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Sceleratin Nitrogen Oxide as Aversive Agent in Conditioning Livestock to Avoid *Senecio latifolius*

Abstract

Sceleratine nitrogen oxide, when administered together with a dichloromethane extract of *Senecio latifolius*, successfully conditioned cattle and sheep to avoid milled freeze dried *S. latifolius* mixed with maize meal. This treatment was effectively applied in conditioning steers to refuse eating *S. latifolius* grown in pots.

Keywords

Senecio latifolius, sceleratine-NO, aversive agent, conditioning, livestock

Cover Page Footnote

Footnote LD Snyman: Retired veterinary research scientist (snymanleendert@gmail.com) Professor CJ Botha of the Faculty Veterinary Science, University Pretoria, is acknowledged for reviewing the veterinary aspects of the manuscript

INTRODUCTION

Ingestion of *Senecio latifolius* and some other *Senecio* spp. causes seneciosis, which is estimated to be the second largest cause of cattle mortalities due to plant poisoning or mycotoxicosis in South Africa (Kellerman *et al.* 1996). Cattle, especially young animals newly introduced to *S. latifolius* infested pastures, may die suddenly within the first few days after ingesting relatively large quantities of the plant. Acute and chronic poisoning of sheep also occurs regularly in areas of abundant plant growth. Young plants in the two-leaf stage of growth appears to be more palatable and most toxic to livestock (Kellerman *et al.* 1988).

The application of conditioned feed aversion potentially might be a means to prevent large scale poisoning of livestock newly introduced to *S. latifolius* infested pastures. Sceleratine-NO, the toxic principle of *S. latifolius*, has been shown to be aversive when administered to a sheep (Snyman 2023) and thus could potentially be used as an aversive agent in conditioning livestock to avoid *S. latifolius*. The sheep, however, did not refuse eating *S. latifolius* presented as part of a maize meal mixture (Snyman 2023), suggesting that sceleratine-NO as such may not be usable for establishing aversion to *S. latifolius*. However, a sheep averted to *S. latifolius* refused eating a dichloromethane extract of *S. latifolius*. This was ascribed to the sensory characteristics of *S. latifolius* present in the extract. Thus, the hypothesis was made that livestock treated with a combination of sceleratine-NO and a dichloromethane extract of *S. latifolius* will associate the aversive effect of sceleratine-NO with the sensory characteristics of *S. latifolius* extracted by dichloromethane and refuse intake of *S. latifolius*. This approach would be in accordance with the mechanism of learning in diet selection by herbivores (Provenza *et al.* 1992).

In this study, the aversive effect of sceleratine-NO administered together with a dichloromethane extract of *S. latifolius* was investigated to determine their capability in conditioning sheep and cattle to refuse *S. latifolius* containing maize meal mixtures and *S. latifolius* grown in pots.

MATERIALS

Plant Material. *Senecio latifolius* was collected in the eMkhondo (= Piet Retief) (27.0245°S, 30.7925°E) district and re-established on a plot on the premises of the Onderstepoort Veterinary Research Institute. *Senecio latifolius* used for pen trials with sheep and cattle was collected at the pre-bloom stage where after it was freeze-dried, milled, and stored at – 20 °C in a conventional deep freezer. Plants from this cultivation were also transplanted to pots to be used for pen trials with steers.

Experimental Animals. All procedures with animals were carried out according to the South African National Standard (*The Care and Use of Animals for Scientific Purposes* [SANS 10386:200X]. Animal trials were approved by the

animal ethics committee of the Agricultural Research Council–Onderstepoort Veterinary Institute.

Adult Dorper wethers of approximately 50 kg and Bonsmara and Nguni steers (15 – 18 months old), naïve to *S. latifolius*, were used as experimental animals. The animals were individually housed in pens and fed *Eragrostis curvula* hay *ad lib*.

Apparatus. Freshly collected plant material was lyophilized with a Christ laboratory freeze drier (Martin Christ, Germany), and ground with a Wiley cutting mill (Arthur H. Thomas Co., Philadelphia) to pass a 1 mm sieve. Evaporation of solvents was carried out at 40 °C using a Büchi R–100 rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland).

Chemicals. Redistilled analytical grade (Merck Darmstadt, Germany) methanol and dichloromethane were used.

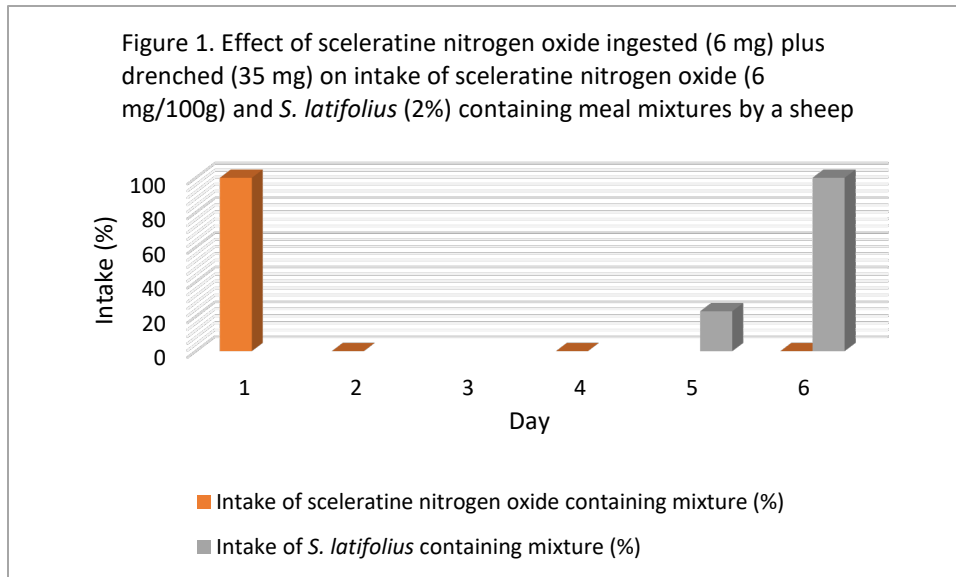
METHODS AND RESULTS

Preparation of scleratine-NO and dichloromethane extract containing maize meal mixtures. The scleratine-NO and dichloromethane extract containing maize meal mixtures presented or dosed to the animals were prepared as follow. The needed amount of scleratine-NO to be mixed with maize meal for the various trials described below was dissolved in 50 ml methanol, mixed, and the methanol evaporated. For mixtures containing both scleratine-NO and a dichloromethane extract, scleratine-NO was mixed with maize meal as described above whereafter the dichloromethane extract (prepared as described by Snyman 2023) was added, mixed and the dichloromethane evaporated. Dichloromethane of mixtures containing only the dichloromethane extract was also evaporated before presentation to animals.

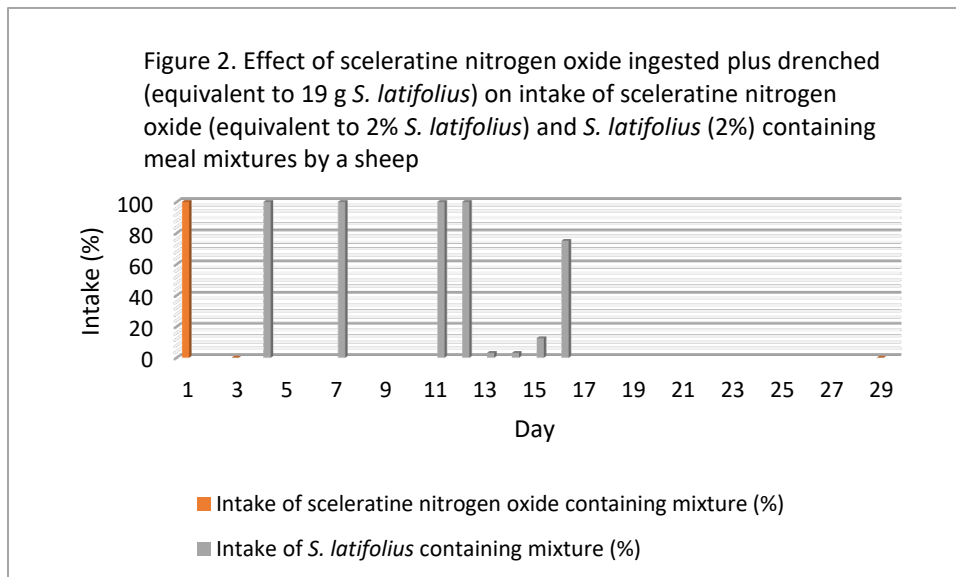
Establishing aversion to milled freeze-dried *S. latifolius*. Establishing and testing for aversion was performed as previously described (Snyman 2023).

Treatment with scleratine–NO. A sheep was offered 6 mg scleratine-NO in 100 g maize meal on Day 1, which was totally consumed. After consumption, 35 mg scleratine-NO dissolved in 100 ml water was orally drenched, using a drench gun. Scleratine-NO ingested plus drenched (41 mg) was equivalent to consumption of 33 g dried *S. latifolius* which is considered as an aversive dose (Snyman 2023). When challenged with the scleratine-NO containing meal mixture (6 mg/100g) on days 2, 4 and 6 the mixture was totally refused. Offering the sheep 100 g of a 2% *S. latifolius* maize meal mixture on days 5 and 6 resulted in partial consumption on Day 5 followed by total consumption on Day 6. The results are shown in Fig. 1.

In a second trial a sheep was offered a scleratine-NO containing maize



meal mixture (50 g) equivalent to 2% *S. latifolius*. After consumption of the mixture the sheep was orally drenched with sceleratine-NO dissolved in water at the equivalent to 18 g *S. latifolius*. The sceleratine-NO containing meal mixture (equivalent to 2% *S. latifolius*) was totally refused when presented to the sheep on

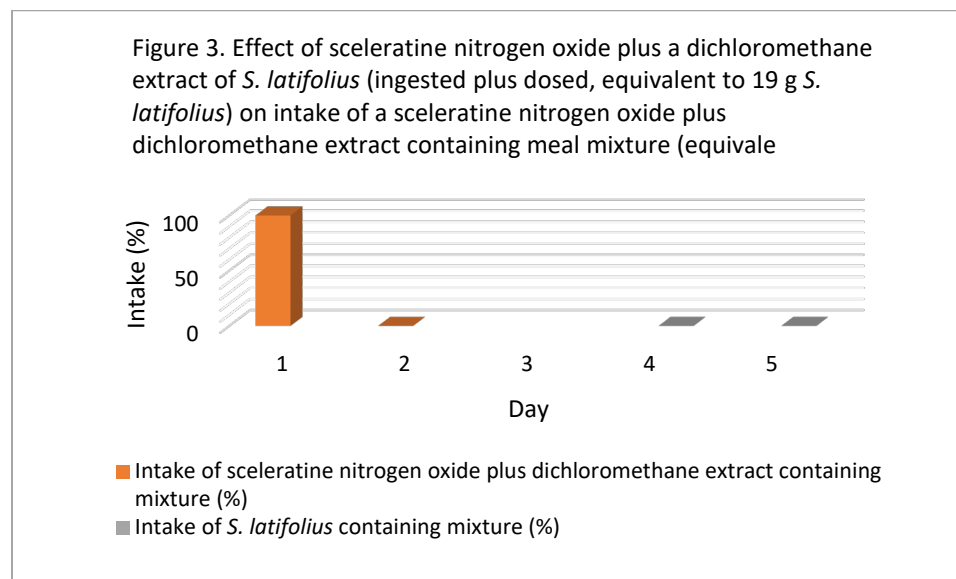


days 3 and 29. However, a 2% *S. latifolius* containing maize meal mixture (100 g) presented on days 4, 7, 11, and 12 was totally consumed on each of these days. Reduced consumption of the mixture recorded on days 13, 14, 15 and 16 might be

ascribed to temporary aversion induced by the preceding intake of *S. latifolius*. The results are shown in Fig. 2.

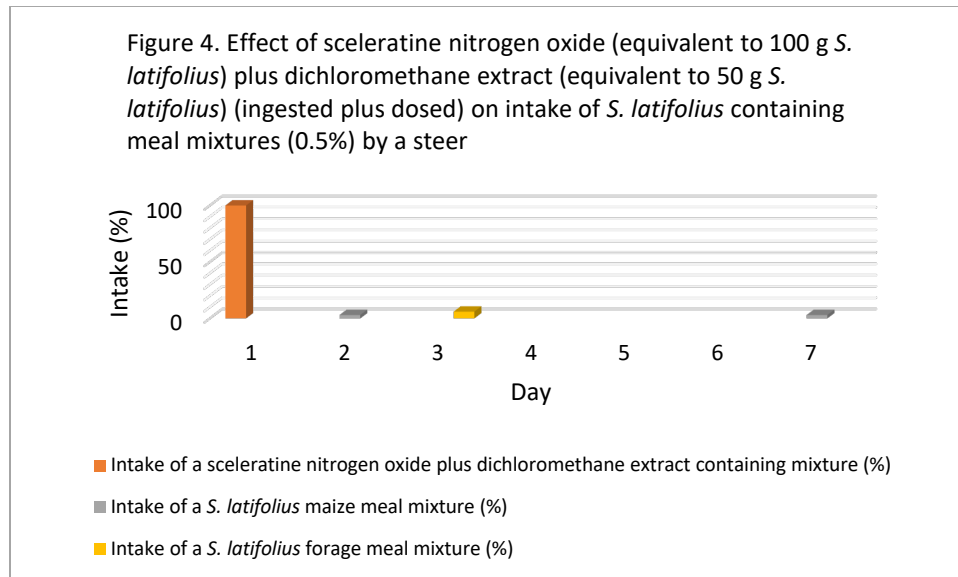
Treatment with a dichloromethane extract. A dichloromethane extract equivalent to 1 g *S. latifolius* was mixed with 50 g maize meal (equivalent to 2% *S. latifolius*) and offered to a sheep. The sheep eagerly consumed the mixture. The sheep was subsequently dosed *via* a stomach tube with an additional amount of the dichloromethane extract (mixed with maize meal and shaken up with water) at the equivalent of 18 g *S. latifolius*. When offering the dichloromethane extract containing meal mixture the following day, the mixture was again completely consumed.

Treatment with scleratine-NO plus a dichloromethane extract. In subsequent trials the aversion inducing capability of scleratine-NO with respect to *S. latifolius* was investigated when ingested together with a dichloromethane extract of *S. latifolius*. In a first trial a sheep was offered scleratine-NO plus a dichloromethane extract of *S. latifolius* mixed with 50 g maize meal at the equivalent of 2% *S. latifolius*. The mixture was totally consumed. The sheep was subsequently dosed *via* a stomach tube with scleratine NO plus a dichloromethane



extract mixed with 50 g maize meal at the equivalent of 18 g *S. latifolius*. The mixture was shaken up with water before dosage. When offering the scleratine-NO plus dichloromethane extract containing maize meal mixture (equivalent to 2% *S. latifolius*) the next day it was totally refused. The sheep also refused a 2% *S. latifolius* containing maize meal mixture (50 g) on days 4 and 5. The results are shown in Fig.3. In a following trial a steer was offered scleratine-NO plus a dichloromethane extract, mixed with 1 kg maize meal, both at the equivalent of

0.6% *S. latifolius*. The mixture presented on Day 1 was totally consumed. The steer was subsequently dosed *via* a stomach tube with sceleratine-NO at the equivalent of 100 g *S. latifolius* in combination with a dichloromethane extract equivalent to 50 g *S. latifolius*. The sceleratine-NO and dichloromethane extract dosed were



previously mixed with 100 g maize meal and shaken up with 1 L water. The steer was then challenged with 0.5% *S. latifolius* in 1 kg maize meal on Day 2, 0.5% *S. latifolius* in 1 kg forage meal on Day 3, and 0.5% *S. latifolius* in 1 kg maize meal again on Day 7. The steer totally refused each of the presentations. The results are shown in Fig. 4.

Establishing aversion to *S. latifolius* grown in pots. Twenty-seven steers were treated for aversion to *S. latifolius* plants grown in pots. Each of the steers (individually penned) was dosed *via* a stomach tube with sceleratine-NO equivalent to 100 g *S. latifolius* (DM) plus a dichloromethane extract equivalent to 50 g *S. latifolius* (DM) after food and water had been withheld overnight. The sceleratine-NO and dichloromethane extract dosed were mixed with 100 g maize meal as earlier described and shaken up in 1 L water before dosage. Hay and water were made available 8 h. following treatment. Steers were exposed to a *S. latifolius* plant grown in a pot (Fig. 5) 24 h. after treatment. Three treated steers were simultaneously compared with 3 untreated control steers at a time. Each of the treated steers were housed in a pen next to a control steer. The steers were made



Figure 5. Steer exposed to a *S. latifolius* plant grown in a pot.

accustomed to the environment by keeping them in the pens for a week prior to treatment. Two replications during the early part of the summer (October –April) were executed each year for 3 consecutive years. Total consumption of the plants was recorded after steers had been exposed to the plants for 24 h. and again after being exposed for an additional 48 h. (72 h. total). The results shown in Table 1 were statistically analyzed using Fisher’s Exact Test.

Table 1. Number of treated and control steers that totally consumed a *S. latifolius* plant throughout different periods of exposure during early and late summer.

Period of exposure (hours)	Number of steers that totally consumed the <i>S. latifolius</i> plant					
	Early summer (October – December)			Late summer (January – April)		
	Control (n=18)	Treated (n=18)	Significance of difference	Control (n=9)	Treated (n=9)	Significance of difference
24	9	0	P<0.01	*	*	*
72	11	1	P<0.01	5	4	P>0.05

* Not recorded

During early summer significantly less treated steers totally consumed the plant during both 24 h. and 72 h. exposure periods compared with controls. Except for

one of the treated steers that partially consumed the plant, all treated and control steers either totally consumed or totally avoided the plant. Nine of the 11 control steers totally consumed the plant within the first 24 h. exposure period. No difference between treated and control steers could be detected during late summer.

Challenging steers averted to *S. Latifolius* grown in pots with *S. latifolius* established on a pasture. Directly after termination of the pen trials executed during early summer of Year 3, three averted steers of each of the last two replications that refused eating *S. latifolius* grown in the pots were introduced to



Figure 6. Averted steers on an established *S. latifolius* pasture

S. latifolius established at a site on the premises of the institute (Fig. 6). Steers of the former replication were moved on to the established *S. latifolius* pasture for 3 days followed by steers of the latter replication, also for 3 days. They received *Eragrostis curvula* hay *ad lib*. No other plant material than *S. latifolius* was available to eat. The steers were monitored for clinical signs of seneciosis. None of the steers exhibited clinical signs of *S. latifolius* poisoning after 3 days. Serum aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) -values also remained within normal reference range.

DISCUSSION

The results show that treatment with scleratine-NO as an aversive agent in combination with a dichloromethane extract of *S. latifolius*, containing the sensory characteristics of the plant, effectively conditioned steers to avoid *S. latifolius* grown in pots during early summer. Only one of 18 treated steers totally consumed the *S. latifolius* plant within the 72 h. period compared to 11 of the control steers.

The results are a positive indication that conditioned feed aversion might potentially be used as a means to prevent livestock from being poisoned on *S. latifolius* infested pastures during early summer. This possibility is supported by the observation that 6 steers averted to *S. latifolius* grown in pots also did not develop clinical signs of poisoning after being kept on an established *S. latifolius* pasture for 3 days. These results should be followed up by field trials proving the use of sceleratine-NO together with a dichloromethane extract as aversive agent in conditioning livestock to avoid *S. latifolius*.

A possible explanation that no aversion to *S. latifolius* could be demonstrated for treated steers exposed to plants during late summer, might be ascribed to the fact that the sensory characteristics of the dichloromethane extract, obtained from *S. latifolius* collected during early summer, did not match the sensory characteristics of late summer *S. latifolius*. Further research needs to be done to clarify this matter.

It is interesting to note that treated and control steers, with the exception of one treated animal (early summer), either totally consumed or totally refused the plant during a 72 h exposure period, indicating an all or nothing approach. Nine of 11 control steers exposed to the plant during early summer totally consumed the plant within 24 h. Total intake/refusal suggests that refusal of *S. latifolius* by both averted and control animals took place merely by smelling.

The treatment regimen applied in these trials seemed to be safe as none of the animals exhibited clinical signs reminiscent of *S. latifolius* poisoning. Using pure sceleratine-NO as aversive agent ensures a controlled, safe and practical means of treatment. In contrast, using the plant material for establishing aversion might be risky as the toxicity of *S. latifolius* might vary under different conditions (Kellerman *et al.* 1988).

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