of fruit (BBCH 71-75)
'Trakiai'	Leaves	0.030 ± 0.009	0.045 ± 0.003	0.074 ± 0.003
	Stems	0.046 ± 0.007	0.076 ± 0.020	0.026 ± 0.001
'Vilčiai'	Leaves	0.048 ± 0.010	0.034 ± 0.002	0.025 ± 0.002
	Stems	0.044 ± 0.014	0.064 ± 0.016	0.032± 0.001
'Vilniai'	Leaves	0.068 ± 0.015	0.096 ± 0.024	0.108 ± 0.012
	Stems	0.062 ± 0.012	0.107 ± 0.037	0.084 ± 0.013
N1702	Leaves	0.064 ± 0.027	0.042 ± 0.014	0.103 ± 0.010
	Stems	0.054 ± 0.017	0.085 ± 0.038	0.083 ± 0.012

LSD_{01} (A) = 0.012; LSD_{01} (B) = 0.007; LSD_{01} (C) = 0.134
LSD_{01} (AB) = 0.029; LSD_{01} (AC) = 0.037
LSD_{01} (BC) = 0.009; LSD_{01} (ABC) = 0.027

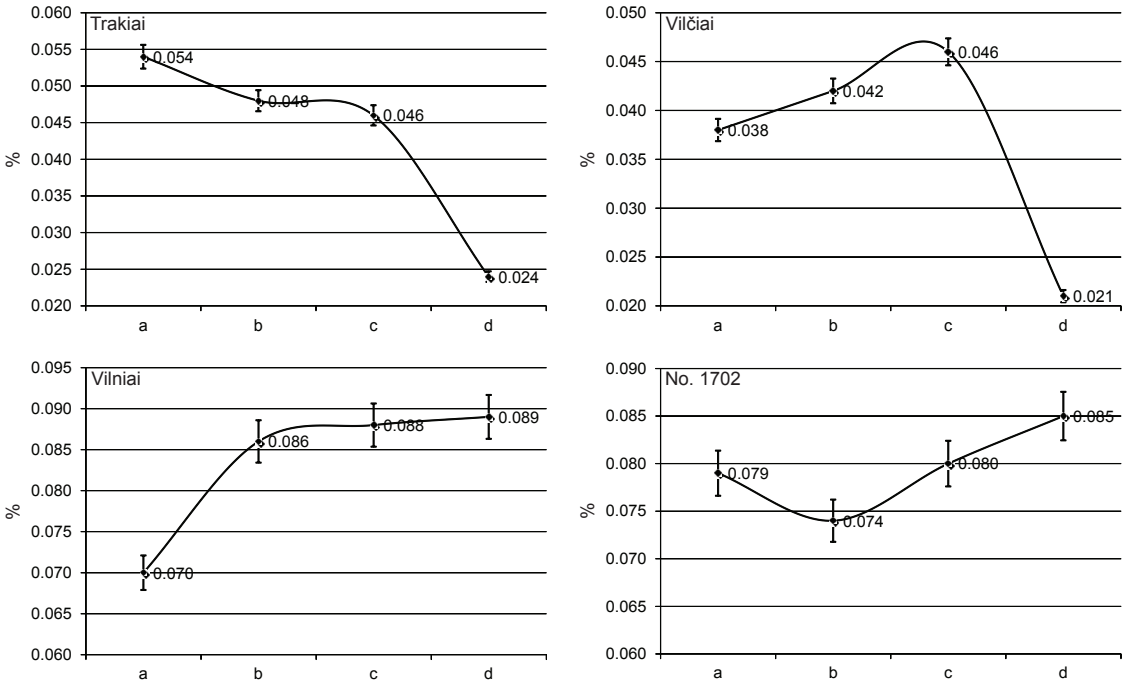

Fig. 1. Alkaloid concentration variations during different vegetation periods: a) inflorescence emergence (BBCH 51-55), b) flowering (BBCH 64-67), c) development of fruit (BBCH 71-75) and d) seed ripening (BBCH 85-88) (T. Vokė, 2006-2008, average data).

the yellow forage lupine (*Lupinus luteus* L.) varieties 'Trakiai' and 'Vilčiai' were similar. The variety 'Vilniai' contained on average more alkaloids than the narrow-leaved selection line N1702. In 2006-2008, from the very first developmental stages, plants of different varieties differed in leaf color, branching, growth dynamics. In the flowering phase (BBCH 64-67), the vegetative organs were finally formed, and morphological differences among the varieties became pronounced. We determined alkaloid levels in vegetative (leaves and stems) and generative (flowers, pods and seeds) organs of plants of four genetic types. The distribution of alkaloids in different vegetative and generative organs of lupine plants is shown in Fig. 2. There are reports that the same plant may contain both similar and different alkaloids (Kurlovich, 2002; Barbachi, 2000). Throughout vegetation, alkaloids levels undergo changes, their peak occurring during flowering. At the end of vegetation, alkaloids accumulate in seedsand roots (Gataulina 2002, Brummund 1988). In our study, the highest average alkaloid levels were found in pods (0.114 ± 0.007) and flowers (0.114 ± 0.006) in narrow-leaved lupines, and the lowest level was measured in the seeds (0.022 ± 0.003) of yellow lupines. The main functions of the overground of stem is to develop the largest possible area, to sustain the weight of flowers and fruits and to intermediate in transporting nutrients from roots to leaves, flowers and fruits as well as from leaves to roots, flowers and fruits. Therefore, the stem contains both conductive and supportive tissues. Besides, stems often serve as nutritive stores (Carey and Wink, 1994). Therefore, our study showed that

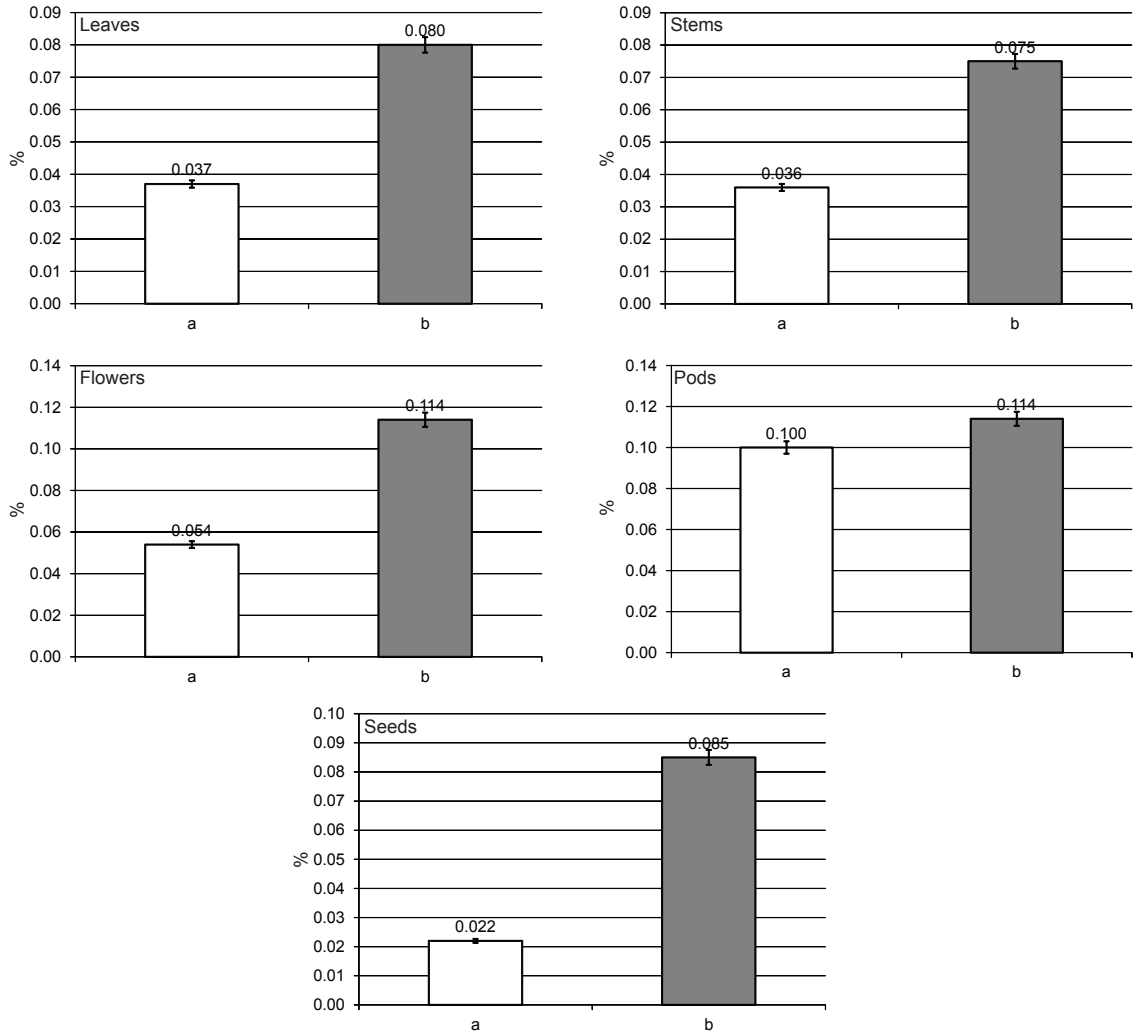

Fig. 2. Alkaloid concentration in different vegetative and generative organs of lupine: a) yellow lupins (*Lupinus luteus* L.); b) narrow-leaved lupins (*Lupinus angustifolius* L.) (Vokė, 2006-2008, average data).

alkaloid levels in stems were lower than in pods which are the basic nutritive organs of a plant. Leaves absorb CO_2 from the environment and from roots, via circulatory tissues, receive water and mineral salts. Leaves, with the aid of solar energy, synthesize from this raw material various organic matters and supply them also to the other organs of a plant (Przybrowski and Packa, 1994; Breitmaier, 2002). Since alkaloid concentration in a plant constantly changes throughout the growth period, the maximum stocks of alkaloids in leaves are accumulated before flowering and they gradually decline together with the qualitative composition of alkaloids with respect to the whole alkaloid complex (Carey and Wink, 1994). Alkaloid concentration in lupines depends on numerous factors such as species variety, age (developmental stage), environment and geographical location. Alkaloid concentration in plants has been found to impact the central

nervous system of living organisms, with low levels acting as stimulators and higher levels as suppressors. Therefore, the aim of lupine selection in Lithuania could be the development of competitive narrow-leaved forage lupine varieties with low alkaloid concentration. The Voke Branch of the Lithuanian Research Centre for Agriculture and Forest has accumulated valuable local material which needs further, more comprehensive selective and genetic studies. Based on the available national genetic fund of lupines, we could suggest for cultivation the most suitable lupine specie, subspecies and varieties adapted to the Lithuanian climatic conditions and improved in terms of their biochemical properties (increased protein concentration and lowered alkaloid levels).

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