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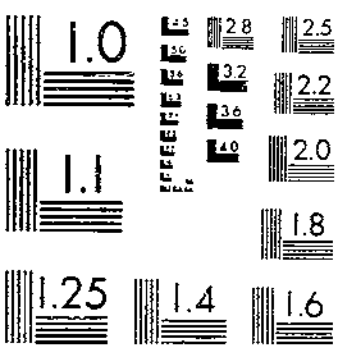

MICROCOPY RESOLUTION TEST CHART
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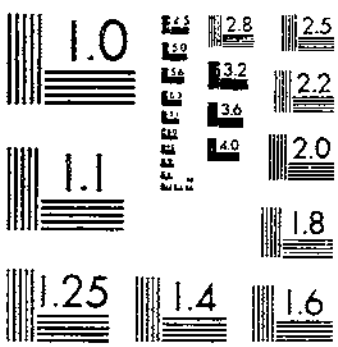

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NATIONAL BUREAU OF STANDARDS-1963-A

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WASHINGTON, D. C.

WILD TOBACCOS (NICOTIANA TRIGONOPHYLLA DUNAL AND NICOTIANA ATTENUATA TORREY) AS STOCK-POISONING PLANTS

By C. DWIGHT MARSH, *Associate Physiologist in Charge of Investigations of Stock Poisoning by Plants*, A. B. CLAWSON, *Associate Physiologist*, and G. C. ROE, *Junior Physiologist, Pathological Division, Bureau of Animal Industry*

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HISTORICAL SUMMARY

The possibility that *Nicotiana trigonophylla* may produce poisoning in livestock was first brought to the attention of the Department of Agriculture by F. L. Schneider, Bureau of Animal Industry inspector in charge, at Albuquerque, N. Mex., in a letter written November 10, 1923. He sent, for determination, a specimen of the plant, which T. W. Crump, of his force, had thought to be the cause of some losses of cattle. While presumably all the species of tobaccos are poisonous, there were no recorded cases of livestock having been poisoned by this plant. Schneider, therefore, was asked for further details in regard to the supposed cases of poisoning.

In reply he sent a letter, written by Doctor Crump, December 13, giving the data which led him to suspect that this plant caused the death of the animals. He had visited a locality near Holbrook, Ariz., where he found about 12 head of cattle dead in an area which did not exceed a quarter section. An examination showed two small buttes around the bases of which *N. trigonophylla* was growing. Much of the plant had been eaten to the ground.

One sick cow seemed to be paralyzed in the fore quarters, but otherwise had no marked symptoms. She was killed for an autopsy, which was negative. An uneaten patch of the plant was marked, and the cattle in the vicinity were driven away about a mile. On visiting

the locality the next day it was found that the cattle had come back and eaten the tobacco on the marked patch.

Two yearling steers were found about 50 yards from the place. One was lying down, and on being approached attempted to rise. He got upon his feet with great difficulty, shaking and shivering all over. The eyes were staring, the head jerked from side to side, and there were severe, clonic spasms of the muscles of the neck and shoulders. He staggered a short distance and fell. The other steer attempted to rise, but was unable to get upon his feet. He had been vomiting during the night. Breathing was slow and labored and the pulse rapid and weak. There was muscular twitching all over the body, and he rolled his eyes back at times until only the whites were visible. There was some salivation. None of the animals were bloated. In the same neighborhood where the cattle were poisoned Crump found a number of skeletons of sheep, which presumably had been killed by the same plant.

While there were no published cases of poisoning from eating *N. trigonophylla*, Crump's experience seemed so definite that it appeared wise to undertake some experimental work. As the plant is widely distributed in the Southwest, it seemed a probable source of danger to livestock.

Another loss was reported in a letter from F. L. Marney, of St. Johns, Ariz., who stated that he thought a loss of 30 head of cattle was caused by the plant.

In 1925 the United States Forest Service, in correspondence from Supervisor O. F. Arthur, of the Lincoln National Forest, N. Mex., was informed that local stockmen considered this plant poisonous to cattle and that a stockman named York, several years before, had corralled a cow and fed her upon this plant with fatal result.

Although nothing in regard to the poisoning of livestock by *N. trigonophylla* has been published, there have been statements in regard to stock poisoned by other species of tobacco. The literature in regard to ordinary tobacco, *N. tabacum*, is very extensive, and it is not necessary to quote the data in detail. A large part of the investigative work, however, has been done on extracts of tobacco and on the extracted nicotine. There have been many cases of poisoning of domestic stock by the administration of tobacco juice and by the use of tobacco extracts as dips. Only a few cases of the poisoning of animals by eating the tobacco plant have been recorded. These cases, however, correspond in their symptoms to the poisoning cases due to the extracts. The fact that there have been few cases of poisoning from eating the plant is, doubtless, explained by the unpalatability of the plant itself, so that livestock do not ordinarily eat it unless very hungry.

In regard to the species *N. suaveolens*, which occurs in Australia, fairly positive data have been accumulated. Apparently the first reference to the poisonous properties of this species was by Bancroft in 1887 (*2*).[1] He states that an alkaloid has been separated and that the plant is most poisonous. He does not, however, report any experimental work on animals.

[1] Figures in parentheses (italic) refer to "Literature cited," p. 22.

An article in the Veterinary Journal (*7*) states that in certain districts in New South Wales a large percentage of the horses became affected with blindness and that careful investigation, followed by experimental work, showed that the blindness was caused by the native tobacco, *N. suaveolens*. The author draws the parallel between the symptoms exhibited by the horses and human amblyopia—dimness of vision—produced by the excessive smoking of tobacco.

Husemann (*9*) gives a somewhat extended review of this work on horses. Kirk (*10, p. 227*) says that the plant is poisonous to stock and that the effects on sheep are drooping head, dull eyes, swollen tongue, and paralysis of the loins. Stewart (*16*) gives brief details of the poisoning of cattle by *N. suaveolens*. In one case he states that of 1,095 cattle, 100 were sick and 4 died. He recommends the eradication of the plant. Maiden (*11, p. 32*) states his belief that this plant is responsible for the death of large numbers of sheep. Cleland and McDonald (*4, p. 738–739*) report the experimental feeding of sheep, with no poisonous effects. The feeding was continued for a considerable period. It appears that the plant, which grows widely in Australia, is generally recognized as more or less poisonous to stock.

N. glauca, or "tree tobacco," grows in the southwestern part of the United States, from Texas to southern California, its range extending into Mexico.

Ewart and Tovey (*7, p. 45, 83*) speak of *N. glauca* as poisonous in Australia, stating that it causes blindness in livestock. Walsh, in 1909, (*17, p. 47–48*), writing of the South African plants, says that this plant has poisoned cattle, and is especially poisonous to ostriches. In one case, of a bunch of about 50 ostriches, nearly two-thirds were killed. Schneider (*13, p. 125*) says that it is "said to have caused the fatal poisoning of cattle that eat it, only, however, when other food was scant." Salisbury (*12, p. 79*) speaks of the plant as very poisonous in Australia. In 1916 Cleland (*3, p. 220*) quotes other authors, especially in regard to its poisonous effects upon ostriches, but adds no new facts.

In 1924 Standley (*15, p. 1378*) says "This plant is abundant in some parts of Mexico. It is reputed to be very poisonous."

In correspondence with the United States Department of Agriculture, Mrs. Belle Miller, of Saluda, N. C., wrote that she knew of serious losses of cattle from this plant in San Luis Obispo County, Calif., several years before.

The foregoing statements cover most of the facts which have been published in regard to the poisonous effects of the wild species of Nicotiana upon livestock in the United States.

TOBACCO, NICOTIANA[2]

DESCRIPTION OF THE GROUP

The tobaccos are rank, acrid-narcotic, mostly sticky-hairy herbs (one shrub), annual or perennial. The leaves are alternate, entire or wavy margined. The flowers are in the terminal or branched racemes, funnelform or salverform, five lobed. The five stamens are inserted in the tube. The calyx is persistent, inclosing the fruit or capsule. The capsule is two celled (except *N. quadrivalvis*), many seeded.

[2] This description was prepared by W. W. Eggleston, of the Bureau of Plant Industry.

FIG. 1.—*Nicotiana trigonophylla*, showing plant with leaves, blossoms, and fruit. This plant, growing in the Southern States, is from a foot to more than 3 feet in height

Several of these species were cultivated by the Indians. *N. rustica*, of the Eastern States, and *N. quadrivalvis*, of the Northwest, are known only in cultivation or as "escapes" from cultivation.

KEY TO THE SPECIES OF WILD TOBACCOS OF WESTERN UNITED STATES

Shrub or small tree; smooth; whitened; flowers yellow. Introduced from Argentina ..... *N. glauca.*
Annual herbs; sticky-hairy; strong scented; flowers white to greenish.
  Leaves clasping, eared at base; flowers open during day.
    Leaves sharp to blunt-pointed, flowers about one-fourth inch broad; flower tube somewhat compressed at throat ..... *N. trigonophylla.*
    Leaves taper pointed; flowers about one-half inch broad; flower tube open at throat ..... *N. palmeri.*
  Leaves not clasping; flowers opening at night.
    Pod two celled.
    Flower limb about one-half inch broad.
      Calyx lobes very unequal, the longer twice the length of the tube ..... *N. clevelandi.*
      Calyx lobes subequal; shorter than the tube ..... *N. attenuata.*
    Flower limb about 1 inch broad ..... *N. bigelovii.*
    Pod four or more celled ..... *N. quadrivalvis.*

DESCRIPTION AND DISTRIBUTION OF N. TRIGONOPHYLLA

An erect, simple-stemmed, or branched, sticky-haired, annual, 1 to 3 feet high. The leaves are sessile, or the lower leaves have winged, clasping, eared stalks, 2 to 5 inches long broadly oblong to egg-shaped; the upper leaves are oblong to lance-shaped, sharp or blunt at end. The flowers are in racemes or branched racemes, greenish white or yellowish, opening in daytime, salverform, three-fourths of an inch long. The calyx teeth are sharp, lance-shaped, one-half as long as the flower tube.

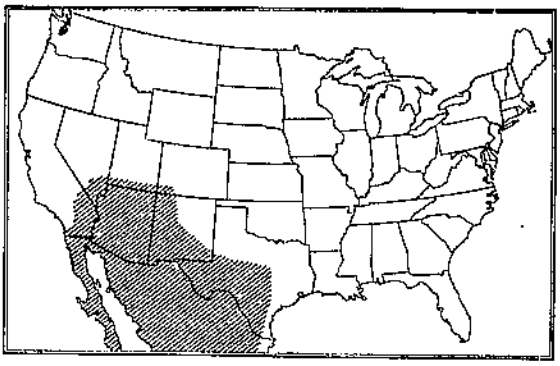

FIG. 2.—Distribution of *Nicotiana trigonophylla*

N. trigonophylla is found in desert and semidesert valleys along the washes in central Texas (Austin), southwest Colorado, southern Nevada, southern California, and south to Central America. Figures 1 and 2 show the plant and its distribution, respectively.

DESCRIPTION AND DISTRIBUTION OF N. ATTENUATA

An erect, branching, sticky-hairy, annual, 1 to 4 feet high. The leaves are stalked, egg-shaped to lance-shaped, long pointed 1½ to 4 inches long. The numerous flowers are in branched racemes, night flowering, salverform, 1 to 1½ inches. The calyx teeth are triangular, lance-shaped, one-third as long as flower tube.

N. attenuata is found in dry, sandy, stream beds and flats from Park County, Colo., to Grant County, N. Mex., and southern California. Figures 3(*a*) and 3(*b*) show the plant, and Figure 4 its distribution.

The plant is known in California as coyote tobacco. The Klamath Indians, according to Coville (*6, p. 104*), called it "käch-kul." Old inhabitants of Utah report that the Indians there called it "quop" or "toquop."

EXPERIMENTAL WORK ON N. TRIGONOPHYLLA

PRELIMINARY CHEMICAL EXAMINATION

A few grams of the dried and powdered whole plant of *N. trigonophylla*[3] were macerated with Prolius' solution overnight, and the extract gave a positive test for alkaloids with Mayer's reagent. A larger quantity of the material, 900 grams, was next moistened with 95 per cent alcohol containing some acetic acid and percolated with alcohol, about 4 liters in all. The resulting extract was neutralized with ammonia and concentrated to small bulk by distillation in vacuo and the residue taken up with dilute acetic acid. This was made strongly ammoniacal and shaken out with chloroform, three portions of that solvent removing the alkaloid completely. There remained after volatilizing the solvent a dark-brown oil with green fluorescence and pyridine odor. It gave a crystalline picrate melting at 218° C., a white precipitate with gold chloride, and an orange-colored crystalline precipitate with platinum chloride. The characteristic picrate is sufficient to identify the alkaloid as nicotine. The quantity of nicotine in the plant was determined, using the silicotungstic method of Shedd (*14*), and found to be 0.39 per cent. As compared with some other analyses of unfermented tobacco plants, this indicates a rather low content of nicotine. That alkaloid is an extremely toxic compound, and its presence in *N. trigonophylla* should make that plant an object of suspicion in cases of stock poisoning where it is abundant.

FIG. 3(*a*).—*Nicotiana attenuata*, showing growing plant with leaves and flowers. This plant grows from 1 to 4 feet in height and is found from British Columbia to California and New Mexico. It is known in California as "coyote tobacco," called by the Klamath Indians "käch-kul," and by some of the Utah Indians "quop" or "toquop"

[3] This examination was made by O. F. Black, of the Bureau of Plant Industry.

FEEDING EXPERIMENTS

The feeding experiments were carried on at the Salina (Utah) Experiment Station during the summers of 1924 and 1925, part of the material used being collected near Flagstaff, Ariz., by Doctor Crump and part from Mount Graham, near Safford, Ariz., by W. W. Eggleston, of the Bureau of Plant Industry.

The animals used experimentally included cattle, sheep, chickens, and one horse.

Table 1 gives a summarized statement of the experimental work. All of the feedings were of air-dried plant, but it seemed best to state the dosage in terms of green plant. In making the computations 78 per cent was allowed for loss of moisture in drying.

FIG. 3(*b*).—*Nicotiana attenuata*, showing more clearly the form of the leaves and flowers

TYPICAL CASES OF POISONING

Cattle 998 and 1017 and sheep 868 may be considered as cases typical of the results of feeding *Nicotiana trigonophylla*.

Steer 998 was given by forced feeding, between 9.35 and 9.59 a. m., on August 26, 2 per cent, estimated as green plant, of its weight of ground material of leaves, buds, fine stems, and flowers moistened with water. This was given while the animal was in the chute, and the material was fed in 24 minutes. When the animal was released he walked unsteadily, wabbling from side to side, and showed great nervousness. In walking, he stumbled, and there was a tendency to drop on

the phalangeal portion of the forefeet. Trembling was soon noticed in the shoulder region. The pulse became very weak, and at 10.28 he was lying down, the whole body trembling. The eyes were staring. The third eyelid (nictitating membrane) was drawn back over the eye, and there was definite salivation. At 10.30 he was unable to get upon his feet. Whenever he attempted to get up the trembling was very violent, and he was forced to lie down. Figure 5 shows his condition at about the time he was almost completely prostrated. There was frequent urination, and his pulse was very weak. The weakness gradually increased. There was more or less dyspnœa, and he died about 2.30 p. m. The autopsy followed immediately, but no pronounced lesions were shown.

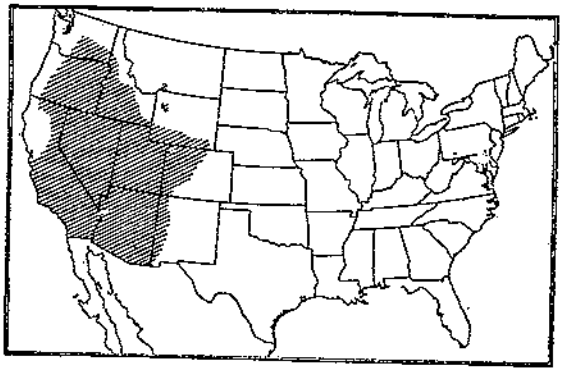

FIG. 4.—Distribution of *Nicotiana attenuata*

Heifer 1017, on September 16, received by forced feeding 0.75 per cent of its weight of the plant moistened with water. The feeding commenced at 10.30 in the morning and was finished at 10.40. Fifteen minutes later, symptoms of poisoning were noticed. The animal was very nervous, the muscles of the shoulder were twitching, the nictitating membrane was drawn over the eye, and confused vision was noticeable. Trembling in the rest of the body followed, becoming quite violent, and there was frequent urination. Figure 6 shows the animal at 11.18 a. m., September 16. The eyes were staring and partly covered by the nictitating membrane. The animal stumbled as she walked, moving with short, quick steps. There was some salivation and great weakness, although no complete prostration. The trembling continued for 22 hours, during which time the animal while walking appeared stiff-legged, and the pulse was very weak. From this time there was continued improvement, but symptoms indicating weakness continued during the day of September 21. After this she seemed to have recovered.

On August 6, between 10.43 and 10.58 a. m., sheep 868, a ewe about 4 years old, received 3 per cent of her weight of the plant moistened with water. Twelve minutes after the feeding was completed she began to show signs of weakness, stumbling as she walked, the weakness being especially pronounced in the forelegs. Trembling in the flank and in the shoulders followed, and there was a tendency to bend the feet backward from the fetlock joint and to walk on the anterior surface of the feet. The trembling continued and at 11.26 a. m. was very violent. The eyes were staring and there was slight salivation. The ewe showed considerable hyperesthesia. At 2.45

TABLE 1.—*Summary of feeding experiments with Nicotiana trigonophylla*

Animal		Date of feeding	Method of feeding	Part of plant used	Percentage of weight fed [1]	Place and date of plant collection	Result	Remarks
Designation	Weight							
Cattle:	*Pounds*	1924			*Per cent*			
1,031	457	Aug. 18	In hay	Leaves, buds, flowers, and fine stems.	0.93	Safford, Ariz., May 9, 1924.	Slight effect.	
1,005	608	Aug. 20	do	do	1.25	do	do	
995	350	Aug. 23	Balling gun.	do	2.00	do	Death	
1,005	618.5	Sept. 2	do	do	1.00	do	Very sick	
1,022	717	Sept. 8	do	do	.50	do	Slight effect.	
1,017	688.5	Sept. 10	do	do	.75	Flagstaff, Ariz., July, 1924.	Very sick.	
Sheep:								
817	41	July 3	do	do	.50	Safford, Ariz., May 9, 1924.	Not sick	Lamb.
819	41	July 5	do	do	1.00	do	do	Do.
830	45.75	July 7	do	do	1.50	do	Sick	Do.
874	78.5	July 9	do	do	1.50	do	Not sick	Mature sheep.
840	70	July 11	do	do	2.00	do	Sick	Do.
848	96.5	July 18	do	do	2.50	do	do	Do.
803	61	July 24	do	Stems	1.00	do	Not sick	Lamb.
823	66.5	July 30	do	Leaves, buds, flowers, and fine stems.	1.50	do	Slight effect.	Do.
868	117	Aug. 6	do	do	3.00	do	Sick	Mature sheep.
818	52	Aug. 21 to Sept. 10, incl.	do	do	17.00	do	Slight effect.	Lamb, toleration experiment. Symptoms after first dose 1.5 per cent of weight.
860	57	Sept. 10	do	do	2.00	do	Sick	Lamb.
826	100.5	Sept. 11	do	do	3.00	do	do	Mature sheep.
810	115	Sept. 15	do	do	4.00	Flagstaff, Ariz., July, 1924.	Death	Do.
847	80.5	Sept. 19	do	do	3.25	do	do	Do.
		1926						
971	136.5	Sept. 1	do	Stems	1.08	Flagstaff, Ariz., Aug., 1924.	Somewhat sick.	Do.
908	125	Sept. 2	do	do	1.7	do	do	Do.
976	115	Sept. 8	do	do	1.59	do	do	Do.
981	140	do	do	do	1.42	do	do	Do.
Horse:		1925						
146	990	Aug. 13	With hay.	Leaves, buds, flowers, and fine stems.	.55	Flagstaff, Ariz., July, 1924.	Not sick	
146	984	Aug. 18	do	do	1.05	do	Slight effect.	
146	984	Aug. 24	do	do	.68	do	do	
Chicken:								
53	2.1	Aug. 10	Force fed.	do	.57	do	Not sick	
52	2.2	Aug. 11	do	do	1.14	do	do	
53	1.97	Aug. 17	do	do	2.23	do	do	
52	2.7	Aug. 18	do	do	3.41	do	do	
53	2.14	Aug. 21	do	do	4.55	do	do	
52	2.8	Aug. 27	do	do	5.66	do	do	

[1] Feedings were of air-dried plant calculated as green plant per 100 pounds of animal. In every case the feedings were on the single day stated, except that lamb 818 was used in a toleration experiment.

p. m., when she was turned out for water, the trembling was so violent that she was obliged to lie down. The respiration was very rapid at this time, and she panted, with her mouth wide open. Figures 7 to 9 show the attitudes assumed by the animal during the illness. The next morning, August 7, the animal had almost entirely recovered.

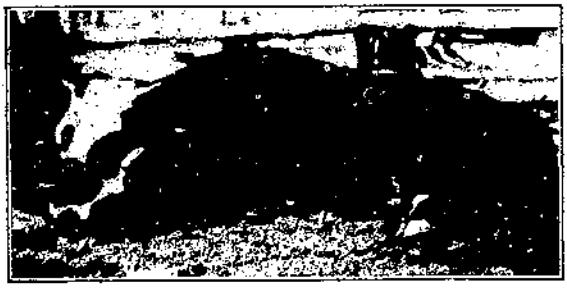

FIG. 5.—Steer 998, August 26, when completely prostrated

In order to determine whether horses may be poisoned by the plant, one horse was fed three times, and in two of the experiments showed definite symptoms.

As ostriches have been reported poisoned by *N. glauca* in South Africa, it seemed wise to determine whether chickens may be affected. Six experiments were conducted with chickens. Although these received much more proportionately than the other animals—in one case 5.68 per cent of its weight—they showed no bad effects. While the experiments were not numerous enough to prove that chickens are immune from tobacco poisoning, it may be inferred that they are much less susceptible than horses, sheep, or cattle.

FIG. 6.—Heifer 1017, September 16, 11.16 a. m. The animal was uneasy and trembling, and the staring eyes, partly covered by the nictitating membrane, were very noticeable

DISCUSSION AND GENERAL CONCLUSIONS

SYMPTOMS

The symptoms of Nicotiana poisoning are summarized by Fröhner (*8, pp. 204–205*), as follows:

> Retching, vomiting, salivation, tympanitis, diarrhea, colic, great muscular weakness, staggering, falling down, inability to stand, lameness, trembling, clonic spasms, opisthotonos, spasms of diaphragm, contraction of eye muscles, dullness and stupor, pulse in the beginning slow, later unusually rapid and irregular, violent palpitation of the heart, cold extremities, dyspnœa.

It will be noted that the symptoms of the experimental animals at the Salina station corresponded quite closely to those listed by Fröhner. Especially noticeable were muscular weakness, a stiff-legged gait, and, in severe cases, complete prostration. The trembling was very marked and was noted in nearly all the experimental animals. The

pulse was typically weak and rapid, and this, especially in the sheep, was correlated with rapid respiration.

In the experiments of 1924 it was noted that sheep 868 had a rise in temperature the second day after the feeding and that there was

FIG. 7.—Sheep 868, August 6, poisoned by leaves, buds, flowers, and fine stems of *N. trigonophylla*

FIG. 8.—Sheep 868, August 6, in another attitude

FIG. 9.—Sheep 868, August 6, in still another abnormal position

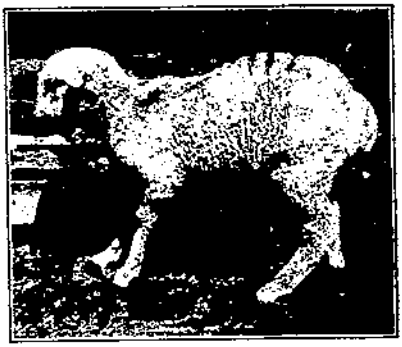

FIG. 10.—Sheep 912, September 10, at 11.15 a. m., staggering from weakness resulting from poisoning by *N. attenuata*

FIG. 11.—Sheep 912, September 10, at 11.22 a. m., finding it difficult to stand

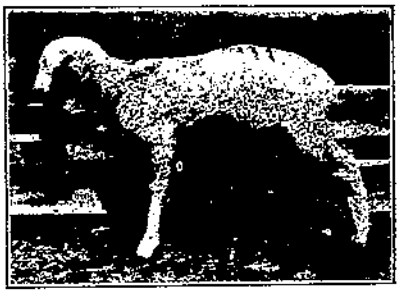

FIG. 12.—Sheep 912, September 10, at 11.37 a. m. It maintained a standing position by stretching the hind legs out as illustrated

an indication of the same effect in sheep 849, although the record for this animal was incomplete. This was not at the time considered significant, as it occurred in only two animals, and positively in only

one, but the experiments with *N. attenuata* in 1925, as shown on page 18, indicated that the plant produced a distinct effect of this kind. As the temperature records of the 1924 experiments were, in most cases, not continued after the apparent recovery of the animals, in the four feedings of 1926 care was taken to continue the temperature records for several days. In two of these animals there was a distinct rise in temperature, in one case in 24 hours, and in the other on the third day after feeding. There was an indication of the same effect in the other two, but it was not shown positively. It seems probable that the delayed high temperature may be characteristic of many cases of poisoning by Nicotiana. Frequent urination was noted in three cases and salivation in five. Vomiting occurred in only two cases. It may be remembered that Crump spoke of vomiting as occurring in his field cases. When attempting to move, some of the sheep and one of the cattle sometimes bent the feet backward from the fetlock joint and knuckled over to such an extent that they walked on the anterior surface of the feet. This was especially marked in the forefeet. The staring eyes and the contraction of the nictitating membrane, which were noted in two of the cattle, are matters of considerable interest, particularly because of the reported effect of *N. glauca* in causing amaurosis in horses in Australia.

TIME BETWEEN FEEDING AND THE DEVELOPMENT OF SYMPTOMS

Table 2 shows the time which elapsed between the conclusion of the feeding and the development of symptoms.

TABLE 2.—*Time elapsing between the feeding and the development of symptoms*

Animal	Dose expressed as a percentage of animal weight	Time before symptoms developed	Animal	Dose expressed as a percentage of animal weight	Time before symptoms developed
Cattle 1022	0.5	3 hours.	Sheep 826, mature	3.0	25 minutes.
Cattle 1017	.75	15 minutes.	Sheep 847, mature	3.25	6 minutes; death.
Cattle 1005	1.0	20 minutes.	Sheep 810, mature	4.0	Immediately; death.
Cattle 998	2.0	Immediately; death.	Sheep 971, mature	1.98	30 minutes.
Cattle 1031	.93	1 hour 30 minutes.	Sheep 998, mature	1.7	15 minutes.
Cattle 1006	1.25	15 minutes.	Sheep 976, mature	1.59	40 minutes.
Horse 146	1.05	4 hours 20 minutes.	Sheep 981, mature	1.42	43 minutes.
Do	.68	5 hours 15 minutes.	Sheep 823, lamb	1.5	17 minutes.
Sheep 849, mature	2.0	42 minutes.	Sheep 830, lamb	1.5	2 hours 31 minutes.
Sheep 848, mature	2.5	20 minutes.	Sheep 869, lamb	2.0	16 minutes.
Sheep 868, mature	3.0	12 minutes.	Sheep 818, lamb	1.5	9 minutes.

In considering this table it should be noted that as the observations on steer 1031 and sheep 830 were not carried on so carefully as were the observations on the other animals, probably the symptoms occurred in these two animals somewhat earlier than the time recorded in the table. If the records of these two animals are disregarded, the average time for the development of symptoms in cattle was 46 minutes; in mature sheep, 23 minutes; in lambs, 14 minutes; and in all the sheep, 21 minutes. The important thing to notice in these

data is that the symptoms appeared a very short time after the administration of the plant. As may be seen in Table 2, the symptoms in steer 998 and sheep 810 appeared immediately after the feeding. Generally speaking, too, there is a correlation between the time required for the development of symptoms and the size of the dose; that is, the animals receiving the larger quantities of the material developed the symptoms more quickly than those which received smaller quantities. So far as shown by Table 2, apparently the symptoms appeared somewhat sooner in sheep than in cattle, but too much stress should not be put on this point, as the number of cases was rather small. The average time in the horse experiment—4 hours and 47 minutes—was very much longer, which may be significant.

DURATION OF SICKNESS

Table 3 shows the time during which the sickness continued.

TABLE 3.—*Duration of sickness*

Animal	Dose expressed as a percentage of animal weight	Time sick
Cattle 1022	0.5	One observation.
Cattle 1017	.75	120 hours.
Cattle 1005	1	48 hours.
Cattle 998	2	3 hours 39 minutes to death.
Cattle 1031	.93	One observation.
Cattle 1000	1.25	1 hour 25 minutes.
Horse 140 (Aug. 18)	1.05	15 minutes.
Horse 146 (Aug. 24)	.68	One observation.
Sheep 849, mature	2	1 hour 25 minutes
Sheep 848, mature	2.5	8 hours 5 minutes.
Sheep 868, mature	3	10 hours 35 minutes.
Sheep 826, mature	3	21 hours 23 minutes.
Sheep 847, mature	3.25	35 minutes to death.
Sheep 810, mature	4	37 minutes to death
Sheep 971, mature	1.98	3 hours 53 minutes
Sheep 988, mature	1.7	3 hours 1 minute.
Sheep 976, mature	1.59	1 hour 52 minutes.
Sheep 981, mature	1.42	1 hour 40 minutes.
Sheep 823, lamb	1.5	2 hours 30 minutes.
Sheep 830, lamb	1.5	3 hours 17 minutes.
Sheep 869, lamb	2	90 hours.
Sheep 818, lamb	1.5	27 minutes.

In Table 3 is shown the time from the first observation of symptoms to the last recorded note. This gives rather roughly the time during which the sickness continued. Inasmuch as these data are drawn from the notes of the observers it is probable that the time indicated is, in many cases, distinctly less than the actual time during which the sickness continued. The minimum period of sickness was, of course, in those cases in which a single observation was made.

The maximum period during which cattle showed symptoms was five days (heifer 1017). In the case of sheep, the maximum period was 3 days and 18 hours (sheep 869). Sheep 869 developed during its sickness a case of torticollis and this condition existed when the animal was returned to its owner, about two and a half weeks after the feeding. The animal never recovered, and died January 20, 1925, about four months after the experimental feeding.

The average period of sickness of the cattle which recovered was 33 hours and 53 minutes. In the case of mature sheep recovering, it was 56 hours and 19 minutes; and in the case of lambs, 24 hours and 3 minutes.

In considering Table 3 it will be noticed that while some of the animals recovered in a very short time, others showed the effects of the poisoning for a period not only of hours but of days.

In the lethal cases steer 998 died 3 hours and 39 minutes after feeding, while sheep 847 died in 35 minutes and sheep 810 in 37 minutes.

There is no clear correlation between the duration of sickness and the dosage.

AUTOPSY FINDINGS

Only three animals were killed, steer 998 and sheep 847 and 810. All showed some regurgitated stomach contents in the trachea, and this material in steer 998 and sheep 810 extended into the bronchi. The gall bladders of both sheep were empty, but the gall bladder of steer 998 was full and distended. There were no other abnormal conditions. The animals evidently died of respiratory failure.

MICROSCOPIC CHANGES IN TISSUES

The changes in the tissues were few and most of them of no great importance. Material was studied from two sheep, 810 and 847, and from steer 998. This consisted of the following organs: Pancreas, liver, heart, kidneys, spleen, and lungs.

The pancreas did not show severe injury, but there was a uniform condition of slight irritation.

The liver probably showed the most pronounced changes. Radiating from the central and sublobular veins, and, to some extent, from the portal canals, were masses and cords of hepatic cells, the cytoplasm of which took the eosin stain very intensely. As a rule the nuclei of these cells reacted very strongly to hematoxylin and in most cases appeared to have shrunk some. Between these cords or masses of intensely eosin-staining cells and occupying all other parenchymal space in the organ the cells exhibited a cytoplasm which varied from a thin, even-staining condition to an almost total peripheral accumulation. This type of cell usually contained a normal and well-stained nucleus. Accompanying this condition of the hepatic cells there were, in some instances, edema and congestion.

The tissues from both ventricles of the heart showed a mild, interstitial myocarditis and more or less edema. Both right and left kidneys exhibited a condition of mild irritation and varying degrees of edema. A uniform, pneumonic condition was found in the lungs, which varied in degree but did not become severe. The spleens were about normal but gave some indication of irritation.

Tissue from the intestinal tract did not show pronounced changes; most of it appeared normal, but some exhibited slight irritation. Changes in other tissues were slight and probably indicated nothing more than the initial stage of mild irritation or hyperactivity.

TOXIC AND LETHAL DOSAGES

Table 4 shows that the minimum toxic and lethal dosages were as follows:

TABLE 4.—*Toxic and lethal dosages*

Animal	Minimum toxic dose	Minimum lethal dose
Cattle	0.5 per cent animal weight	2 per cent animal weight.
Sheep, mature	1.42 per cent animal weight	3.25 per cent animal weight.
Sheep, lambs	1.5 per cent animal weight	Not determined.
Horse	0.68 per cent animal weight	Do.

While too broad inferences can not be drawn from the comparatively small number of cases, it seems fairly evident that the plant is distinctly more toxic to cattle than to sheep. The minimum toxic dose for lambs is practically the same as that for mature sheep, although one would naturally expect that the younger animals would be somewhat more susceptible.

So far as indicated by the feeding of one horse, apparently the susceptibility of horses is about like that of cattle.

None of the chickens were poisoned, although they received as much as 5.68 per cent of their weight of green plant. It is evident that chickens are not especially susceptible to poisoning by this plant, and that if they can be poisoned, the toxic dose is more than 5.68 per cent of their weight.

TOLERATION OF POISONOUS EFFECT

Observation of tobacco users indicates that toleration of nicotine poisoning is quite readily acquired. It would be expected that a similar result would follow with the use of *N. trigonophylla*. In this connection the experiments with sheep 818 and 869 are suggestive.

Sheep 818 received every other day 1.5 per cent of its weight of the plant until it had received a total of 15 per cent. After each feeding there was a slight effect even to the last, but at no time was the animal seriously affected. On the second day after a total of 15 per cent had been fed, the animal was given a dose equal to 2 per cent of its weight. This quantity affected it only slightly. As a matter of fact, the observers questioned whether any toxic effect was produced at all.

Sheep 869, which was the twin of sheep 818 and of very nearly the same weight, was given a dose of *N. trigonophylla* equal to 2 per cent of its weight on the day on which sheep 818 received a 2 per cent dose. Sheep 869 was made quite sick, having all the typical symptoms, and the symptoms continued for 3 days and 18 hours. There seems to be little doubt in this case that the continued feeding of sheep 818 had produced a certain toleration of the effect of the plant.

ANIMALS POISONED BY N. TRIGONOPHYLLA

The experiments show conclusively that cattle, sheep, and horses may be poisoned by this plant. Inasmuch as chickens were not affected, although receiving as much as 5.68 per cent of their weight, more than four times the minimum toxic dose for sheep, and more than ten times the minimum toxic dose for cattle, they may be considered as practically immune to the effects of the plant.

PREVENTION OF POISONING

So far as treatment of poisoned animals is concerned there apparently is very little to be done. They should be kept quiet, and if there is a tendency to constipation, probably treatment with a laxative like linseed oil would be helpful. The main reliance, however, must be placed upon keeping them from eating the plants. It is to be presumed that the plants are distasteful and are not likely to be eaten except when other feed is short. Inasmuch, however, as the plant grows in somewhat thick patches, cattle or sheep confined to a rather limited area, where other forage is short, evidently may eat enough of it to cause serious harm. It should not be a matter of great difficulty to largely eliminate the plant from any given range. *N. trigonophylla* is an annual and grows in somewhat limited areas. Therefore, if it is cut down before the formation of the seed, the plant can be easily destroyed.

EXPERIMENTAL WORK ON N. ATTENUATA

There are no reported cases of poisoning of livestock by *N. attenuata*. The only publication in regard to the poisonous properties of *N. attenuata* is the determination of the nicotine, by Couch (*5*). The following statement is quoted from his summary: "Nicotine has been isolated from *N. attenuata* and has been shown to be chemically and pharmacologically identical with nicotine from *N. tabacum*. *N. attenuata* contains the following amount of nicotine in anhydrous samples: Leaves, 1.45 per cent; stems, 0.48 per cent; roots, 0.25 per cent." Inasmuch, however, as this species of wild tobacco is rather widely distributed, and as its range overlaps that of *N. trigonophylla*, it was thought desirable to get the main data in regard to its poisonous effects. Moreover, because of the fairly close resemblance of the two species, it seems probable that, in reports of distribution, they may have been confused sometimes with each other.

FEEDING EXPERIMENTS

In the experimental work, which was carried on at the Salina Experiment Station, there were eight feedings of sheep and one feeding of chickens. The results are summarized in Table 5.

All the feedings were of dry plant, and 78 per cent was allowed as loss of moisture by drying. The plant was collected at Panguitch, Utah, on August 2, 1925.

Sheep 912 was a yearling ewe in good condition, weighing 88 pounds at the time of the experiment.

On September 10, between 10.54 and 11.10 a. m., she was given by balling gun 3.7 per cent of her weight of *Nicotiana attenuata* leaves and flowers. Immediately after the feeding, symptoms of discomfort were observed, and there was a slight trembling about the neck. At 11.15 she was trembling, stood humped up, and was showing some weakness in the legs. At 11.27 her temperature was 101.1° F., pulse 140, and respiration 20. Figures 10, 11, and 12 show attitudes of the animal between 11.15 and 11.37. Respiration was somewhat

irregular. At 11.43 her temperature was 102.4°, her pulse 148 and weak, she was trembling all over, and the weakness was very much more pronounced. At 11.55 the respiration was 16, shallow and quick, and the pulse 148 and weak. At 12.03 p. m., while the animal remained on her feet, she staggered when walking, the hind legs being particularly weak, and the fetlock joints had a tendency to "break down." At 12.40 the temperature was 104.2°, pulse 160, and respiration 18. The respiration was irregular. At 1.37 the tempera-

TABLE 5.—*Summary of feeding experiments with Nicotiana attenuata*

Animal		Date of feeding	Method of feeding	Part of plant used	Percentage of animal weight fed	Result
Designation	Weight in pounds					
Sheep:		1925			*Per cent*	
927	69.5	Aug. 17	Balling gun	Leaves, buds, flowers, and fine stems.	2.0	Not sick.
928	90	Aug. 18	do	do	2.25	Slight effect.
929	101.5	Aug. 20	do	do	2.5	Do.
891	81	Aug. 26	do	do	3.4	Sick.
883	81	Aug. 31	do	do	3.6	Slight effect.
902	84.5	Sept. 8	do	do	3.5	Do.
912	88	Sept. 10	do	do	3.7	Sick.
920	84	Sept. 16	do	Stems	3.6	Death.
Chicken:						
57	2.13	Sept. 23	Force fed	Leaves, buds, flowers, and fine stems.	5.0	Sick.

ture was 103.5°, pulse 160, respiration 40, the respiration being somewhat spasmodic. At 3 o'clock the animal was turned out to water and walked fairly well, although somewhat stiffly. The trembling still continued. At 5 p. m. the temperature was 102.7°, pulse 120, respiration 32. The character of the pulse was improved, and the respiration was fairly deep and regular.

During September 11 the temperature continued between 103° and 104°, pulse between 82 and 104, respiration between 24 and 28. The pulse had become strong, but the respiration continued irregular in character.

On the morning of September 12 the temperature was 104.2°, the pulse 72, weak but regular. In the afternoon the temperature had dropped to 102.6°, the pulse was 84, and respiration was 20.

On September 13 the morning temperature was 104.1°, pulse 72, respiration 24. In the afternoon the temperature was 103°, the pulse 60. On September 14 the morning temperature was 104° and the pulse was 68.

During the days from September 11 to September 14 no trembling was noted and the general condition of the animal was fairly good. The abnormal characteristics were the weak pulse and the distinctly high temperature. Figure 13 shows the temperature curve.

On September 15 the morning temperature was 101.7°, pulse 60, respiration 21. The afternoon temperature was 102.3°, pulse 88, respiration 36. The character of the pulse, respiration, and temperature was that of a normal sheep. The animal at this time was considered recovered.

DISCUSSION AND GENERAL CONCLUSIONS

SYMPTOMS

In general, the symptoms of poisoning by *N. attenuata* resembled very closely those produced by *N. trigonophylla*.

Most prominent were depression, irregular respiration, rapid and weak pulse, trembling, and muscular weakness resulting in a staggering gait. In some cases the symptoms included salivation and soft feces. The general appearance of a poisoned animal is shown in the pictures of sheep 912, Figures 10, 11, and 12. The temperature was inclined to be high, sometimes not appearing until two or three days after the experimental feeding. This high temperature, too, in some cases continued to be the only distinct symptom for a considerable period. The temperature curves of sheep 912 and 902 (figs. 13 and 14) show very clearly this effect.

In some of the cases of poisoning by *N. trigonophylla*, a similar effect upon temperature was noted.

TIME BETWEEN FEEDING AND THE DEVELOPMENT OF SYMPTOMS

Table 6 shows the time between feeding and the development of symptoms.

TABLE 6.—*Time elapsing between feeding and the development of symptoms*

Animal	Dose expressed as a percentage of animal weight	Time before symptoms developed	Animal	Dose expressed as a percentage of animal weight	Time before symptoms developed
Sheep 928	2.25	27 minutes.	Sheep 902	3.5	16 minutes.
Sheep 920	2.5	5 minutes.	Sheep 912	3.7	Immediately.
Sheep 894	3.4	30 minutes.	Sheep 920	3.6	Do.
Sheep 883	3.	Immediately.	Chicken 57	5.	Do.

In some cases symptoms appeared immediately after the feeding. The longest period which elapsed before the appearance of any symptom was 30 minutes. It was found in the *N. trigonophylla* cases (Table 2) that if sheep 830 was excluded for the reasons stated on page 12, the longest period of time before the development of symptoms in sheep was 43 minutes.

The effect in the two species was almost identical. The average time before the development of symptoms in the *N. attenuata* experimental sheep was 11 minutes. In the cases in which *N. trigonophylla* was used the average period was 21.2 minutes. As in the *N. trigonophylla* cases, in general, the shorter time was correlated with the larger dose.

DURATION OF SICKNESS

Table 7 shows the time during which the sickness continued.

TABLE 7.—*Duration of sickness*

Animal	Dose expressed as a percentage of animal weight	Time sick
Sheep 928	2.25	18 hours 21 minutes.
Sheep 929	2.5	99 hours 47 minutes.
Sheep 894	3.4	44 hours 42 minutes.
Sheep 883	3.0	5 hours 16 minutes.
Sheep 902	3.5	80 hours 56 minutes.
Sheep 912	3.7	94 hours 9 minutes.
Sheep 920	3.6	104 hours 44 minutes to death.
Chicken 57. (Apparently sick until killed a week later; notes indefinite.)		

The maximum period of illness in the sheep that recovered was 99 hours and 47 minutes, and the average in these animals was 57 hours and 12 minutes. Sheep 920, which died, was sick 104 hours

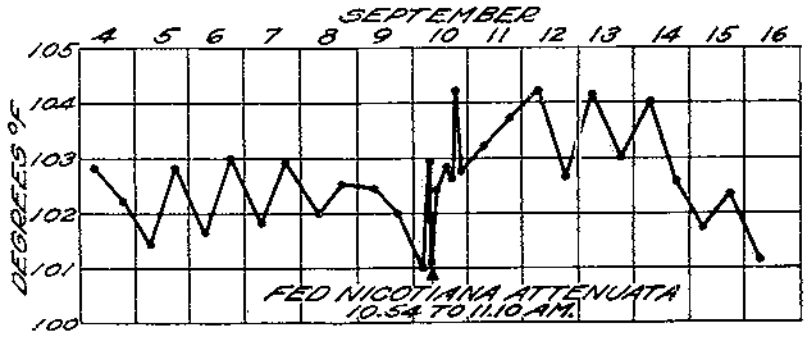

FIG. 13.—Temperature curve of sheep 912. Note the higher temperature after receiving the feeding of *N. attenuata* September 10

and 44 minutes. In regard to chicken 57 the notes were incomplete, and it can only be said that the apparent effect of the plant continued for a long period.

AUTOPSY FINDINGS

The autopsy of sheep 920 did not indicate much in the way of definite lesions as the result of poisoning by the plant. There were some petechiæ on the heart, the lungs were congested, the fourth stomach was somewhat reddened, and the surface vessels of the

ileum were congested. The posterior mediastinal lymph glands were congested and hemorrhagic.

MICROSCOPIC CHANGES IN TISSUES

In the microscopic examination of tissues comparatively few changes were observed which are of significance.

Tissues of sheep 920 were examined. The pancreas showed slight irritation. The liver exhibited the most pronounced changes. The cytoplasm of the hepatic cells immediately surrounding the portal canals, the central and sublobular veins, and the region just below the capsule took the eosin stain very strongly, probably indicating a swollen condition. The nuclei of these cells were usually shrunken and stained almost black with hematoxylin. Mild congestion existed throughout the entire organ. The spleen showed slight irritation. The abomasum was normal, as were also the duodenum and ileum. The ventral parts of both lungs were affected with acute pneumonia in an advanced stage. This pneumonic condition extended into the

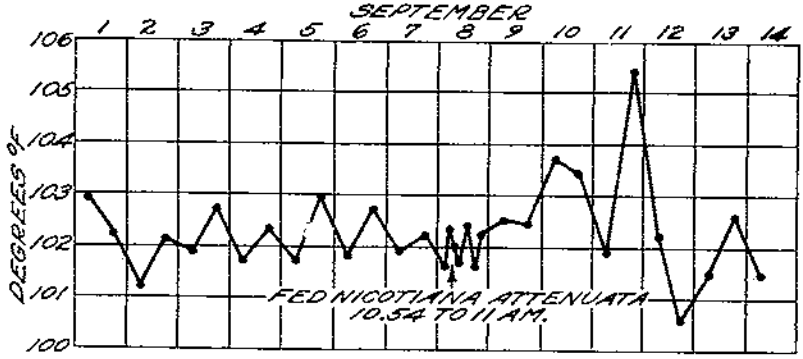

FIG. 14.—Temperature curve of sheep 902. Note the higher temperature occurring the second day after the experimental feeding

dorsal lobes, but was less severe there, and finally merged into a condition of congestion. The suprarenals were practically normal. Slight irritation was found in both kidneys. The posterior mediastinal lymph gland displayed pronounced hyperplasia and congestion, while a mesenteric lymph gland examined exhibited slight irritation. Mild interstitial myocarditis was present in the walls of both ventricles of the heart.

A comparison of tissues of animals poisoned by *N. trigonophylla* and tissues of those poisoned by *N. attenuata* did not indicate fundamental differences. Such as were noted appeared to be differences of degree and not of type of injury.

TOXIC AND LETHAL DOSAGES

The minimum toxic dose for sheep was 2.25 per cent of animal weight of green plant, and the lethal dose was 3.6 per cent. There was only one lethal case, sheep 920. As sheep 912 was made sick on 3.7 per cent, it is presumable that 3.6 is close to the minimum lethal dose. It should be noted, however, that sheep 920 received stems,

while sheep 912 received leaves and flowers. While it is supposed that there is little difference in toxicity between the leaves and stems, there were no experiments to make sure of this. It is, therefore, possible that the stems are more toxic than the leaves. Only one chicken was used, and that was made sick by a 5 per cent dose. Since in the work with *N. trigonophylla* no effect was produced by 5.68 per cent, it is probable that 5 per cent is about the minimum toxic dosage.

So far as the experiments go, with the comparatively small number of animals, it may be inferred that *N. attenuata* is somewhat less toxic than *N. trigonophylla*. There is, however, no material difference between the two species.

ANIMALS POISONED BY *N. attenuata*

As the experimental work with sheep showed clearly that the symptoms and dosage of *N. attenuata* differed only slightly from those obtained in the work with *N. trigonophylla*, it did not seem necessary to try out the plant with horses and cattle. The single experiment with a chicken indicated the same lack of susceptibility which was found in *N. trigonophylla*. It may be concluded that cattle, horses, and sheep may be poisoned by *N. attenuata* and that chickens are practically immune.

PREVENTION OF POISONING

The statement on page 16 in regard to the prevention of poisoning by *N. trigonophylla* applies equally well to *N. attenuata*. The latter species is also an annual, and its eradication should be a comparatively simple matter. Animals well supplied with good forage are not likely to eat enough of either species to do harm, but on closely grazed ranges these plants may become dangerous.

SUMMARY

The experimental work with *Nicotiana trigonophylla* showed that it is distinctly poisonous to cattle, sheep, and horses, producing typical tobacco symptoms.

Generally speaking, the poisonous effects appear very soon after the feeding and in some cases continue for a considerable period.

Of the animals used in these experiments the cattle were much more susceptible than the sheep. The susceptibility of the horses was about the same as that of the cattle. Fatal results are more liable to occur with cattle than with the other animals experimented upon.

The poisoning of chickens is a very improbable occurrence.

The remedy for livestock poisoning by *N. trigonophylla* on the range lies in prevention, by taking care that animals have a sufficient supply of other forage, and in the destruction of the plant.

The symptoms produced by *N. attenuata* are closely identical with those produced by *N. trigonophylla*.

The toxic and lethal dosages of *N. attenuata* are somewhat greater than are those of *N. trigonophylla*.

There is a possibility of the poisoning of chickens by *N. attenuata*, but the dosage required is so heavy that it probably never occurs, except as produced experimentally.

While there are no recorded cases of poisoning by *N. attenuata*, it is a possible source of serious losses of livestock on overgrazed ranges.

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