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# ALKALOID CONTENT VARIATIONS IN *LUPINUS LUTEUS* L. AND *LUPINUS ANGUSTIFOLIUS* L.

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*Abstract* - Testing of lupine varieties for alkaloids was performed in the period 2006-2010 at the Voke Branch of the Lithuanian Institute of Agriculture in the course of a competitive trial of feeding lupine (*Lupinus* sp.). Samples of feeding yellow lupine (*Lupinus luteus* L.) varieties as well as narrow-leaved lupine (*Lupinus angustifolius* L.) were used. The alkaloid concentration was assessed in the periods of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development of fruit (BBCH 71-75) and ripening of seed (BBCH 85-88). Exsiccates of leaves, stems, flowers, pods and seeds were used as test material. The results revealed similar average levels of alkaloids in yellow forage lupine (*Lupinus luteus* L.). Analysis of the average alkaloid levels showed that in all phenological stages in the stems of yellow lupine (*Lupinus luteus* L.) alkaloid levels were lower than in the narrow-leaved (*Lupinus angustifolius* L.). At the stage of flowering (BBCH 64-67), the average alkaloid level in the leaves was lower than in the stems. In the course of our investigation, the highest average alkaloid levels were determined in the pods (0.114 ± 0.007) and flowers (0.114 ± 0.006) of narrow-leaved lupines, and the lowest level in the seeds (0.022 ± 0.003) of yellow lupines.

*Key words*: Alkaloids, forage lupines, vegetation periods, vegetative and generative organs

## INTRODUCTION

Prior to 1926, lupines were used exclusively as siderates. G.G. Gataulina hypothesized about the natural existence of low-alkaloid lupines; however, studies in this field were hampered due to the absence of reliable and rapid methods for determining alkaloid plants (Gataulina, 2002). R. Sengbush (Central German Institute of Genetics) in 1928 proposed a method that was applied for the analysis of 1.5 mil alkaloid plants; three non-alkaloid mutants of yellow lupine and two non-alkaloid mutants of narrow-leaved lupine were determined (Kurlovich, 2002). The alkaloid absence was revealed to be an inherited trait, and the yields of the obtained individuals equaled the alkaloid containing plants. These plants were used for selection, which resulted in the first well-known varieties of Munchenberg sweet lupines. Biochemical mutation underlies low alkaloid concentration in lupines. The first forage varieties of lupines were created by applying the method of individual selection from alkaloid plant populations where low-alkaloid mutants rarely but still occurred (Kurlovich, 2002). Alkaloid content is a dominant trait determined by four genes in yellow lupine, by five genes in narrow-leaved and by eight genes in white lupine (Phan et al., 2007). As a result of cross-pollination of low-alkaloid and alkaloid lupine varieties, the alkaloid in $F_2$ was produced, and a splitting into alkaloid and non-alkaloid generations occurs at a ratio 3:1. The function of alkaloids in plants is not fully clear yet. Alkaloids are alleged to protect plants from pests that 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 the soil, become a barrier to microorganisms (Peneva, 2006). However, none of the above theories comprehensively explains the significance of alkaloids to plants, because some plants accumulate alkaloids while others do not. Alkaloids are unevenly distributed in plant organs: in some plants they mostly accumulate in seeds or leaves, roots or cortex, in the parenchymal tissue or in cells. Both similar and different alkaloids can accumulate in the same plant. Alkaloid concentration undergoes changes in the course of the vegetation period; the peak concentration coinciding with the plant flowering. At the end of vegetation, alkaloids accumulate in the seeds and roots (Hondelmann, 1984). Alkaloid concentration in a plant is determined by numerous factors – age, environmental impacts and geographical situation, the mode of soil fertilization (Breitmaier, 2002). Lupine (*Lupinus* sp.) is a universal plant characterized by multiple useful properties; it could be used as fodder or for soil fertilization. Low-alkaloid lupine species, such as yellow fodder lupine (*Lupinus luteus* L.) and narrow-leaved forage lupine (*Lupinus angustifolius* L.), are used as fodder. Lupines, however, do not produce alkaloids in order to supply them to man or animals. In plants, various alkaloids function as insecticides, herbicides, fungicides or pest deterrents (Lee et al., 2008; Gataulina, 2002). The hypothesis exists that lupine alkaloids may destroy toxic fungi in forage and thus favor forage assimilation (Ralphs et al., 2011; Hondelmann 1984). Some investigations demonstrate that low levels of alkaloids exert no effect on human and animal organisms, while in larger quantities they may provoke acute ailments or even death. Lupine alkaloids are characterized by toxic as well as pharmacological properties. In yellow fodder lupine, the alkaloid concentration may range from 0.005% to 1.7% and in narrow-leaved from 0.005% to 3.0%. In lupines, the alkaloid levels varying between 0.025-0.099% are considered low. Breeding alkaloid-free lupine varieties or varieties with low amounts of alkaloids offers new possibilities for lupine application not only in forage production but also in the food industry. The aim of the present study was to determine the variations of alkaloid concentration in the vegetative and generative organs of different *Lupinus luteus* L. and *Lupinus angustifolius* L. varieties at various developmental phases.

## MATERIALS AND METHODS

The study was performed in 2006-2010 at the Voke Branch of the Lithuanian Institute of Agriculture. The experimental plots were established on sandy loam on carbonaceous fluvioglacial gravel eluviated soil (IDp), according to FAO-UNESCO classification *Haplic Luvisols* (*LVh*), with the following agrochemical parameters: pH – 5.6-6.2, humus – 1.37-2.5%, mobile $P_2O_5$ – 130-250 mg $kg^{-1}$ and mobile $K_2O$ – 146-254 mg $kg^{-1}$. Competitive trials of the varieties were performed according to a selection scheme (Maknickiene, 2007).

### *Plant material*

(i) The samples from feeding yellow lupine (*Lupinus luteus* L) 'Trakiai' and 'Vilčiai' varieties, as well as the narrow-leaved lupine (*Lupinus angustifolius* L.) 'Vilniai' variety, and cropper of No. 1702, were taken. Selection line No. 1702 was selected by individual selection method from collection sample No. 3186 (A factor).

(ii) Alkaloid concentrations were estimated in the periods of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development of fruit (BBCH 71-75) and ripening of seed (BBCH 85-88 (B factor).

(iii) The test material included exsiccates of leaves, stems, flowers, pods and seeds (C factor).

### *Concentration of alkaloids*

Freeze-dried plant material was finely ground at room temperature and 15 ml 5% (w/v) trichloroacetic acid were added to 200 mg of the plant material. The suspension was stored at room temperature for 2 h followed by centrifugation at 3000 rpm for 15 min. A 12 ml aliquot of the supernatant was subsequently alkalinized with 25% (v/v) ammonia to pH ~11 and extracted twice with 25 ml dichloromethane. The pH

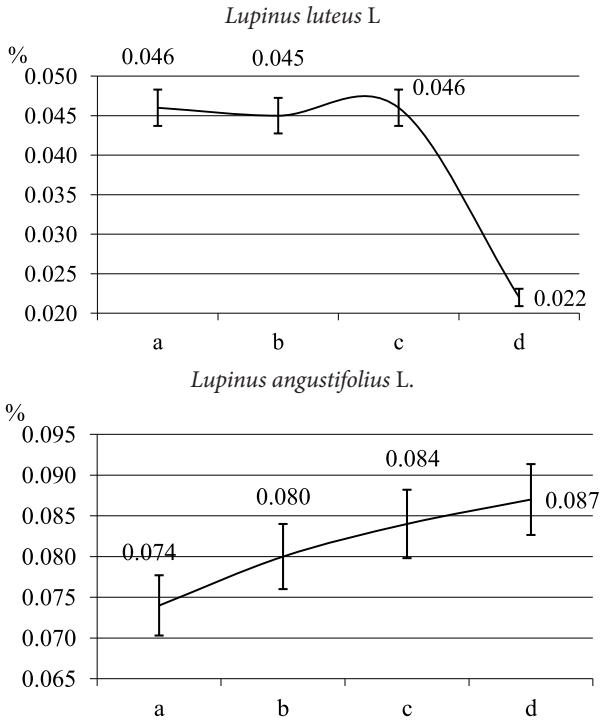


**Fig. 1.** Variations of alkaloid concentration during particular vegetation periods (B factor): a) inflorescence emergence (BBCH 51-55), b) flowering (BBCH 64-67), c) development of fruit (BBCH 71-75), d) ripening of seed (BBCH 85-88) (average data, $LSD_{01}AB$ – *0.029*)

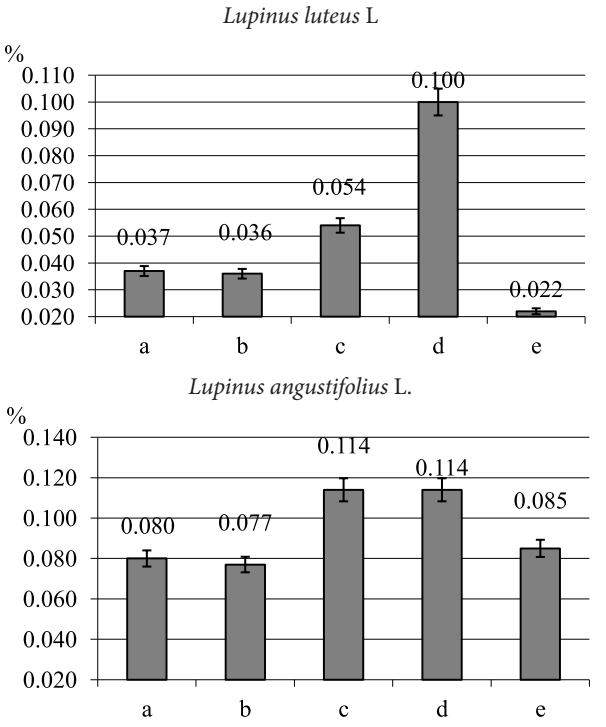


**Fig. 2.** Alkaloid concentration in separate vegetative and generative organs (C factor) of lupine (A factor): a) leaves, b) stems, c) flowers, d) pods, e) seeds (average data, $LSD_{01}AC$ – *0.037*)

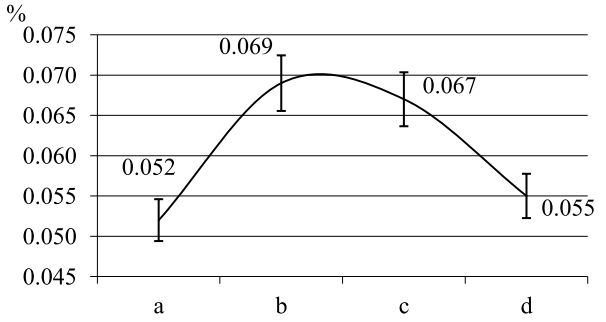


**Fig. 3.** Alkaloid concentration at particular vegetation periods (B factor) in vegetative organs (C factor): a) inflorescence emergence (BBCH 51-55), b) flowering (BBCH 64-67), c) development of fruit (BBCH 71-75), d) ripening of seed (BBCH 85-88) (average data, $LSD_{01}BC$ – *0.009*)

was then raised to 14 by adding 10 M of 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 dried over anhydrous sodium sulphate and concentrated by heating ($40^0$C) under a continuous stream of nitrogen. The residue was reconstituted in methanol (100 µl) containing caffeine (10 µg) as an internal standard. Chromatographic analysis was performed employing the modified method of Lee et al. (2007). The alkaloid quantities were recalculated as a percentage of the dry-matter concentration.

### *Statistics*

Statistical analysis of the data was done using a StatView ANOVA program. The obtained data were assessed by the method of dispersion analysis, em-

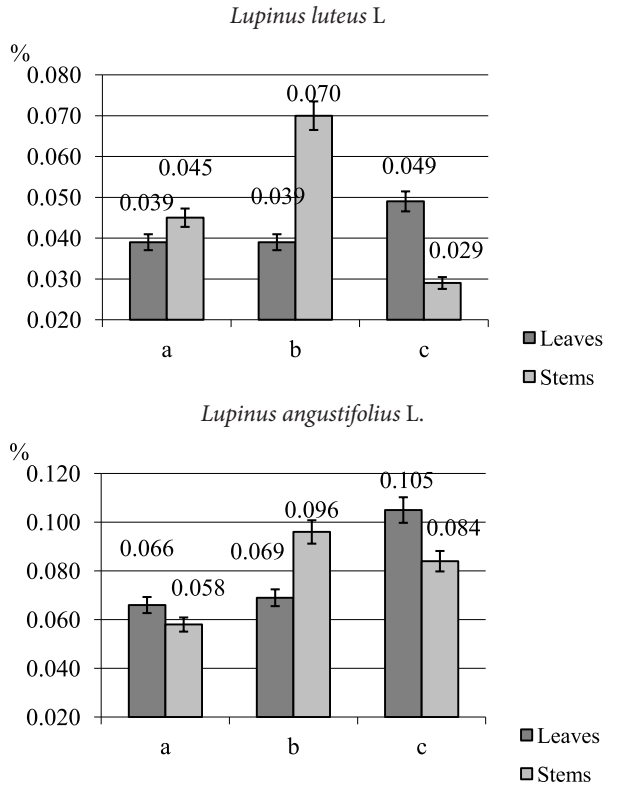


**Fig. 4.** Alkaloid concentration in leaves and stems of some lupine species (A factor): a) inflorescence emergence (BBCH 51-55), b) flowering (BBCH 64-67), c) development of fruit (BBCH 71-75) (average data, $LSD_{01}ABC$ – *0.027*)

ploying the ANOVA ($LSD_{0.1}$) statistical data processing software (Tarakanovas, 2002).

## RESULTS

During the period of 2006-2010 two forage lupine species (with two genotypes each) were tested; the alkaloid levels in vegetative and generative parts of the plants were separately ascertained at the stages of inflorescence emergence (BBCH 51-55), flowering (BBCH 64-67), development of fruit (BBCH 71-75) and ripening of seed (BBCH 85-88).

The quantitative distribution of alkaloids at different stages of lupine development is presented in Fig. 1. At the stages of flowering (BBCH 64-67) (0.080 ± 0.003), development of fruit (BBCH 71-75) (0.084 ± 0.002) and ripening of seed (BBCH 85-88) (0.087 ± 0.002), the average alkaloid level in *Lupinus angustifolius* L. was highest. Species and genotype also influenced the alkaloid levels.

In 2006-2010, starting with the very first developmental stages, plants of diverse varieties differed in leaf color, branching and growth dynamics. At the flowering (BBCH 64-67) phase, the vegetative organs were fully formed, and morphological differences among the varieties became obvious. The alkaloid levels in the vegetative (leaves and stems) and generative (flowers, pods and seeds) organs of plants ascribed to four genetic types were determined. The distribution of alkaloids in different vegetative and generative organs of lupine plants is shown in Fig. 2. Some reports indicate that the same plant may contain similar as well as different alkaloids (Kurlovich, 2002; Barbachi, 2000). Throughout the vegetation period alkaloid levels change, their peak occurring during the plant flowering. At the end of vegetation, alkaloids accumulate in the seeds and roots (Gataulina, 2002; Brummund, 1988). In the course of this study, the highest average alkaloid levels were recorded in the pods (0.114 ± 0.007) and flowers (0.114 ± 0.006) of narrow-leaved lupines, and the lowest level in the seeds (0.022 ± 0.003) of yellow lupines.

The quantitative distribution of alkaloids in different stages of lupine development is presented in Fig. 3. At the stages of flowering (BBCH 64-67) (0.069 ± 0.003) and development of fruit (BBCH 71-75) (0.087 ± 0.002), the average alkaloid levels were highest.

The results have shown variations in alkaloid content in different lupine genotypes. The highest alkaloid content was recorded in *Lupinus angustifolius* L. leaves (0.105 %) at the development of fruit phase (BBCH 71-75) (Fig. 4). Analysis of the average alkaloid levels revealed that during all phenological stages alkaloid levels in the stems of yellow lupine were lower than in the stems of narrow-leaved lupine. At the stage of flowering (BBCH 64-67), the average alkaloid level in the leaves was lower than in the stems.

## DISCUSSION

In lupine plants distinct periodical changes of alkaloid levels occur. In plants, alkaloids are the intermediate forms of nitrogen metabolism in which these compounds are rendered harmless and accumulate (Kurlovich, 2002; Barbachi, 2000). The hypothesis 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) exists.

Leaves absorb $CO_2$ from the environment and from roots and receive water and mineral salts via circulatory tissues. From this raw material, various organic matters are synthesized by the leaves, employing solar energy, and supplied to other organs of a plant (Przybrowski and Packa, 1994; Breitmaier, 2002). Alkaloid concentration in a plant constantly changes throughout the growth period, and the maximum stocks of alkaloids in leaves are accumulated before flowering; later they gradually decline together with the qualitative composition of alkaloids with respect to the whole alkaloid complex (Carey and Wink, 1994).

The main functions of the aboveground stem is to develop the largest possible area in order to sustain the weight of flowers and fruits and to mediate in transporting nutrients from the roots to leaves, flowers and fruits, as well as from leaves to roots, flowers and fruits. Therefore, the stem is formed of both conductive and supportive tissues. In addition, stems often serve as nutritive stores (Carey and Wink, 1994); therefore, as this study has revealed, alkaloid levels in the stems are lower than in the pods, which are the basic nutritive organs of a plant.

Alkaloid concentration in lupines depends on many factors – plant variety, age (developmental stage), environment and geographical location. It has been determined that different alkaloid concentrations in plants affect the central nervous system of living organisms, i.e. low alkaloid levels act as stimulators while higher levels act as suppressors. Consequently, the aim of lupine selection in Lithuania could be the creation of competitive narrow-leaved forage lupine varieties with low alkaloid concentration. Valuable local material that needs further exhaustive selective and genetic studies has been accumulated in the Voke Branch of the Lithuanian Research Centre for Agriculture and Forestry. Based on the available national genetic fund of lupines, the most suitable lupine species, subspecies and varieties, adapted to the Lithuanian climatic conditions and advanced regarding their biochemical properties (increased protein concentration and lowered alkaloid levels), could be suggested for cultivation.

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