

Effect of 2,4-D on Hymenoxon Concentration and Toxicity of Bitterweed [*Hymenoxys odorata*] Force-Fed to Sheep

M.C. CALHOUN, D.N. UECKERT, C.W. LIVINGSTON, JR., AND B.J. CAMP

Abstract

Bitterweed (*Hymenoxys odorata*) growing at two locations was sprayed with 2,4-D (1.1 kg acid equivalent/ha) during the spring of 1977. Subsequently, plants were collected, dried, and stored when they showed definite signs of herbicide phytotoxicity (epinasty and turgidity). Hymenoxon concentrations were determined on the dried plant material and it was force-fed to penned sheep, in two experiments, to determine the effect of foliar spraying with 2,4-D on bitterweed toxicity. Bitterweed administration decreased voluntary feed intake and increased serum concentrations of urea nitrogen (UN), creatinine (C) and glutamic-oxalacetic transaminase (GOT). Hymenoxon concentrations (air-dry basis) were $2.33 \pm .18\%$ and $1.64 \pm .05\%$, for unsprayed and 2,4-D sprayed bitterweed, respectively, in Experiment 1 and $1.24 \pm .02\%$ and $1.08 \pm .05\%$, respectively, in Experiment 2. Spraying bitterweed did not affect feed intake and serum levels of UN, C and GOT and there were not interactions between bitterweed levels and 2,4-D treatments.

Bitterweed (*Hymenoxys odorata*) poisoning of sheep is a severe problem in Texas (Hardy et al. 1931; Boughton and Hardy 1937; Rowe et al. 1973). Death losses of sheep attributed to bitterweed poisoning on the Edwards Plateau average 1 to 6% annually (Sulzmeier 1961). Numerous studies have examined practices for reducing bitterweed toxicity problems, including use of grazing systems (Merrill and Schuster 1978), herbicide treatments (Ueckert et al. 1980) and various supplemental feeds and feed additives (Rowe et al. 1973). The toxic principle in the bitterweed plant, hymenoxon, has been described in several studies (Kim et al. 1974; Kim et al. 1975; Ivie et al. 1975). Although grazing a combination of cattle and sheep, use of deferred rotation grazing systems, and herbicide treatments have been effective in reducing bitterweed problems, there is not an effective means of preventing losses when sheep are consuming bitterweed.

Substantial shifts in the concentrations of several plant poisons have been observed after herbicidal treatment. Amine salts of 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) increased total alkaloids when applied to tall larkspur (*Delphinium barbeyi* Huth) (Williams and Cronin 1963). Spring parsley leaves [*Cymoptus watsonii* (Coulter and Rose) Jones] were much less phototoxic to chicks 4 weeks after treatment with 4-amino-3,5,6-trichloropicolinic acid (picloram) or an approximate 4 to 1 mixture of (2,4-dichlorophenoxy)acetic acid (2,4-D) and picloram (Williams

1968). When timber milkvetch (*Astragalus miser* var. *oblongifolius*) was treated with esters of either 2,4,5-T or silvex the concentration of miserotoxin rapidly decreased. After 4 weeks, treated plants contained only one-third as much miserotoxin as the controls (Williams 1970). Recently, Leo B. Merrill (personal communication) observed that spraying bitterweed with 2,4-D at 1.1 kg/ha (acid equivalent) appeared to increase its palatability and sheep consumed the weed without apparent harm. This observation was made initially in 1974, when a ewe flock was allowed to intensively graze a bitterweed pasture 1 day after spraying with 2,4-D. It was estimated each ewe consumed a minimum of 45 kg of bitterweed during a 50 to 60 day period following spraying.

Boughton and Hardy (1937) determined that the acute median lethal dose (LD₅₀) of fresh green bitterweed seedlings growing during a year of normal rainfall was approximately 1.3% of a sheep's body weight. The acute LD₅₀ of bitterweed growing under drought conditions was 0.5% of a sheep's body weight. Rowe et al. (1973) reported acute LD₅₀ values for air-dried bitterweed of 3.6 to 8.5 g/ka (0.36 to 0.85% of live weight). In addition, the bitterweed toxin has been demonstrated to be cumulative when less than the acute LD₅₀ is consumed for a number of days (Rowe et al. 1973).

Therefore, the level of intake observed by Merrill should have been lethal to sheep. Merrill repeated his study in 1975 and 1976, with similar results. However, because of low palatability of bitterweed in unsprayed plots, sheep consumed little of the unsprayed bitterweed and no deaths occurred among sheep grazing unsprayed bitterweed. The purpose of this research was to test the hypothesis that spraying bitterweed with 2,4-D will decrease its toxicity to sheep.

Materials and Methods

Bitterweed Collection

Bitterweed for the first experiment was collected on the Bill Pfluger Ranch in south-central Tom Green County, Texas. Four separate plots ranging in size from .02 to .04 ha were sprayed with 2,4-D on March 7, 1977, with a livestock sprayer. The dimethylamine salt of 2,4-D was applied at a rate of 1.1 kg (acid equivalent)/ha in a water carrier (1,403 L/ha) with 0.1% nonionic spreader and activator. Four days later (March 11), when the bitterweed showed definite signs of herbicide phytotoxicity (epinasty and turgidity), bitterweed on sprayed plots was hand clipped, and dried and stored as previously described (Calhoun et al. 1981). Approximately equal amounts were collected from each of the four plots and from unsprayed rangeland adjacent to the sprayed plots.

Bitterweed for the second experiment was collected from the H and H Cattle Co. Ranch, in east-central Sterling County, Texas. The ethylhexyl ester of 2,4-D was applied with a tractor-mounted, small plot sprayer at 1.1 kg (a.e.)/ha in a (1:14 v/v) diesel oil-water emulsion carrier (140 L/ha) with 0.05% dispersant-activator-emulsifier. A 6 m × 92 m plot was sprayed on April 20, 1977. At 5 days post-spraying, bitterweed was collected from the sprayed plot and from an adjacent unsprayed area. The sprayed bitterweed

Authors are, respectively, associate professor and professors, Texas Agr. Exp. Sta., San Angelo, 76901; professor, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station 77843.

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showed slight to moderate epinasty of floral parts; however, signs of herbicide phytotoxicity at this location were slight, compared to the 4-day post-spraying signs at the Pfluger Ranch.

The bitterweed sprayed and collected from both locations were 15 to 23 cm tall. Few bitterweed flowers were present at the Pfluger Ranch, but the bitterweed was in full flower at the H and H Cattle Co. location.

In addition to the above, 10 individual bitterweed plants were harvested at random from the 2,4-D sprayed area at H and H Cattle Co. on May 9, 1977, (19 days post-spraying) and 10 from the adjacent unsprayed area. Hymenoxon concentrations of the individual plants were measured to provide an estimate of the range in hymenoxon concentrations in individual plants growing on a common site, as well as an estimate of the effects of 2,4-D on hymenoxon concentrations.

Samples of fresh bitterweed from sprayed and unsprayed plots were collected on March 11 (Experiment 1) and on April 25 (Experiment 2), frozen, and later analyzed by gas-liquid chromatography for residues of 2,4-D (Lisk 1967). Hymenoxon concentrations of the dried bitterweed were also determined (Hill et al. 1979).

Animals and Feeding

The procedures for handling and feeding the sheep and administration of bitterweed in these experiments were the same as previously described (Calhoun et al. 1981). Lambs were individually restrained and fed (*ad libitum*) a 70% concentrate ration that was available from 8:00 a.m. to 4:00 p.m. each day. Lambs were then released for the remainder of the day for exercise and to facilitate observation of clinical signs of bitterweed poisoning. In the first experiment, 12 lambs were assigned to the unsprayed material and 12 to the 2,4-D sprayed bitterweed. The daily bitterweed dosages were 0, 0.066, 0.132 and 0.264% of the sheep's live weight (based on air-dry weight of bitterweed). Three lambs were assigned to each level.

In the second experiment (bitterweed from H and H Cattle Co.), 12 lambs were assigned to unsprayed bitterweed; however, only enough 2,4-D sprayed material was available to feed three sheep at the highest level (.264%). Three were also assigned to be controls (0 level) for this group. The bitterweed was administered as a single dose (by stomach tube in a water suspension) at approximately 8:30 a.m. each day for a 10-day period (Calhoun et al. 1981).

Blood samples (for serum) were collected by jugular venipuncture initially and again when the majority of the lambs on the highest bitterweed dose showed definite signs of toxicity (on the 6th day in the first experiment and on the 7th day in the second experiment). Blood serum samples were analyzed at the Texas Veterinary Medical Diagnostic Laboratory at College Station for urea nitrogen, creatinine, and glutamic-oxalacetic transaminase using an automated serum analyzer.

All lambs were weighed to the nearest 0.45 kg at the beginning and end of each experiment. Feed intake was recorded daily for each lamb. Lambs were observed for signs of bitterweed toxicosis

at 8:30 a.m., 4:30 p.m. and 1 hr after administration of the daily dose of bitterweed. More frequent observations were made when deemed desirable as experiments progressed. Lambs that died were necropsied to ascertain cause of death.

The analysis of variance for a completely random design was used for the statistical treatment of the data (Steel and Torrie 1960). For both experiments, the model included 2,4-D treatment, bitterweed levels and the interaction between 2,4-D treatment and bitterweed levels. Regression analysis was used to separate the linear, quadratic and residual components. However, for those criteria not fitting a linear or quadratic response, i.e., those changing at only the highest bitterweed level, Duncan's multiple range test was used for comparison of treatment means.

Results

Experiment I

Concentration of 2,4-D on the external surface of bitterweed plants collected at 4 days after spraying averaged 20.5 ppm. Herbicide concentration in the plant tissues was 71.2 ppm. Thus, approximately 78% of the intact 2,4-D was absorbed. Hymenoxon concentrations of the harvested material were $2.33 \pm 0.18\%$ and $1.64 \pm 0.05\%$, for unsprayed and sprayed bitterweed, respectively. The mean live weight of the 24 lambs was 29.1 ± 0.9 kg.

Administration of bitterweed, by stomach tube, as a single dose each morning produced an immediate but temporary reaction, especially at the highest dose. The sheep's ears drooped and the head and neck were extended. After the third daily dose, sheep on the highest level appeared listless or depressed and, unless disturbed, remained lying down most of the time. Vomition of rumen contents was observed but was not considered to be a serious source of loss of the bitterweed-water suspension. By the fourth day sheep on the highest bitterweed level were foaming at the mouth, reluctant to get up and unsteady when standing. Signs of toxicity were similar for sheep given sprayed and unsprayed bitterweed.

Six sheep administered the highest bitterweed level died, regardless of herbicide treatment. The three given unsprayed bitterweed died on days 7, 8 and 9; whereas, those getting sprayed bitterweed died on days 6, 7 and 9. Post-mortem examination revealed gross pathological changes typical of bitterweed poisoning in each case. Immediate cause of death was generally aspiration pneumonia. Sheep on the two lower bitterweed levels (0.066 and 0.132%) showed little effect of bitterweed.

Administration of bitterweed decreased feed intake ($P < .01$) (Table 1). The response was linear to the point where eating ceased (essentially no feed was eaten by sheep given the highest bitterweed dose). Feeding bitterweed sprayed with 2,4-D did not affect feed intake, compared to that of animals fed unsprayed bitterweed, and there was no interaction between bitterweed level and 2,4-D treatment relative to feed intake (Table 1). When the feed intake data for sheep fed sprayed and unsprayed bitterweed was combined, the

Table 1. Effect of spraying with 2,4-D on voluntary feed intake and serum concentrations of urea nitrogen, creatinine, and glutamic-oxalacetic transaminase of lambs force-fed harvested, air-dry bitterweed (*Hymenoxys odorata*). (Experiment 1)

Criterion	Unsprayed bitterweed % of live weight ¹				2,4-D sprayed bitterweed % of live weight ²				S.D.
	0	.066	.132	.264	0	.066	.132	.264	
Feed intake, kg/day ²	1.26 ^{c3}	0.94 ^b	0.26 ^a	0.01 ^a	1.46 ^c	1.10 ^{bc}	0.20 ^a	0.01 ^a	.25
Urea nitrogen, mg/dl	16.0	12.2	19.6	53.9	14.8	17.4	25.9	70.6	
Urea nitrogen, log ₁₀ mg/dl	1.19 ^{ab}	1.08 ^a	1.28 ^{ab}	1.73 ^c	1.16 ^{ab}	1.22 ^{ab}	1.41 ^b	1.78 ^c	.15
Creatinine, mg/dl	0.71	.65	0.86	1.76	0.54	0.84	0.97	3.12	
Creatinine, log ₁₀ (mg/dl*10 ²)	1.85 ^a	1.81 ^a	1.93 ^a	2.23 ^{bc}	1.71 ^a	1.92 ^a	1.98 ^{ab}	2.38 ^c	.16
Glutamic-oxalacetic transaminase, IU/l	177.0	105.0	148.0	454.0	115.0	118.0	126.0	687.0	
Glutamic-oxalacetic transaminase, log ₁₀ IU/l	2.22 ^a	2.02 ^a	2.17 ^a	2.62 ^b	2.05 ^a	2.07 ^a	2.09 ^a	2.73 ^b	.19

¹Air-dry basis

²Averaged for the 4-day period days two through five.

³Means on the same line with differing superscripts are significantly different at the $P < .05$ level.

Table 2. Effect of spraying with 2,4-D on voluntary feed intake and serum concentrations of urea nitrogen, creatinine, and glutamic-oxalacetic transaminase of lambs force-fed harvested, air-dry bitterweed (*Hymenoxys odorata*). (Experiment 2)

Criterion	Unsprayed bitterweed % of live weight ¹				2,4-D sprayed bitterweed % of live weight		S.D.
	0	.066	.132	.264	0	.264	
Feed intake, kg/day ²	1.21 ^c	1.01 ^c	0.52 ^b	0.21 ^a	1.22 ^c	0.10 ^a	.20
Urea nitrogen, mg/dl	16.7	15.9	21.5	43.0	12.6	27.0	
Urea nitrogen, log ₁₀ mg/dl	1.22 ^{ab}	1.20 ^{ab}	1.33 ^{ab}	1.58 ^c	1.10 ^a	1.41 ^{bc}	.14
Creatinine, mg/dl	0.83	0.78	1.02	3.08	0.81	1.54	
Creatinine, log ₁₀ (mg/dl)•10 ²	1.92 ^a	1.89 ^a	2.01 ^{ab}	2.34 ^b	1.91 ^a	2.11 ^{ab}	.22
Glutamic-oxalacetic transaminase, IU/l	163.0	113.0	296.0	382.0	120.0	438.00	
Glutamic-oxalacetic transaminase, log ₁₀ IU/l	2.20 ^{ab}	2.05 ^a	2.36 ^{ab}	2.51 ^b	2.07 ^a	2.61 ^b	.23

¹Air-dry basis

²Averaged for the 4-day period days two through five.

³Means on the same line with differing superscripts are significantly different at the $P < .05$ level.

equation for the relationship between voluntary feed intake in kg/day averaged for the 4-day period (days 2 thru 5) (Y) and bitterweed dose as a percentage of live weight (air-dry basis) (X) was $Y = 1.43 - 8.52X$ ($r^2 = .75$, $P < .01$). Feed intake ceased at a calculated bitterweed dose of 0.17% of live weight.

Serum concentrations of urea nitrogen, creatinine, and glutamic-oxalacetic transaminase were elevated at the highest bitterweed dose ($P < .05$) (Table 1). Spraying bitterweed with 2,4-D had no effect on concentrations of these serum constituents, and there were no interactions between bitterweed level and 2,4-D.

Experiment 2

The calculated absorption of 2,4-D was 51% for the bitterweed sprayed on April 20 and collected on April 25 in the second experiment. The hymenoxon level of the unsprayed bitterweed plants was $1.24 \pm .02\%$; whereas, sprayed bitterweed measured $1.08 \pm .05\%$. The 18 lambs used for this feeding trial weighed $28.6 \pm .7$ kg.

Signs of bitterweed toxicity were less dramatic than were observed in the first experiment and none of the sheep died. There were no observable differences between lambs given unsprayed and 2,4-D sprayed bitterweed. Ingestion of bitterweed decreased voluntary feed intake (Table 2). Spraying bitterweed did not affect feed intake and there was no interaction between bitterweed level and 2,4-D treatment. The relationship between voluntary feed intake (kg/day) averaged for the 4-day period (days 2 thru 5) (Y) and bitterweed level as a percentage of live weight (X) was $Y = 1.20 - 4.08X$ ($r^2 = .86$, $P < .01$).

Bitterweed at the highest level increased serum concentrations of

urea nitrogen, creatinine, and glutamic-oxalacetic transaminase ($P < .05$) (Table 2). Spraying with 2,4-D did not affect concentrations of these serum constituents and there were no interactions between bitterweed level and 2,4-D treatments.

Variation in Hymenoxon

Hymenoxon content of individual unsprayed bitterweed plants collected on May 9 in the second experiment averaged $1.21 \pm .07\%$ (Table 3). The 10 plants sprayed with 2,4-D averaged $.65 \pm .07\%$ hymenoxon—a significant reduction compared to that of unsprayed plants.

Discussion and Conclusion

There was considerable variation in hymenoxon content of bitterweed both within and among locations. Unsprayed individual plants in full flower in the second experiment varied from .71 to 1.47% hymenoxon (air-dry basis). Unsprayed bitterweed in the vegetative stage collected in the first experiment contained 2.33% hymenoxon. The factors which influence toxicity of bitterweed are not completely understood; but, an increase in plant maturity and moisture stress have been reported to make the plant more toxic to sheep (Boughton and Hardy 1937). However, in this study plants in earlier phenological stages were more toxic to sheep than bitterweed in later phenological stages. These data strongly suggest real differences in the toxicity of bitterweed plant populations at different locations.

It is evident from this research that spraying with 2,4-D can significantly reduce the hymenoxon content of bitterweed. The reduction in hymenoxon would help explain field observations of reduced potential for poisoning after spraying. However, additional research is required to resolve the difference observed in this study between chemically determined hymenoxon levels and actual toxicity of the material when harvested and force-fed to sheep under controlled experimental conditions.

Previous experience (Calhoun et al. 1981), as well as the dose response relationships observed in this study, suggest that voluntary feed intake should have been higher when 2,4-D-sprayed bitterweed was administered. There is no apparent explanation for this not occurring. However, hymenoxon is not the only toxic compound that has been isolated from bitterweed. The chemistry and interrelationships of the sesquiterpene lactones is complex (Herz 1973) and the effect of 2,4-D on the plant metabolism of these compounds is unknown.

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Table 3. Hymenoxon concentration (%) of individual bitterweed plants and effect of 2,4-D on hymenoxon concentrations.¹

Sample	Unsprayed (%)	2,4-D Sprayed (%)
1	1.23	0.68
2	0.93	0.92
3	1.19	0.37
4	1.35	0.68
5	1.33	1.05
6	1.27	0.61
7	0.71	0.55
8	1.43	0.69
9	1.17	0.49
10	1.47	0.45
Avg.	1.21 ²	0.65
S.E.M.	0.073	0.066

¹Hymenoxon concentrations are expressed on an air-dry basis.

²Difference between means is significant ($P < .01$).

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POSITION ANNOUNCEMENT

Range Resources Division
School of Renewable Natural Resources
College of Agriculture
University of Arizona.

Title: Extension Range Specialist and Professor, Range Management

Rank and Salary: Commensurate with qualifications

Closing Date: October 15, 1982, or until a suitable candidate is found

Availability Date: January 1, 1983

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This position also provides the opportunity to spend 30% time conducting a research program in the individual's area of interest and to work with graduate students.

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