

BULLETIN NO. 112

JANUARY, 1917

112
1917

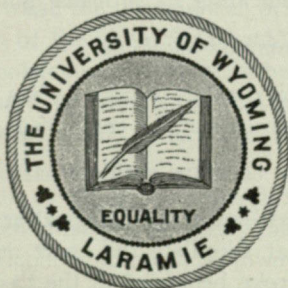
UNIVERSITY OF WYOMING
AGRICULTURAL
EXPERIMENT STATION

LARAMIE, WYOMING

The Poisonous Properties of the
Two-Grooved Milk Vetch

(*Astragalus bisulcatus*)

By O. A. BEATH and E. H. LEHNERT



Bulletins will be sent free upon request

Address: Director Experiment Station, Laramie, Wyoming

SUMMARY.

- I. Milk vetch has been proved to be poisonous to cattle. Suspicion is held regarding its effect upon sheep.
- II. Eighty to ninety per cent of the animals affected die.
- III. Water easily removes the active poison from green or air-dried material.
- IV. All parts of the plant contain poison with a slight excess in the leaves.
- V. The poison is neither precipitated by basic acetate of lead nor decomposed at the boiling point of water.
- VI. It can be deprived of its toxicity by boiling with dilute acids (indicating its probable glucosidic character).
- VII. The poison is non-alkaloidal.
- VIII. A definite crystalline substance has been isolated, giving chemical reactions common to glucosides.
- IX. Thus far no chemical antidote has been obtained.
- X. As indicated by the physiological action of the poison, drugs that stimulate the heart and nervous system should prove beneficial in the case of vetch-poisoning.

The Poisonous Properties of the Two-Grooved Milk Vetch

(*Astragalus bisulcatus*)

PART I. CHEMICAL.

BY O. A. BEATH.

INTRODUCTION.

A brief account of the two-grooved milk vetch (*Astragalus bisulcatus*) is put in the form of a preliminary bulletin at this time, largely to warn stockmen of its poisonous nature. Authorities on poisonous plants make no mention of this particular vetch as being suspected of producing toxic effects. As a consequence it is safe to conclude that certain conditions undoubtedly prevail which cause it to be extremely variable in its toxicity. Moisture unquestionably influences the activity of the toxic principle, inasmuch as it is readily soluble in water. Certain stockmen have used this vetch for forage purposes without any apparent ill effects, thereby illustrating the small degree of danger when the plant is well dried.

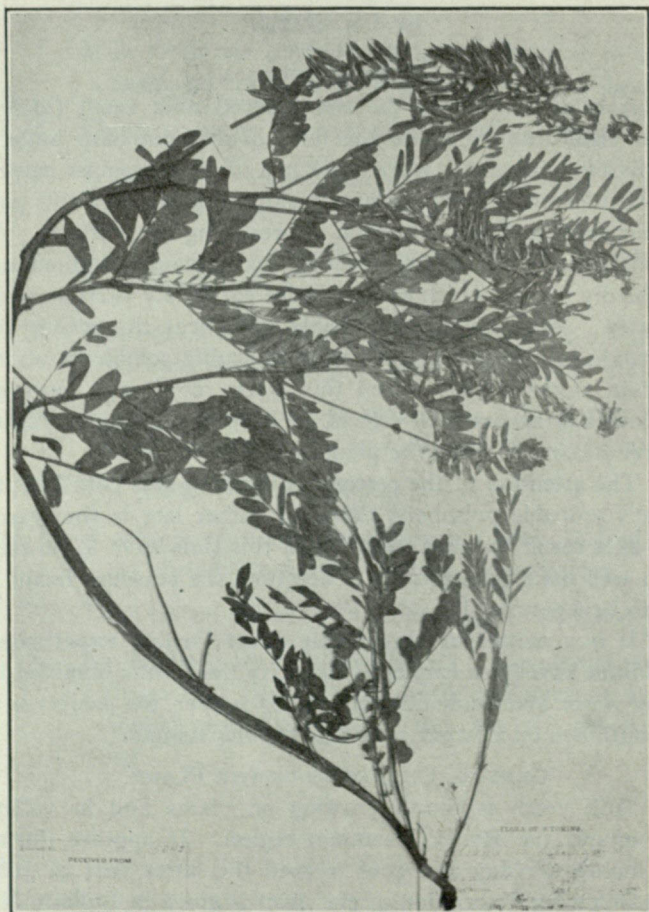
The attention of the research laboratory was called to the plant's probable poisonous character rather late in the season and as a result the data available at this time have to do only with well matured material. Therefore, the seasonal factor is yet to be determined.

It is a matter of regret that direct feeding experiments could not have been employed to verify the results obtained by using water and alcoholic extracts. However, the project is to be continued on a larger scale this coming summer.

GENERAL CHARACTER OF THE PLANT.

Milk vetch is found growing on plains and in valleys throughout the Rocky Mountain region. It appears during the month of May and goes to seed the latter part of July. The following description of the plant is given in Bulletin No. 76 of the Wyoming Experiment Station:

This is one of the large, coarse vetches, usually growing in dense clumps and becoming 2 to 3 feet high. It produces purple blossoms in profusion and these are followed by an abundance of pods from one-half to nearly an inch long and having two rather deep furrows on the upper side. The plant is rather rank scented, especially when young, and, unless other forage is scarce, is not browsed down until the pods begin to mature.



The Two-Grooved Milk Vetch.

All parts of the plant contain poison apparently quite evenly distributed throughout.

Data gathered from stockmen would indicate that this species of vetch is poisonous to cattle and sheep. Nothing is known in regard to its effect on horses. The laboratory has obtained no direct evidence that sheep have been killed by eating it, although a number of suspicious cases are on record.

During July, 1916, E. B. Foster of Casper reported a loss of nine calves and ten cows out of a herd of forty-one head. He states, "No two acted in the same way. However, all evidenced signs of craziness. On some the effect would hardly be noticed until one went near them, and then they would suddenly act frightened and in attempting to get away would stumble and fall or have a fit." All animals which showed signs of milk-vetch poisoning died.

EXPERIMENTAL.

The plant material submitted for investigation was secured from Mr. Foster of Casper, Wyo. Green plants could have been obtained in the vicinity of Laramie, but it seemed advisable to deal with those authentically known to have given trouble.

After drying, the seeds, pods, leaves, and stems, respectively, were coarsely ground and placed in air-tight containers.

Test for Hydrocyanic Acid.

Five hundred grams of air-dried material were placed in a flask and saturated with water. Steam was then passed into the flask for a period of three hours. The distillate gave negative results for hydrocyanic acid. Tests were made upon each part of the plant. The residue remaining in each case was acidified with strong sulphuric acid and again treated as above. Negative results were obtained for combined hydrocyanic acid.

The distillates had an odor particularly disagreeable. The distillate from the seeds yielded a small amount of volatile oil which proved to be non-toxic in character.

Test for Active Enzymes.

One hundred grams of finely ground leaves and stems were digested with three liters of water at a temperature of 37.5° C. for a period of 48 hours. The aqueous portion was separated and mixed with an excess of alcohol. A profuse precipitate was formed which soon settled to the bottom of the flask. The alcohol was removed and the solids remaining were washed with a little cold alcohol and subsequently dried. A portion added to a dilute solution of amygdalin failed to produce hydrolyses to any appreciable extent. This indicates the absence of *Beta*-glucose or allied enzymes capable of hydrolyzing *Beta*-methyl glucosides. Not having any representative of the *Alpha*-methyl glucosides the presence of *Alpha*-glucose could not be ascertained.

Water Extracts.

In carrying out the work of determining the character of milk vetch it was quickly demonstrated that water was a better solvent for the removal of the poisonous principle than alcohol, ether, chloroform, or acidulated water.

The concentrated water extracts were invariably dark, resembling plant resins obtained by alcoholic percolation. Residues obtained by alcoholic treatment were dark and in general did not differ in appearance from those derived by maceration with water. If to a concentrated alcoholic extract a large volume of water was added, a bulky grayish precipitate was thrown down. This, upon closer examination, proved to be calcium sulphate.

With the exception of three extracts, all were given internally to full-grown rabbits. The three administered otherwise were given to an old ewe, a yearling calf, and a female dog.

The extracts used in the tests were prepared as follows:

No. 1.

Seeds and pods (dry)	3 oz.
Alcohol (95%)	10 oz.

Mixture digested 24 hours at a temperature of 37.5° C., concentrated to small bulk and diluted with 2 oz. of water.

No. 2.

Leaves (dry) 3 oz.

Alcohol (95%) 10 oz.

Treated the same as No. 1.

No. 3.

Seeds and pods (dry) 3 oz.

Water 15 oz.

Digested for 24 hours at 37.5° C. Aqueous portion separated and concentrated to 2 oz.

No. 4.

Leaves and stems (dry) 46 oz.

Water 350 oz.

Same treatment as No. 3, concentrated to 75 oz.

No. 5.

Stems (dry) 6 oz.

Water 12 oz.

Treated as in No. 3, concentrated to 5 oz.

No. 6.

Leaves and stems (dry) 87 oz.

Water 150 oz.

Same treatment as in No. 3, concentrated to 25 oz.

No. 7.

Whole plant (dry) 22 oz.

Water 150 oz.

Digested 48 hours at 37.5° C. Aqueous portion separated and concentrated to 25 oz. Treated with slight excess of basic lead acetate. Lead precipitate removed, washed, and suspended in large volume of water. Lead precipitated as lead sulphide. Remaining solution concentrated to 5 oz. Labeled Solution "A".

That portion of the original water extract not precipitated by basic lead acetate was treated with hydrogen sulphide to remove lead and concentrated to a volume of 5 oz. Labeled Solution "B".

No. 8.

Leaves, stems, and pods (dry) 3 oz.
 Water 10 oz.

Digested 48 hours at the boiling point of water. Filtered. Concentrated to 2 oz.

No. 9.

Leaves, stems, and pods (dry) 3 oz.
 Water 10 oz.
 Sulphuric acid (5%) 1 oz.

Digested 48 hours at 90° C. Filtered. Sulphuric acid removed. Solution concentrated to 2 oz.

Results as to Activity of Extracts.

Extract No. 1. Volume, 2 oz.

Given internally to full-grown rabbit at 11 a. m. No symptoms at 5 p. m. Died during the night.

Extract No. 2. Volume, 2 oz.

Given to full-grown rabbit at 11 a. m. No symptoms at 5 p. m. Died during the night.

Extract No. 3. Volume, 2 oz.

Given internally to full-grown rabbit at 11 a. m. Decided symptoms at 1:30 p. m. Restoratives administered. Died at 6 p. m.

Extract No. 4. Volume, 75 oz.

Given by drench to an old ewe October 4, at 2:45 p. m. No symptoms indicated.

Extract No. 5. Volume, 5 oz.

Given by drench to dog at 10 a. m. Decided symptoms of nausea at 10:30 a. m. The dog vomited repeatedly with marked straining. In a few hours the symptoms disappeared.

Extract No. 6. Volume, 25 oz.

Given to yearling calf October 31, at 11 a. m. No symptoms observed until the following day, at 9:45. Animal was unable to rise, and had a temperature of 94.5° F., pulse 74, respiration 12. Died at 2 p. m., November 1.

Extract No. 7. Volume, 5 oz.

Solution "A" given to full-grown rabbit at 1 p. m. Died at 6 p. m. Solution "B" given to rabbit at 1:30 p. m. No indications of poisoning.

Extract No. 8. Volume, 2 oz.

Given internally to full-grown rabbit at 11 a. m. At 1 p. m. the usual symptoms were observed, such as non-co-ordinated movements and loss of control. Rabbit died at 4 p. m.

Extract No. 9. Volume, 2 oz.

Given internally to full-grown rabbit at 11 a. m. No symptoms indicated.

Conclusions.

1. The data so far obtained indicate that a definite active poison is present.

2. In no case did a rabbit live when given freshly prepared extracts.

3. Although the old ewe showed no effects from a dose of extractive material equivalent to fifteen pounds of the green plant, yet it is not safe to presume from this experiment that the vetch is not poisonous to sheep, for (1) only one sheep was tested and (2) green plants may produce an effect different from that of the extract of the dried ones.

4. On account of the ease with which a dog can empty its stomach by vomiting when irritants are given, we have concluded that in our experiment with the dog not enough of the poison was absorbed to manifest itself.

FUTURE WORK.

Having studied the general nature of the active poison, our next step will be to conduct feeding experiments, typifying range conditions as far as possible, and to study the problem of milk vetch poisoning from the point of view of symptoms and specific antidotes.

PART II. PHYSIOLOGICAL.

BY E. H. LEHNERT.

HOW THE POISON ACTS.

From experiments carried on with extracts and other preparations from this plant, our observations both before and after death have led to a few definite conclusions. When sufficient poison to cause death is introduced into the system, whether through the stomach or intravenously, it acts quickly, that is it is quickly absorbed, and very soon produces symptoms of paralysis, by its action on the nervous system; the action of the heart is also interfered with, and the poison no doubt kills by acting on this organ. When the poison is administered by the stomach, postmortem examination shows that digestion is stopped immediately, as invariably this organ remains full of food, indicating that the poison is quickly absorbed from the stomach and that the stomach is paralyzed.

REMEDIES AND METHODS OF TREATMENT.

Attempts at determining an antidote for the poison were made, and, although few in number, certain conclusions have been reached. Thus far no chemical antidote has been obtained, but from the obvious action of the poison on the heart and nervous system as a depressant, antidotes of a physiological nature would be those that stimulate these organs, such as alcohol, ammonia, strychnine, nitro-glycerine, and digitalis, belladonna or atropine. In our experiments we were able to counteract the poison for a considerable time, although in no case were we able entirely to neutralize its action. It appears, however, very probable that the remedies mentioned should prove very valuable where the amount of poison taken into the system is not too great.

In the administration of remedies in case of poisoning, the following doses and methods would apply: Immediately give to full-grown animal (cow), as a drench, 1 to 2 lbs. of

Epsom salts, dissolved in water; vary dose with size, as in all cases the doses given are for mature animals.

Alcohol should be administered in the form of whiskey, brandy, or gin, in 2 to 4-oz. doses, diluted with an equal quantity of water, the dose repeated every hour.

Aromatic Spirits of Ammonia is one of the best quick-acting heart stimulants, and should be given once an hour in two-ounce doses, diluted with three volumes of water.

Belladonna fluid extract may be used as a heart and nerve stimulant in hourly doses of 2 drachms in a little water. This drug would work well combined with either whiskey or aromatic ammonia.

Digitalis. The tincture digitalis may be given, diluted with water, in 2 to 3 drachm doses every 3 to 4 hours.

For quick action, drugs should be administered hypodermatically, that is, injected under the skin. For this purpose the drugs are very concentrated and put up in tablet form, very soluble in water, and should be administered with the hypodermatic syringe, of which there are many varieties on the market at prices from \$2.00 up. For hypodermatic uses in poisoning by vetch, I would suggest a compound tablet consisting of strychnine, nitro-glycerine, and digitalin or atropine, the quantity of strychnine being not over 2 grains; the quantities of other ingredients will not matter materially, as the compounders put them up in the right proportion to give best results. In using the hypodermatic remedy alone, it should be administered once an hour, using clean boiled water to dissolve the tablet, and disinfecting the skin at the point of puncture with any common disinfectant or tincture of iodine. In the majority of cases the worst stages should be over in 3 to 4 hours, and if the vital organs can be kept active during this period, recovery is possible.

UNIVERSITY OF WYOMING

Agricultural Experiment Station

LARAMIE

BOARD OF TRUSTEES

Officers

TIMOTHY F. BURKE, LL. B.....	President
MARY B. DAVID.....	Vice President
C. D. SPALDING.....	Treasurer
FRANK SUMNER BURRAGE, B. A.....	Secretary

Executive Committee

A. B. HAMILTON	T. F. BURKE	W. S. INGHAM
----------------	-------------	--------------

<i>Appointed</i>	Members	<i>Term Expires</i>
1911.....	HON. ALEXANDER B. HAMILTON, M. D.....	1917
1911.....	HON. LYMAN H. BROOKS.....	1917
1913.....	HON. CHARLES S. BEACH, B. S.....	1917
1895.....	HON. TIMOTHY F. BURKE, LL. B.....	1919
1913.....	HON. MARY B. DAVID.....	1919
1914.....	HON. MARY N. BROOKS.....	1919
1911.....	HON. W. S. INGHAM, B. A.....	1921
1913.....	HON. C. D. SPALDING.....	1921
1915.....	HON. J. M. CAREY, LL. B.....	1921
EDITH K. O. CLARK, State Superintendent of Public Instruction.....		Ex Officio
PRESIDENT C. A. DUNIWAY, Ph. D., LL. D.....		Ex Officio

STATION COUNCIL

C. A. DUNIWAY, Ph. D.....	President
HENRY G. KNIGHT, A. M.....	Director and Agricultural Chemist
F. S. BURRAGE, B. A.....	Secretary
C. D. MOIR.....	Clerk
A. NELSON, Ph. D.....	Botanist and Horticulturist
F. E. HEPNER, M. S.....	Research Chemist
J. A. HILL, B. S.....	Wool Specialist
J. C. FITTERER, M. S., C. E.....	Irrigation Engineer
A. D. FAVILLE, M. S.....	Animal Husbandman
T. S. PARSONS, M. S.....	Agronomist
KARL STEIK, M. A.....	Engineering Chemist
J. W. SCOTT, Ph. D.....	Parasitologist
O. A. BEATH, M. A.....	Research Chemist
P. T. MEYERS, B. S. A.....	Assistant Agronomist
E. H. LEHNERT, D. V. S.....	Veterinarian