

Investigation of The Relationship Between The Level of Alkaloids and Degree of Pest Damage in *Lupinus Polyphyllus* Lindl.

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ABSTRACT: Wild alkaloidal perennial Washington lupin (*Lupinus polyphyllus* Lindl.) has spread widely in many countries and is considered an invasive plants which are proposed in many countries to destroy. Our research is aimed at its cultivation and rational use. One of the ways in this direction is the use of low alkaloidal (sweet) forms. The first sweet forms of lupins were created almost 90 years ago by Reinhold von Sengbusch in Germany. The Russian Institute of Plant Industry (VIR) also developed its research as well as an instruction to identify alkaloid-free lupins. It was immediately published with the foreword by N.I. Vavilov (Ivanov *et al.*, 1932). Several thousand plants were analyzed by this method and the first sweet form of *L. polyphyllus* was bred in 1932 at VIR. The above-mentioned publication of VIR as well as the discovery of German scientists was a cornerstone of the modern breeding work with low-alkaloid fodder (sweet) lupins in the whole world. One of the authors of this article worked in the Russian Institute of Plant Industry (VIR) with collection of lupins more than 30 years and produced the first fodder commercial cultivar of *L. polyphyllus* named 'Pervenets' (the first sweet variety) for the conditions of the Northwest Russia (Kurlovich *et al.* 2002). Variety 'Truvor' was developed for the conditions of Ukraine. Now we are researching sweet and bitter forms of the perennial Washington lupin in Finland, New Zealand and UK. It has found practical use as a green feed for sheep in New Zealand. We present some of our research results in this paper. We noticed that low alkaloid plants are exposed to pest. This makes it easy to detect sweet plants, but they are difficult to replicate and keep from pests. The developed approaches may also be applied to many cross-pollinated American lupin species (subgen. *Platycarpus* (S. Wats.) Kurl.), such as *L. mutabilis* weet., *nootkatensis* Donn., *L. arboreus* Sims., *L. perennis* L., *L. elegans* H.B.K., *L. hartwegii* Lindl. and other forms promising for agricultural production (Kurlovich *et al.* 2002, 2008).

Keywords: *Lupinus polyphyllus*, alkaloids, Dragndorf's reagent, gas chromatography, pests, biodiversity, sheep feeding

INTRODUCTION

Wild alkaloidal perennial multifoliate or Washington lupin (*Lupinus polyphyllus* Lindl.), coming from North America, has spread widely in many countries and is considered invasive plants which are proposed in many countries to destroy. However, this plant is high in protein (38-40% in dry matter). Our research is aimed at its cultivation and rational use. Now that reduced-alkaloid forms have been identified, future use of this species for fodder production, green manure and silage look possible. We noticed that low alkaloid plants are exposed to pests. This makes it easy to detect sweet plants, but they are difficult to replicate and keep from pests. Alkaloids

are useful for plants and are poisonous to most animals. They protect plants from pests. However, the digestive tract of sheep can digest alkaloids, as evidenced by the experience of New Zealand. This material was presented as an oral presentation at the XV International Lupin Conference 2019 "Developing Lupin crop into a modern and sustainable food and feed source", March 18-21, 2019. Cochabamba – Bolivia.

Object: Accessions of different alkaloid content from the collection of the N.I.Vavilov Institute of Plant Genetic Resources (VIR), forms of lupin with different alkaloids from Finland, UK and New Zealand (Loxton *et al.* 2015). The plant of *Lupinus polyphyllus* is represented in Figure 1.



Figure 1. The plant of *Lupinus polyphyllus* Lindl.

Methodology

Qualitative evaluation of alkaloid activity was carried out by a conventional traditional method with the help of indicator paper impregnated with a dragendorff solution (Ivanov *et al.*, 1932). Gas chromatography was used for the quantitative analysis of alkaloids. The extraction and analyses were conducted following the common procedure (Kamel *et al.* 2016). The main part of the biochemical analyzes performed in the Department of Biochemistry the Russian Institute of Plant Industry (VIR). Part of the analysis is performed in the laboratory of Pharmacology and Neuroscience, Genoa, Italy. We used the scale of the descriptor list for lupin while measuring the degree of plant susceptibility to pests: 1 – No symptoms or very weak (< 2.5%); 3 – Slight (2.5-10%); 5 – Intermediate (11-25%); 7 –Severe (26-50%); 9 - Very severe (>50%). Visual methods were used in analyzing pests based on direct inspection, counting and photographing of pests and plant organs damaged by them. Pests and diseases were determined by our methodological guidelines (Kurlovich and Golubev, 1990).

Soya UK examined low alkaloidal *Lupinus polyphyllus* (2015 – 2019) in the following direction: Perennial lupin was sown in 1919 on the area of 0.8 ha. All plants were tested for alkaloids with paper impregnated with Dragndorf's reagent. Gas chromatography will also be used to analyze alkaloids in 2019/2020. Green mass was tested on sheep.

Sawdon Station of New Zealand investigated the effectiveness of feeding green mass of perennial lupin to sheep.

Results

Harmful fauna of lupins is characterized by great diversity in the environments of Finland. It is very likely that in other regions it may be different. Stems, leaves, and flowers are damaged by various leaf-eating insects, especially leaf-eating caterpillars, beetles, aphids, bugs and trips, among which are especially common is alfalfa aphid. The most harmful and more common pests are shown in the following figure.

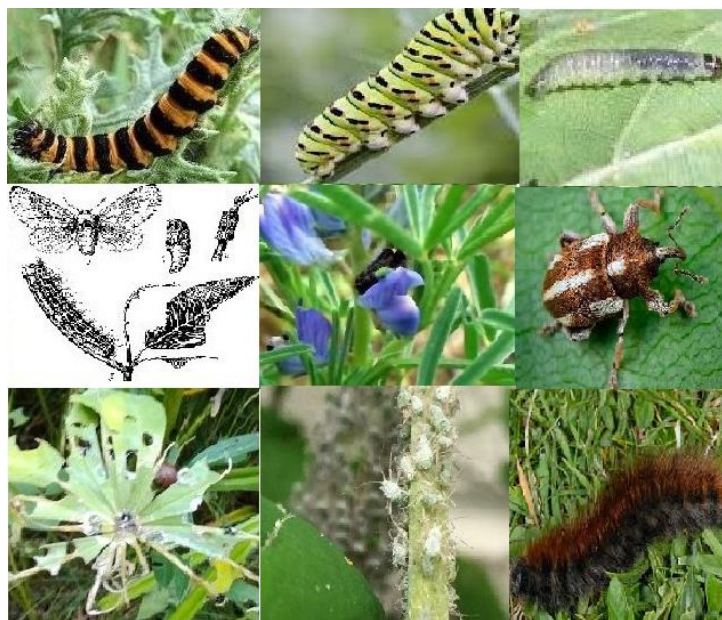
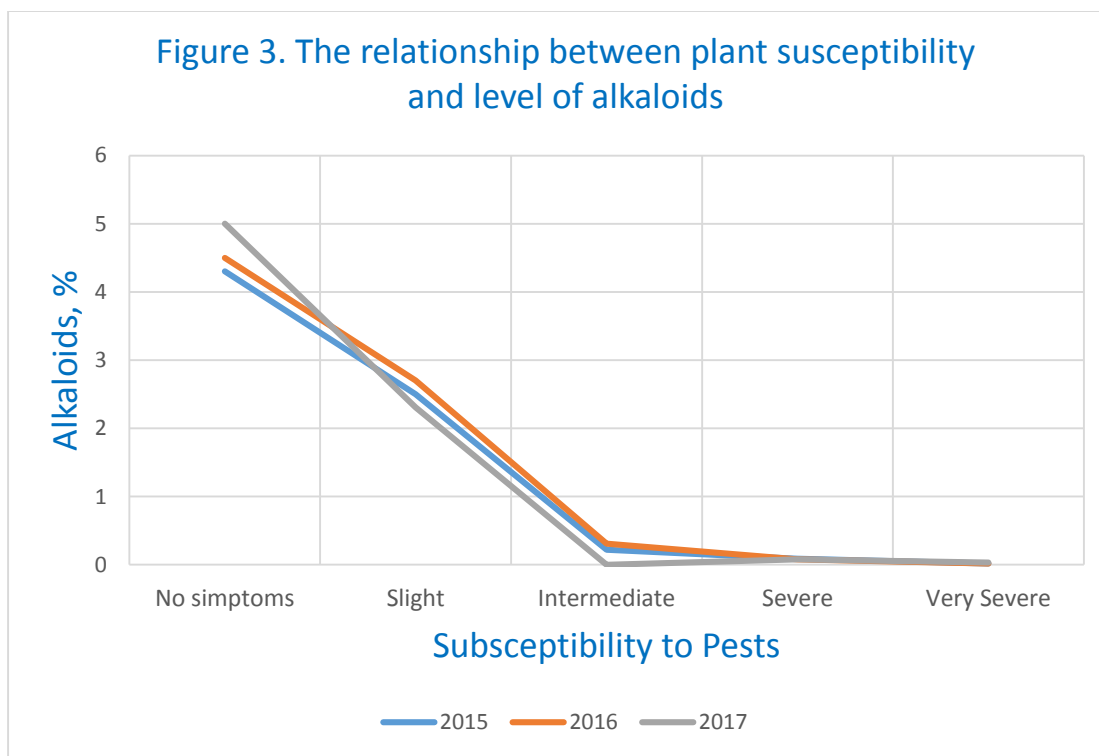


Figure 2. The most harmful pests of *Lupinus polyphyllus* Lindl. in the conditions of Finland.

Different accessions were affected by pests to varying degrees. Some of them showed no signs of damage, while others were strongly afflicted. We drew attention to the fact that the leaves of lupin severely affected by leaf pests had a low content of alkaloids. The relationship between plant susceptibility to pests and the level of alkaloids appeared in our studies as follows: no symptoms of damage – 4.00% of alkaloids, slight – 2.00%, intermediate – 0.20%, severe – 0.09%, and very severe damage – 0.02% (Fig.3).



We managed to collect a small number of seeds from very severely affected plants whose alkaloid content was 0.02%. The residual composition of alkaloids in these seeds was as follows: 58% of 13-hydroxylupanine, 23% of

lupanine, 4.5% of angustifoline, 1.2% of 11, 12-dehydrolupanine, 1% of ethyl-lupanine, and 12.3% of some other unidentified substances.

We can judge about the degree of defeat *Lupinus polyphyllus* by pests comparing amazed leaf by pests and healthy leaf (fig. 4).



Figure 4. The leaf evacuated by pests with a residual alkaloid content of 0.02- 0.04% in comparison with not damaged leaf.

We are trying to multiply seeds from pest affected plants to use them in the future for research and possible breeding.

New Zealand is developing sweet and bitter *L. polyphyllus* for use as a forage crop for sheep. The stand of perennial lupin-grass (*Lupinus polyphyllus*) used in this study had persisted under sheep grazing, modest inputs of fertilizer and lime, and 600-650 mm of rainfall a year, for 8 years before the commencement of measurements in 2011. Over the next 4 years, the stand carried an average of nine ewes/ha from October to May each year. The ewes lambed in October and the lambs were weaned and taken off the stand in February. On average, the annual lambing percentage was 112%, the weight of the lambs at weaning was 27 kg, and the amount of lamb live weight produced was 344 kg/ha a year. The wool yield in September averaged 4.62 kg/ewe (greasy) with a mean fiber diameter of 18.5 μm . In comparison, a similar flock of 200 ewes managed predominantly on alfalfa and clover-based pastures on the farm achieved on average 105% lambing, 31 kg lambs at weaning, and 4.92 kg/ewe of 18.5 μm wool. The average standing biomass in the lupin pasture was 3.0 t of dry matter (DM)/ha at the start of lambing in October, reached 7.8 t DM/ha in December, and decreased to 3.5 t DM/ha in May.

Soya UK also researches the quality of the green mass of perennial lupin with different alkaloids and the effectiveness of its feeding to sheep. Plants are selected with low alkaloids and their reproduction for future research.

Discussion

We noticed that low alkaloid plants are exposed to pests. This makes it easy to detect sweet plants. The degree of damage of the plant by pests can be the marker that allows to identifying low alkaloidal (sweet) forms of lupins. Strongly affected plants are usually low alkaloidal. This marker will significantly accelerate and simplify the search for low-alkaloid forms. In this case, there is no need to test for alkaloids a huge number of plants.

On the other hand, these plants are difficult to multiply and keep healthy. Alkaloids protect lupins from pests. Which is more important for these two conclusions?

Tolerance to diseases and pests is very important for lupins (Mc Naughton, 2015). However, the lack of alkaloids in the plant reduces its tolerance. Sweet forms of lupins were found about 90 years ago. Humanity took it as a great discovery. The era of cultivation of sweet (low alkaloidal) lupins began started from that time. Lupin obtained the status of a valuable fodder plant. But, there were many successes and failures in this way - fusarium, anthracnose, etc. However, the direction of creating fodder low-alkaloidal varieties of lupins has been determined as a basic and it is difficult now to turn from this path. The creation of cultivars resistant to diseases and pests is the only mainline on this path. But, according to the forecasts, due to general warming by the middle of the century, there will be a doubling of insect pests. Insects will destroy approximately 2.5% of the cropper degree of warming (Cline, William, 2007). This means that the activity of pests will grow faster than the productivity of plants. In this connection, the question of future growth of bitter lupins cannot be ignored. People cultivated the bitter lupins from ancient times,

soaking and boiling seeds (Greece, Rome, Portugal). The bitter *Lupinus mutabilis* is growing at present in the American continent, (we've seen it in Bolivia). It is easy to remove alkaloids and use it in medicine, cosmetics, etc. in our time of technological progress. The remaining protein can be used after separation for food and fodder purposes as an alternative to soybeans. Maybe this method is also progressive?

Our research with perennial lupin in New Zealand showed the possibility of effectively using sweet and bitter lupin for feeding sheep. The digestive tract of sheep can digest alkaloids. Sawdon Station is a 7500 ha farm located near Lake Tekapo in the South Island of New Zealand. The farm produces fine Merino wool from about 4500 ewes for the global markets. Following the tradition of early pastoral run-holders, the Merino ewes are raised on a pasture-based system, generally comprised of native grasses and introduced pasture species grown in the difficult high country environment. This system is acceptable for producing fine Merino wool, which is shorn in September, and market weight lambs by February. However, as a global industry competitor in fine wool production, Sawdon Station faces the challenge of improving efficiency and productivity. This challenge is being met, at least in part, by using pasture legumes that are adapted to the environment. Perennial lupin (*Lupinus polyphyllus*) is cultivated in the lower-cost developments. Perennial lupin survives the climate and is adapted to acidic, low phosphorus soils. However, there has been little commercial sowing of perennial lupin in the high country; Sawdon Station is one of only a few farms using perennial lupin in the district. The objective of this study was to quantify the performance of Merinos grazing on a commercial stand of perennial lupin-grass (10 ha) on Sawdon Station over 4 years.



Figure 5. Perennial lupin (*Lupinus polyphyllus*) and sheep on the flock of the Sawdon Station

Lupin in these conditions turned out to be an indispensable cheap feed, which ensured high productivity of sheep. (Loxton et al. 2015).

Conclusions.

Lupinus polyphyllus is not always a weed or an invasive plant, and has potential as a cultivated plant. Now that reduced-alkaloid forms have been identified, future use of this species for fodder production, green manure and silage look possible.

The Express method of the finding of low alkaloid plants of lupins is offered. The degree of damage of the plant by pests can be the marker that allows identifying low alkaloidal (sweet) forms of lupins. Strongly affected plants are usually low alkaloidal.

The negative link between low alkaloid and pest resistance has been revealed. Alkaloids protect lupins from pests. Low alkaloidal plants are exposed to pests. This phenomenon should be considered inbreeding.

Alkaloids are useful for plants and are poisonous to most animals. They protect plants from pests. However, the digestive tract of sheep can digest alkaloids, as evidenced by the experience of New Zealand.

We believe that our observations and experiments will contribute to the development of new low-alkaloid (sweet) and bitter varieties of different American lupin species (subgen. *Platycarpus* (S. Wats.) Kurl.).

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