BIOLOGIJA. 2008. Vol. 54. No. 2. P. 112–115
DOI: 10.2478/v10054-008-0023-7

Alkaloid content variations in lupin (*Lupinus* L.) genotypes and vegetation periods

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Testing for alkaloids in lupine varieties was conducted at the Vokė Branch of Lithuanian Institute of Agriculture during a competitive trial of feeding lupine (*Lupinus* L.) in 2006–2007.

Samples were taken from feeding yellow lupine (*Lupinus luteus* L.), 'Trakiai' and 'Vilčiai' varieties, the narrow-leaved lupine (*Lupinus angustifolius* L.), 'Vilniai' variety and from cropper No. 1702. Alkaloid content was estimated in the periods of bud formation, full blossom, shining legumes and full maturity. The test material included exsiccates of leaves, stems, inflorescences, legumes, and seeds.

According to the results, in the 'Trakiai' and 'Vilčiai' varieties of forage yellow lupins (*Lupinus luteus* L.) total alkaloids were lower than in the 'Vilniai' variety of narrow-leaved lupins (*Lupinus angustifolius* L.) and variety N1702.

The highest content of alkaloids was identified in the periods of full blossom and legumes. A lower content was noted during bud formation, and the lowest in the full maturity period.

High levels of alkaloids are present in legumes, while stems, inflorescences and leaves contain lesser amounts. The lowest content of alkaloids was identified in seeds.

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, works in this field were hampered by absence of reliable and rapid methods of determining alkaloid plants. In 1928, R. Sengbusch from the Central German Institute of Genetics proposed a method which was applied to analyse 1.5 mill. of alkaloid plants, and three non-alkaloid mutants of yellow lupine and two such mutants of narrow-leaved lupine were established [1, 2]. The absence of alkaloids was determined to be an inherited trait, and the obtained individuals by their yields equalled alkaloid plants. These individuals were used in selection work which resulted in the first famous varieties of Munchenberg sweet lupines.

Low alkaloid content in lupines is a runlet of 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 [2]. Alkaloid is a dominant trait which in yellow lupine is determined by four, in narrow-leaved by five and in white lupine by eight genes [1]. Cross-pollination of low-alkaloid and alkaloid lupine varieties was found to produce the alkaloid F_1, and in F_2 a 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 pest whose acid taste prevents grazing [3]. Another theory proclaims alkaloids to be useless products of protein metabolism [4]. Yet another opinion is that alkaloids accumulated in the underground parts of a plant participate in metabolic processes, induce root growth and, on getting into soil, make a barrier to microorganisms [5]. 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 content undergoes changes, the peak coinciding with the flowering. At the end of vegetation, alkaloids accumulate in seeds, roots [6]. Alkaloid content in a plant depends on numerous factors such as age, environmental impacts and geography, also on soil fertilization [7].

Lupine (*Lupinus* L.) 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 protectors [1, 8]. There is also an opinion that lupine alkaloids may destroy toxic fungi in forage and thus favour forage assimilation [6]. There are studies to show that low levels of alkaloids exert no effect on human and animal organisms,

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white in larger quantities they way cause acute ailments or even death. Lupine alkaloids exhibit not only toxic but also pharmacological properties. In yellow fodder lupine, alkaloid content may vary from 0.005% to 1.7% and in narrow-leaved from 0.005% to 3.0%. Low alkaloid levels in lupines are considered to vary within 0.025–0.099%.

The aim of the present study was to determine alkaloid content variations in the vegetative and generative organs of different *Lupinus luteus* L. and *Lupinus angustifolius* L. varieties at different developmental phases.

MATERIALS AND METHODS

The study was carried out in 2006–2007 at the Vokė Branch of the Lithuanian Institute of Agriculture. The experimental plots were established on sandy loam on carbonaceous fluvial-glacial gravel eluviated soil (IDp), *Haplic Luvisols* (*LVh*) according to the FAO-UNESCO classification. Its 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 [9].

The samples were taken from feeding yellow lupine (*Lupinus luteus* L.), 'Trakiai' and 'Vilčiai' varieties, the narrow-leaved lupine (*Lupinus angustifolius* L.), 'Vilniai' variety, and from cropper No. 1702. Selection line No. 1702 was selected by the individual selection method from the collection sample No. 3186. The selected genotype had a low alkaloid content and an intensive pink flower colour. Alkaloid content was estimated in the periods of bud formation, full blossom, shining legumes, and full maturity. The test material included exsiccates of leaves, stems, inflorescences, legumes, and seeds.

Samples were taken from dried and ground parts of lupine plants (leaves, stems, flowers, pods and seeds) from which fatty substances had been extracted with petroleum ether, followed by extraction of active substances with ethanol. After alcohol evaporation we obtained, in the from of a syrup, concentrated extracts which further were separated by first mixing them with a mixture of two-phase ethyl acetate and water-saved wine acid and then using a separating fennel to obtain the organic (ethyl acetate) and inorganic (water) phases. In this way, the neutral and weakly basic alkaloids get into the organic phase. Alkaloid content was determined in four replications by the gravimetric method LST 1560 at the Center of Agrochemical studies of the Lithuanian Institute of Agriculture. The alkaloid quantities were recalculated as a percentage from the dry matter content.

The obtained data were assessed by the method of dispersion analysis, employing the ANOVA [10] statistical data processing software [10].

RESULTS

In 2006–2007, two forage lupine species (four genotypes) were studied, in which alkaloid levels were determined at the stages of budding, full flowering, fruit formation and full ripeness, separately in vegetative and generative parts of the plants.

The results have shown different alkaloid numbers in different lupine genotypes. The highest number was found in stems of the narrow-leaved variety No. 1702 at the fruit formation phase (Table). Analysis of the average alkaloid levels revealed that in the yellow lupine varieties 'Trakiai' and 'Vilčiai' alkaloid levels (0.064 ± 0.003 and 0.054 ± 0.004, respectively) were lower than in the narrow-leaved varieties 'Vilniai' and No. 1702 (0.104 ± 0.031 and 0.085 ± 0.028). The average alkaloid level in leaves was lower than in stems. Also, at the stage of full flowering, alkaloid content (41.34%) was higher than at the stages of budding and fruit formation.

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 [2, 8]. There are data on the possible participation of alkaloids in the processes of breathing, oxidation of various compounds such as ascorbic and citric acids, hydrochinone, pyrogallol, enzyme synthesis [11]. Figure 1 shows the quantitative distribution of alkaloids in different stages of lupine development. The average alkaloid level was highest at the stages of fruit formation (0.113 ± 0.002) and full flowering (0.109 ± 0.002). This level was lower at the stage of budding (0.074 ± 0.002) and lowest at the stage of full

Table. **Alkaloid content in leaves and stems of some lupine species (T. Vokė, 2006–2007 average data)**

Genotype (A)	Apparatus (B)	Phonological growth stages (C), dry matter %		
		Booting	Flowering	Fruit formation
'Trakiai'	Leaves	0.026 ± 0.008	0.057 ± 0.004	0.108 ± 0.003
	Stems	0.055 ± 0.008	0.111 ± 0.040	0.026 ± 0.001
'Vilčiai'	Leaves	0.074 ± 0.015	0.037 ± 0.002	0.013 ± 0.002
	Stems	0.061 ± 0.016	0.091 ± 0.021	0.045 ± 0.001
'Vilniai'	Leaves	0.050 ± 0.013	0.161 ± 0.094	0.133 ± 0.012
	Stems	0.043 ± 0.013	0.135 ± 0.057	0.104 ± 0.013
No. 1702	Leaves	0.051 ± 0.037	0.060 ± 0.014	0.131 ± 0.010
	Stems	0.048 ± 0.037	0.106 ± 0.048	0.114 ± 0.012
LSD_{01} (A) = 0.022; LSD_{01} (B) = 0.008; LSD_{01} (C) = 0.140				
LSD_{01} (AB) = 0.049; LSD_{01} (AC) = 0.045				
LSD_{01} (BC) = 0.008; LSD_{01} (ABC) = 0.027				

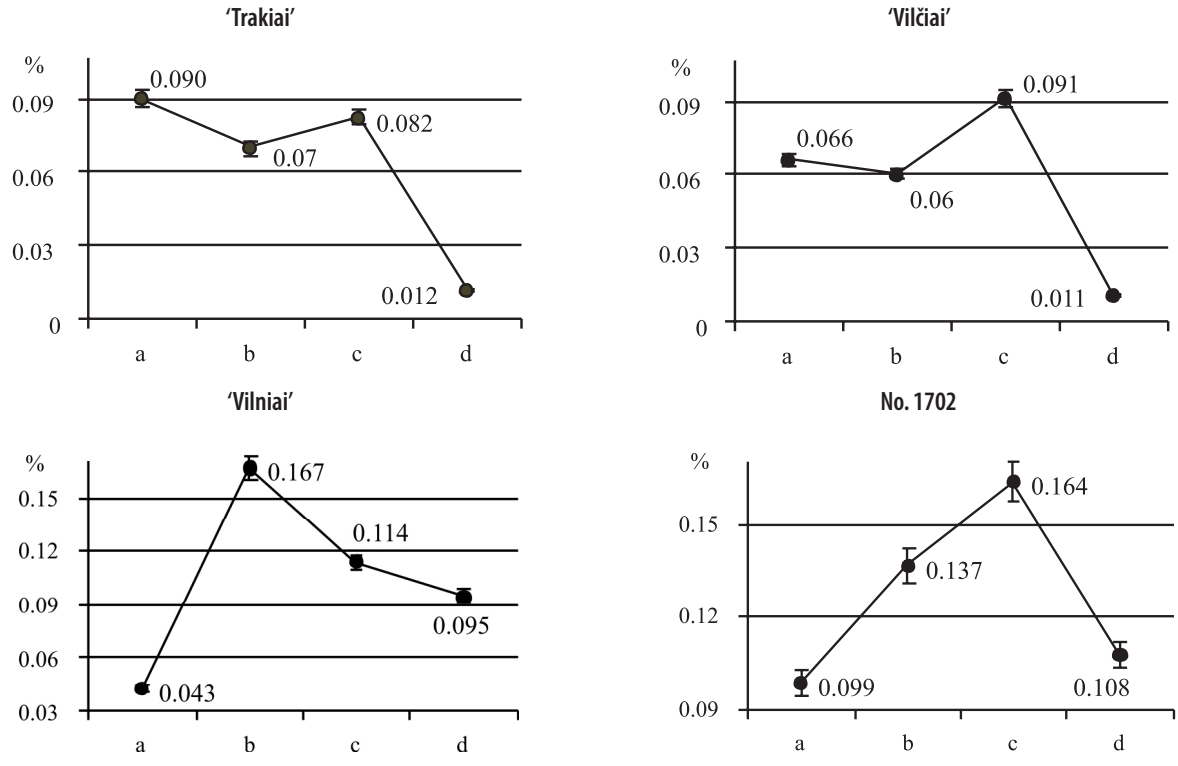

Fig. 1. Alkaloid content variations during particular vegetation periods: a) budding, b) flowering, c) fruit ripening and d) fruit maturity (T. Vokė, 2006–2007 average data)

ripeness (0.057 ± 0.002). Alkaloid levels were influenced also by the species and genotype. The average alkaloid levels were similar in yellow forage lupine varieties 'Trakiai' and 'Vilčiai' (0.064 ± 0.021 and 0.057 ± 0.018). The selection line No. 1702 on average contained more alkaloids (0.127 ± 0.003) than the narrow-leaved variety 'Vilniai' (0.105 ± 0.002).

In 2006–2007, from the very first developmental stages, plants of different varieties differed in leaf colour, branching, growth dynamics. In the flowering phase, 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, siliquae 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 alkaloid [2, 8]. Throughout vegetation, alkaloids levels undergo changes, their peak occurring during flowering. At the end of vegetation, alkaloids accumulate in seeds, roots [1, 12]. In our study, the highest average alkaloid levels were found in siliquaes (0.151 ± 0.007), inflorescences (0.109 ± 0.001), leaves (0.091 ± 0.002), and stems (0.075 ± 0.001), and the lowest level in seeds (0.055 ± 0.003).

DISCUSSION

The main functions of the overground of stem is to develop the largest possible aerial, to sustain the weight of flowers and fruits and to intermediate in transporting the nutrive substances from roots to leaves, flowers, fruits as well as from leaves to roots, flowers and fruits. Therefore, the stem contains both conductive

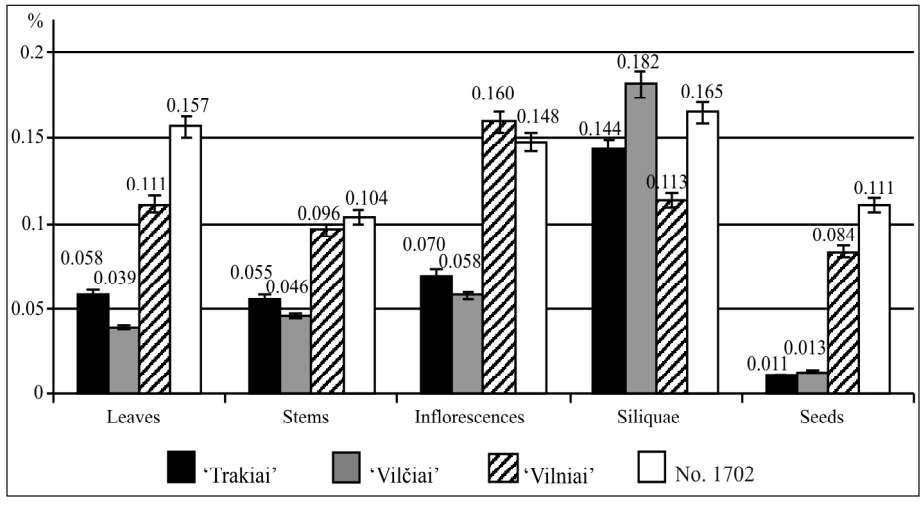

Fig. 2. Alkaloid content in particular vegetative and generative organs of lupine (Vokė, 2006–2007 average data)

and supportive tissues. Besides, stems often serve as nutritive stores [13], therefore, as our study has shown, alkaloid levels in stems are lower than in siliquae which are the basic nutritive organ 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 [7, 14]. Since alkaloid content 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 [15].

Alkaloid content in lupines depends on numerous factors such as species variety, age (developmental stage), environment and geographical location. Alkaloid content 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 development of competitive narrow-leaved forage lupine varieties with a low alkaloid content. The Vokė Branch of the Lithuanian Institute of Agriculture 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 species, subspecies and varieties adapted to the Lithuanian climatic conditions and improved as regards their biochemical properties (increased protein content and lowered alkaloid levels).

Received 19 February 2008
Accepted 7 May 2008

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ALKALOIDŲ KIEKIŲ POKYČIŲ YPATUMAI SKIRTINGUOSE LUBINŲ (*LUPINUS* L.) GENOTIPUOSE IR VYSTYMOSI TARPSNIUOSE

Santrauka

Alkaloidų kiekiai tirti 2006–2007 m. LŽI Vokės filiale atliekant konkursinius pašarinių lubinų veislių bandymus. Ėminiai alkaloidų kiekiams nustatyti buvo paimti iš geltonžiedžių pašarinių lubinų (*Lupinus luteus* L.) veislių 'Trakiai' ir 'Vilčiai', siauralapių pašarinių lubinų (*Lupinus angustifolius* L.) veislės 'Vilniai' ir selekcinio numerio N1702 augalų. Alkaloidų kiekiai tirti esant butonizacijos, pilno žydėjimo, blizgančių ankštelių ir pilnos brandos tarpsniams, tam naudojant išdžiovintas bei susmulkintas lubinų dalis: lapus, stiebus, žiedus, ankštis ir sėklas.

Nustatyta, kad geltonžiedžių pašarinių lubinų veislėse 'Trakiai' ir 'Vilčiai' bendras alkaloidų kiekis buvo mažesnis nei siauralapiuose pašariniuose lubinuose 'Vilniai' ir N1702. Didžiausia alkaloidų sankaupa užfiksuota augalų pilno žydėjimo ir blizgančių ankštelių tarpsniuose. Mažiau alkaloidų nustatyta butanizacijos bei mažiausiai – pilnos brandos tarpsnyje. Daugiausia alkaloidų lubinuose kaupiasi ankštelėse, mažiau – stiebuose, žieduose bei lapuose ir mažiausiai – sėklose.

Raktažodžiai: pašariniai lubinai, alkaloidai, vystymosi tarpsniai, vegetatyviniai ir generatyviniai organai