Bulletin No. 144 - Lupine Studies II. The Silvery Lupine

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Silvery lupine patches along the trail should be avoided.

Lupine Studies II. The Silvery Lupine

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Chemical Study of the Silvery Lupine

BY O. A. BEATH

In 1920 the author presented some preliminary data relating to the distribution, habitat and poisonous character of the silvery lupine (*Lupinus argenteus*) in Wyoming Station Bulletin No. 125. Since that time the investigations have been continued and broadened to include feeding tests and range studies along with the more or less technical laboratory researches.

The chemical study, particularly that part dealing with the alkaloids, has been until the past year very difficult to follow. Plants collected from the same localities have varied from year to year in yielding alkaloidal types of different compositions. This has not been true of the total toxic substances but appears to be confined to particular fractions separated by chloroform. Reference is made to this fact to point out one of the difficulties encountered in the lupine studies.
The toxic dose of the poisonous "fruit" and seeds of the silvery lupine obtained from feeding tests with sheep has not been satisfactorily established. The results of our work follow later in this bulletin. During the past summer a large collection of green fruit was made from a section of the state where this species grows very prolificly and it is hoped that additional information may be obtained as to approximate dosage.

It is evident, from our range observations and studies, that many other lupines play an important part in sheep poisoning. Such species as *L. leucophyllus* (Figure 1) of the western part of the state, *L. laxiflorus*, quite widely distributed over Wyoming, and *L. ornatus* of the central and northern sections, constitute a group which must be suspected of poisoning sheep under present range conditions, for sheep losses have been observed where *L. argenteus* was not present and these other lupines were the only plants at all suspicious. *L. alpestris* (Figure 2) occurring in dense patches at higher elevations in most of the national forests of the state appears to be browsed freely by sheep with very little trouble following. After sheep have grazed over the *L. alpestris* area, nothing is left but the bare stems. Considering the quantity

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*Fig. 2. A typical growth of *Lupinus alpestris*.***
of the plant eaten and the comparatively low death rate reported, it would appear to be one of the less poisonous lupines.

The fruit of the lupines, as is well known, is rich in oils and fats. It appears reasonable to believe that sheep occasionally seek this part of the plant for the fatty substances, especially sheep coming from a range where the forage lacked such oily constituents. Several such cases have come to our attention. Naturally, feeding exclusively upon the more poisonous lupines ends with a certain amount of poisoning. Some of the more important fats and oils are discussed under another heading in this bulletin.

EXPERIMENTAL FEEDING

Relative proportion of seeds and pods.

It is well known that the lupines as a group produce their fruiting stalks gradually. This has been discussed in Bulletin No. 125 with particular reference to the silvery lupine. As the green pods

Fig. 3. Some typical fruiting stalks of the silvery lupine.
or fruit develop the seeds are dispersed, leaving the dried pods intact. Lupine poisoning on the range, therefore, is largely due to sheep eating the fruit (seeds in pods).

For a number of years the author has given considerable study to the weight relationship of pods and seeds. Information concerning the relative toxicity of seeds and pods must be considered in connection with their relative proportions. Figure 3 illustrates fruiting heads at various stages of maturity. The curled pods have dispersed their seeds; the others remain intact. In most cases the basal pods are much larger and more fully developed than those near the apex of the cluster. Figure 4 (left) is an illustration of a typical silvery lupine fruiting head. The same cluster (right) is shown with the seeds and corresponding pods detached.

In selecting lupine fruits for these special examinations, the samples were not taken at random. About 1,000 pounds of whole plants were cut with a sickle, and then the fruiting clusters were hand-picked. The final sample was obtained by the usual method of quartering. While in the green condition the pods were opened
and the seeds removed by hand. Figure 5 illustrates the weight relationship of the sample after air drying. (The seeds were removed in a green condition to prevent loss from worms). After two seasons' results the following data have been obtained:

The average moisture content of the fruit is roughly seventy per cent, depending upon the maturity of the plant.

The green seeds average about five per cent less moisture than the corresponding pods.

On an average, the weight of pods exceeds that of seeds, roughly three times.

The mature pods left after the seeds have been expelled varied in their moisture content from 18 to 23 per cent.

Based on a weight relationship of the pods and seeds as given above, it is evident that the lupine fruit as a whole must be considered in range poisoning.

An inspection of Figure 6 shows that many seeds are quite irregularly and poorly developed. To arrive at an approximation of the ratio of sound seeds to those underdeveloped in an ordinary run of the silvery lupine, a sample of 1,000 grams of dried seeds was selected by proper quartering from five kilos, the original quantity. The separation was roughly made by the use of a screen having oblong openings (2 x 4 mm.). This test gave sixty per cent well developed and forty per cent inferior seeds.

Fig. 5. Weight relationship of lupine seeds and corresponding pods after being air dried.
The samples were kept separate and examined chemically to determine, if possible, any differences in alkaloidal content. The results are discussed under another heading in this bulletin.

Preliminary feeding tests.

During the fall of 1924 the author undertook to determine the relative quantities of dried seeds and pods necessary to produce symptoms of lupine poisoning in sheep. The results, on the whole, indicated a much larger dosage is required than was anticipated. At least two factors enter which must be carefully considered in lupine feeding tests of this sort. One is that the dried material may have materially changed in its toxic composition, and the second factor involves corral fed animals. Lack of feed entirely or of a certain kind, the fatigued condition of the animals in strange or new environments so characteristic of many range poisoning cases, can not be duplicated fairly by artificial feeding. However, the feeding tests at this station were made to add, if possible, additional information concerning the toxicity of the silvery lupine. Nevertheless, the difficulties involved were appreciated.
The pods and seeds were fed separately in order to acquire data as to the toxic relationship of the two since the quantity of material available for the feeding tests was limited, the tests were not carried beyond the point of positive symptoms of poisoning. The results of the preliminary feeding follow:

**Seeds.**

Ewe Lamb No. 1—Fed on dried seeds (whole) at the rate of .6 pounds per hundred weight of animal. Equivalent to 2.1 pounds original seeds. Initial temperature 101.2/5° at 11:00 a.m. At 8:40 a.m. following day temperature reached 104.3/5°. No positive indications of poisoning observed. A portion of seeds were not digested.

Ewe Lamb No. 2—Fed air-dried, finely ground seeds at the rate of .88 pounds per hundred weight of animal. Equivalent to 2.5 pounds of original fresh seeds. Given at 8:35 a.m. and 5:15 p.m. Temperature increased from 103° to 105°. Otherwise the animal appeared normal.

Ewe Lamb No. 3—Fed 1.05 pounds per hundred weight, finely ground seeds, air dried. Equivalent to 3 pounds of original fresh seeds. Given at 8:10 a.m. Initial temperature, 103.3/5°. At 5 p.m. temperature was 105.3/5°. In the afternoon this ewe was not well, her ears drooped, she did not eat and constantly grated her teeth. The following morning her temperature had dropped to 104°. She was still dull and depressed and remained in this condition until the next day, when she gradually began eating hay and appeared normal the morning of the fourth day. The quantity fed in this test appeared to approach the toxic dose of the seeds.

**Pods.**

Ewe Lamb No. 4—Fed finely ground, air-dried pods at the rate of 1.10 pounds per hundred weight of animal; equivalent to 4 pounds of fresh material. Given at 7:35 a.m. At 7:00 p.m. a slight uneasiness was noted. Temperature at this time, 105°. The following morning the animal appeared normal in every way.

Ewe Lamb No. 5—Fed 1.14 pounds of finely ground, air-dried pods per hundred weight of animal. Equivalent to 4.2
pounds of fresh pods. Given at 3:30 p.m. Slight uneasiness noted next morning. Maximum temperature recorded at 7:00 p.m. was 105°. This animal ate hay more or less from the time of the feeding until fully normal. The results indicate that the animal was slightly affected.

Ewe Lamb No. 6—Fed 1.2 pounds of finely ground, air-dried pods per hundred weight of animal. Equivalent to 4.4 pounds of fresh pods. Started feeding at 5:30 p.m. and completed at 6:00 p.m. Initial temperature, 103°. Maximum temperature of 105 3/5° noted at 7:00 p.m. following day. This ewe seemed dull and depressed, the ears were slightly drooped and there was noted some champing of the jaws. Results in this case interpreted as positive, although at no time was the animal seriously affected.

Leaves.

Ewe Lamb No. 7—Fed .88 pounds of air-dried leaves per hundred weight of animal at 5:30 p.m. Initial temperature, 102 4/5°. This quantity was equivalent to 4 pounds fresh leaves. No appreciable change was noted in the temperature for the following twenty-four hours. No toxic symptoms were noted.

DISCUSSION OF FEEDING RESULTS

The material fed was given in some cases by large capsules and in others by the use of a metal pump. Naturally some material was lost during the feeding and the figures given for quantities fed must be looked upon as approximate.

Attempts to have the sheep eat the lupine voluntarily proved fruitless. It is known that when sheep are poisoned on the range the fruits are eaten more or less exclusively, and, of course, in a comparatively short period. So in order to simulate the time of action, forced feeding was followed.

Additional experimental feedings must necessarily be made to determine the exact toxic and lethal dosage of lupine under corral conditions. Our work, while preliminary to further feeding tests, indicates that the equivalent of 3 pounds of fresh seeds (computed from original water content) per hundred weight of sheep, failed to seriously affect the animals, although positive symptoms were noted.
Fig. 7. One illustration of what sheep will browse upon under trailing conditions. *Rudbeckia occidentalis* (Nigger's thumb; cone flower). This plant is very coarse and bitter; not ordinarily eaten.
The equivalent of 4.4 pounds of fresh pods (computed from original water content) per hundred weight of sheep gave definite symptoms of poisoning, although at no time was the animal dangerously affected.

Based on the original water content four pounds of leaves per hundred weight of sheep failed to give the slightest suggestion of toxicity.

Referring again to the weight relationship of the lupine fruit, i.e., seeds and pods (20% and 80%, respectively), and taking the minimum dosage of pods as 4.4 pounds per hundred weight of animal. Then it is evident that in the natural condition of the plant, 4.4 pounds of fruit by weight would consist of .88 pounds of seeds, and 3.52 pounds of pods. The seeds, according to the preliminary feeding tests, have roughly twenty-five per cent greater toxicity than the corresponding green pods, so that one would infer that the minimum toxic dose of whole fruit would be somewhat less than 4.4 pounds per hundred weight of sheep.

It is the opinion of the author that the minimum dosage suggested by the preliminary feeding tests is in excess of that ordinarily eaten by sheep on the range. Further information is needed regarding the toxic dosage of the silvery lupine in order to more satisfactorily explain the apparent discrepancy between corral-fed sheep and actual range observations.

CHEMICAL STUDIES

Alkaloidal Assays.

The alkaloidal assays of the poisonous parts of the silvery lupine have not been satisfactory. This is, in part, due to the fact that chloroform, a necessary solvent, sometimes combines with one of the alkaloids forming an acid alkaloidal salt. Consequently, many assays are reported too low because of the neutralizing action of the acid salt. This reaction is more likely to occur with the pod assays. At this time it is not known whether this reaction is due to the varying composition of the plants from year to year or to methods used in the separations. Plant collections made in 1922 and 1923, and later examined, were found to yield comparatively large quantities of the acid alkaloidal salt. In
1924 both seeds and pods failed to yield the same product as obtained previously, although treated in the same manner.

Samples collected August 15, 1923, and consisting largely of the fruits (not mature) gave alkaloidal contents ranging from 0.104% to 0.211%. Because of these discrepancies repeatedly obtained, no attempt is made in this bulletin to present the correct alkaloidal values of this species of lupine.

Alkaloids of the Silvery Lupine.

The alkaloids so far isolated from the silvery lupine were obtained by the following general procedure: Ground, air-dried material extracted with eighty-five per cent alcohol (acidulated) until exhausted; concentrated from alcohol diluted with water and washed with petroleum ether, ethyl ether and ethyl acetate; then alkaliized with dilute sodium hydroxide and agitated with ethyl either until no further alkaloidal matter was separated. The washing was next followed by chloroform, using a motor agitator for the extraction. After repeated (usually five) agitations, the mother liquor was found to be quite free from alkaloids.

Ether Washings.

For the most part ether removes from the mother liquor only the oily alkaloidal constituents. From the point of view of toxicity, the oily fraction is the most toxic of all the alkaloids present. On an average .020 grams per kg. of rabbit injected intravenously is fatal.

The oily fractions could not be distilled even under low pressures, being unstable and undergoing decomposition by such treatment.

Reference has been made to the fact that about forty per cent of the lupine seeds are irregular and undersized. However, no appreciable difference was found in the total alcoholic extract or the ether washings of the two batches of seed (separation made by sieving).

The toxicity tests of the oily fractions obtained from the ether washings have been reported in Bulletin No. 125.
Chloroform Washings.

By following up the extraction of the mother liquor with chloroform, practically all of the remaining alkaloidal constituents are removed by this solvent. As stated above, it has been in these washings that variable results have been obtained. Plant collections made in 1919, 1922, and 1923, gave a white crystalline compound having an acid reaction. It was found to be non-lethal* to guinea pigs in small doses. The pigs recovered with only slight discomfort. The effect was quite similar to a non-lethal dose of aconite. The same compound treated with sodium carbonate yields a free crystallizable base, melting at 147-8° C. Eighty milligrams introduced intravenously was without effect on a rabbit weighing 2 kilograms.

Plants collected from the same general area in 1924 gave, upon similar treatment, a colorless crystalline base, insoluble in water and melting sharply at 121-2° C. This compound had not been obtained before by the author. It occurred much more abundantly in the pods than in the seeds; only a trace was found in the leaves. Preliminary tests indicate that it is not lethal in small doses. A rabbit weighing 2 kilograms received intravenously 0.080 grams October 24th. No bad effects followed until the morning of the 28th, when the rabbit died. The circumstances lead one to believe that possibly the death was due to a delayed action of the drug.

Results of a more detailed study of the composition and toxicity of the alkaloids of the silvery lupine is to be published elsewhere.

Comparative toxicity tests of the various alkaloidal fractions show the oily portions to be far more potent than the solid crystalline substances. The latter undoubtedly occur in quantities sufficient to exert some toxic effect, but at this time data for definite conclusions are not available.

Non-toxic fatty substances.

The fatty substances occurring in the fruits of the silvery lupine are very difficult to properly separate and identify. Likewise, to express quantitative relationships of the more commonly

*Tests made by Parke, Davis & Company.
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occurring compounds leads at best only to an approximation. To
discuss this phase of the problem here seems, perhaps, to be too
technical to be included, hence methods of procedure and physical
and chemical data are withheld for another special report.

Because of the apparent attractiveness, many times, indicated
by these fatty substances, it occurred to the author that it might
be of interest to give a list of the important ones known to be
present. It should be kept in mind, however, that these substances
are not at all new products. Those listed, and, of course, many
more, have been associated with certain plants for a long time.

In the group, chemically speaking, of solid fatty acids, two
compounds were found to predominate, viz., palmitic and stearic.
The wide occurrence and nutritional value of these substances
needs no elaboration. The group of liquid fatty acids consists
largely of three, viz., oleic, linoleic, and linolenic. The second and
third members of this series are perhaps not as familiar as the
first one, although fairly well distributed in the plant kingdom.

These substances, then, constitute the bulk of the fatty mat-
ter in the fruit of the lupine, and, as indicated, play an important
part in certain areas in supplying a desirable food supplement.
Of course, when eaten in excess, i. e., more or less exclusively at
one feeding, some poisoning is likely to result, from the alkaloids
known to be present.
SUMMARY

I. An earlier bulletin of this station (No. 125) reported on the silvery lupine with respect to the occurrence, distribution, kind of animals affected, physiological action of liquid alkaloids:

II. Preliminary feeding tests indicate that with corral-fed sheep, the equivalent of 4.4 pounds of fresh pods (seeds not included) per hundred weight of animal induced toxic effects; approximately 3 pounds of fresh seeds (equivalent) per hundred weight of animal gave positive lupine symptoms.

III. The silvery lupine is not found in the high mountains, consequently several other poisonous species occurring at the higher elevations are responsible for stock losses.

IV. The large quantities required to produce toxic symptoms make it clear that loss of sheep from lupine poisoning can be greatly cut down if the sheepmen will:

(a) learn to know the poisonous species of lupines, and

(b) take steps to keep the sheep from grazing on them exclusively long enough to get more than a pound of the fruits.

V. The most poisonous part of the silvery lupine is the fruit (pods with inclosed seeds).

VI. The toxicity of the silvery lupine is largely due to the oily or liquid alkaloids, rather than the crystalline forms.