

Article

Not peer-reviewed version

Overview of Cyanide Poisoning in Cattle from Sorghum halepense and S. bicolor Cultivars in North-West Italy

Stefano Giantin , [Alberico Franzin](#) , Fulvio Bruşa , [Vittoria Montemurro](#) , [Elena Bozzetta](#) , [Elisabetta Caprai](#) , Giorgio Fedrizzi , [Flavia Girolami](#) , [Carlo Nebbia](#) *

Posted Date: 6 February 2024

doi: 10.20944/preprints202402.0315.v1

Keywords: Sorghum bicolor; Sorghum halepense; dhurrin; cyanide poisoning; cattle; drought



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Overview of Cyanide Poisoning in Cattle from *Sorghum halepense* and *S. bicolor* Cultivars in North-West Italy

Stefano Giantin ^{1,†}, Alberico Franzin ^{1,†}, Fulvio Bruşa ¹, Vittoria Montemurro ², Elena Bozzetta ², Elisabetta Caprai ³, Giorgio Fedrizzi ³, Flavia Girolami ⁴ and Carlo Nebbia ^{4,*}

¹ Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), S.S. Cuneo, Via Sandro Pertini 11, 12100 Cuneo, Italy; stefano.giantin@izsto.it (S.G.); alberico.franzin@izsto.it (A.F.); fulvio.brusa@izsto.it (F.B.)

² Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV), S.C. Diagnostica Specialistica, Via Bologna 148, 10154 Turin, Italy; vittoria.montemurro@izsto.it (V.M.); elena.bozzetta@izsto.it (E.B.)

³ National Reference Laboratory for Vegetal Toxins in Food and Feed, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Via Pietro Fiorini 5, 40127 Bologna, Italy; elisabetta.caprai@izsler.it (E.C.); giorgio.fedrizzi@izsler.it (G.F.)

⁴ Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; flavia.girolami@unito.it (F.G.); carlo.nebbia@unito.it (C.N.)

* Correspondence: carlo.nebbia@unito.it

† These authors contributed equally to this work

Simple Summary: Both wild (*Sorghum halepense*) and cultivated Sorghum (*Sorghum bicolor*) species are commonly used for animal feeding. However, sorghum plants naturally produce dhurrin, a cyanogenic glycoside releasing cyanide, an extremely toxic molecule potentially causing lethal toxicoses particularly in ruminants. This was the case in a number of farms located in North-western Italy during August 2022, under weather conditions (drought, tropical temperatures) known to increase dhurrin plant content. Sixty-six bovines died after grazing Sorghum pastures (*Sorghum bicolor* or *Sorghum halepense*) or being fed with Sorghum-containing hay (*Sorghum halepense*). The reported clinico-pathological findings clearly indicated cyanide poisoning, and chemical analysis revealed high concentrations of dhurrin in the plant materials. The successful management of the toxicosis should rely on the prompt removal of the contaminated fodder and the administration of the antidote sodium thiosulphate. Dhurrin content should be carefully monitored, particularly in the hot season, and both farmers and veterinarians should be made aware of the risks associated with feeding cattle even with cultivated Sorghum, particularly if grown under adverse climatic conditions.

Abstract: *Sorghum* plants naturally produce dhurrin, a cyanogenic glycoside which may be hydrolysed to cyanide resulting in often lethal toxicoses. Ruminants are particularly sensitive to cyanogenic glycosides due to the active role of rumen microbiota in dhurrin hydrolysis. This work provides an overview of a poisoning outbreak occurred in 5 farms from North-West Italy in August 2022; a total of 66 cows died, and many others developed an acute toxicosis after being fed on either cultivated- (*Sorghum bicolor*) or wild Sorghum (*Sorghum halepense*). Clinical signs were recorded, and all cows received an antidotal/supportive therapy. Dead animals were subjected to necropsy and dhurrin content was determined in Sorghum specimens by a LC-MS/MS method. Rapid onset, severe respiratory distress, recumbency and convulsions were the main clinical features; bright red blood, bitter almond smell and lung emphysema were consistently observed at necropsy. The combined i.v. and oral administration of sodium thiosulphate resulted in a rapid improvement of clinical signs. Dhurrin concentrations corresponding to cyanide levels higher than the tolerated threshold of 200 mg/kg were detected in sorghum specimens from 4 out of 5 involved farms; thereafter, such levels declined reaching tolerable concentrations in September–October. Feeding cattle with wild or cultivated Sorghum green fodder is a common practice in Northern Italy, especially in summer. However, care should be taken in case of adverse climatic conditions, such as severe drought and tropical temperatures (characterising summer 2022), which are reported to increase dhurrin synthesis and storage.

Keywords: *Sorghum bicolor*; *Sorghum halepense*; dhurrin; cyanide poisoning; cattle; drought

1. Introduction

Sorghum is a genus of plants belonging to the family of *Poaceae*, widely used as forage crop as well as human food and for biofuel production. Globally, the most cultivated species is *Sorghum bicolor* (L.) Moench, also known as broomcorn or the great millet, that is particularly spread in the Americas and Africa, which in 2017-2021 accounted together for more than 80% of total production. In 2021, Europe produced 1.9% of global Sorghum, with France being the first country with 386,040 t [1]. In Italy, the second European producer, Sorghum yielding reached 242,855 t in 2023, being the fourth cereal after wheat, corn and rice [2]. Some wild species are also exploited for animal feeding, like the widespread *Sorghum halepense* (L.) Pers., commonly referred to as Johnson grass, originating from the Mediterranean and Western Asia regions and now reported as an invasive weed across all the continents [3].

When used as feed, Sorghum management must be particularly careful because of a cyanogenic glycoside called dhurrin ((S)-4-Hydroxymandelonitrile β -D-glycoside) [4], which is synthesised as a secondary metabolite in its tissues. This molecule contains a cyanide group (CN^-) that can be released upon hydrolysis and is extremely toxic to every eukaryotic cells. CN^- inhibits cellular respiration by binding to the Fe^{+++} of cytochrome oxidase resulting in cell inability to utilize molecular oxygen and ultimately to synthesise ATP [5]. Rumen microbiota is able to rapidly hydrolyse dhurrin, further accelerating cyanide leak, making therefore ruminants much more sensitive to CN^- than monogastric species [6,7]. Such a rapid and massive CN^- release can cause severe, often lethal, poisonings in ruminants, particularly upon the ingestion of large amounts of high dhurrin-containing fodder [6].

In Sorghum plants dhurrin is produced especially during early growth phases [5,8]. Thanks to this glycoside, *Sorghum* species are quite resistant to herbivores, including insects [9]. Mature plants generally contain lower amount of dhurrin and are therefore considered safe for animal feeding; however, dhurrin content is reported to increase under the following conditions [10]:

- Prolonged drought, frost, wilting, chewing and any other condition causing plant cell injury;
- Massive herbicide treatments;
- Extensive use of nitrogen-based fertilizers.

When used as feed, Sorghum can be directly grazed by animals, or harvested for green forage, silage and hay production. Generally, ensiling process leads to a dispersal of CN^- from plant tissues. Still, in some cases high CN^- concentrations could remain in plants that have undergone rapid desiccation and subsequent conservation in large bales [9]. Because of CN^- poisoning potential, Sorghum harvesting and its use requires a cautious management in order to minimise intoxication risks, with special attention when used for feeding ruminants. Young leaves and new shoots, including the sprouts, are the most dangerous parts as they can concentrate large amounts of dhurrin [6].

Although Sorghum toxicity is long known, no poisoning cases in bovines have been reported in Europe in the last decades [11,12], with the exception of two cases in Spain quoted in a review on plant poisoning [13]. A search on grey literature also revealed no results in Europe, but several cases in both the Americas and Australia [14]. Likewise, data on Sorghum poisonings were found -both in scientific databases and online search engines- in extra-European countries, especially in semi-arid regions from South America [15–17] and India [18–20], where Sorghum cultivation for fodder purposes is common.

In August 2022, 66 bovines died in Piedmont (a region in North-western Italy) after being exposed to *S. bicolor* x *S. sudanense* -i.e., *S. bicolor* ssp. *sudanense* (P.) Stapf- cultivar called Suzy [21] or to forage containing *S. halepense*. The aim of this study is to provide a detailed overview of this outbreak, with special emphasis on the diagnosis and the therapeutic management of the toxicosis. Results of dhurrin concentration monitoring from August to November 2022 in both cultivated and wild *Sorghum* samples from the affected farms and elsewhere are also presented. A preliminary short report of the outbreak has been published in Italian in 2023 [22].

1.1. Poisoning cases (August 2022)

Five outbreaks of Sorghum poisoning occurred in August 2022 in Piedmont. Figure 1 shows the epidemiological data concerning the poisoning cases.

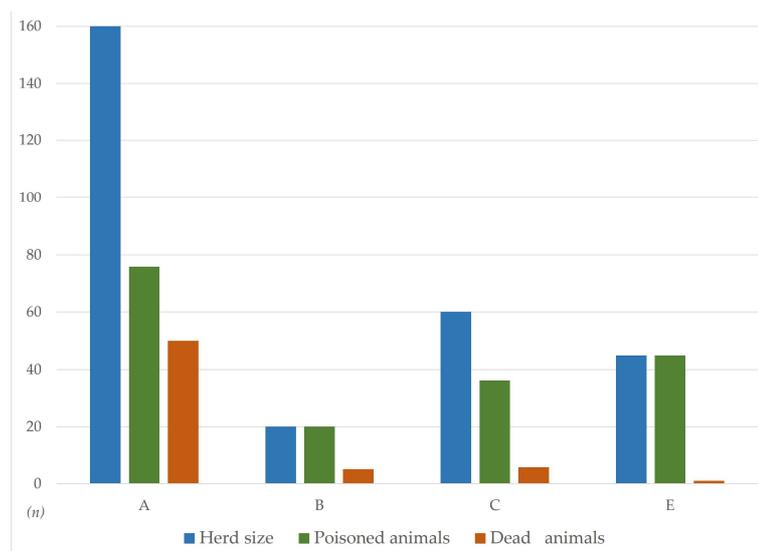


Figure 1. Epidemiological data (herd size, morbidity and mortality rate) of the five reported poisoning cases: A-Sommariva del Bosco; B-Moretta; C-Bra; E-Cossato. Case D-Asti is not shown due to the lack of reliable information.

1.1.1. Case A - 6th of August: Sommariva del Bosco (Cuneo)

A herd of 160 cows, mainly of Piedmontese breed, was allowed free access to a field entirely cultivated with *S. bicolor* x *S. sudanense* cultivar Suzy. As animals were hungry due to overnight fasting, they rapidly ingested Sorghum plants, and namely sprouts with a height range of 30-45 cm. Around half of the animals were poisoned; forty-six of them rapidly died 20-30 min after the ingestion (Figure 2a), while in 4 further individuals death ensued in the following hours. Most of the dead individuals were pregnant. The survived ones were immediately moved away. Based on the clinical picture, the sudden deaths and the gross lesions (see below), a cyanogenic glycoside poisoning was promptly suspected.

1.1.2. Case B - 11th of August: Moretta (Cuneo)

A group of 20 adult mixed breeds cows and bulls (mainly Friesian x Piedmontese or other meat breeds) housed in tie stalls were fed green chop (fresh forage) mainly composed by *S. halepense*. This is a common farming practice in Piedmont especially during the warm season, when green and high-quality forage is scarce. All the affected animals were lactating cows aged more than 3 years; five of them suddenly died after being offered the contaminated feed (Figure 2b). The forage was promptly removed from the troughs after the onset of the clinical signs of poisoning.



(a)



(b)

Figure 2. Case A-Sommariva del Bosco (a) and B-Moretta (b): poisoned/dead animals in lateral recumbency, mostly on the right side.

1.1.3. Case C - 11th of August: Bra (Cuneo)

Sixty adult cows of Piedmontese breed housed in a free stall barn were fed green chop, mainly consisting of Johnson grass. Poisoning signs were noticed during the subsequent night in 36 individuals: 4 of these suddenly died after grass ingestion, while in 2 further individuals death ensued few days later. As in case B, the forage was removed after the first symptoms and no further mortality was recorded.

1.1.4. Case D - 12th of August: Asti

This case occurred in a cow-calf operation farm consisting of about 60 Piedmontese breed heads (cows and calves) housed in a free stall barn. Animals are daytime allowed to graze on pastures in the proximity of the farm for most of the year. Four cows died after the ingestion of *S. halepense*, which was found to contaminate the pasture. This episode was lately and poorly reported to the veterinarians, so that it was not possible to collect reliable epidemiologic information.

1.1.5. Case E - 25th of August: Cossato (Biella)

Farm characteristics are similar to that from case D, i.e. a cow-calf operation farm with about 45 heads mainly of the Piedmontese breed (but also meat crossbreds). For most of the year, animals are free to graze on pastures surrounding the farm. All cows showed the typical signs of cyanide poisoning, mainly respiratory distress and tendency to recumbency; overall, symptoms were less severe than in case A, B and C, resulting in the loss of just one cow. Also in this case, the cause of poisoning was pasture contamination with *S. halepense*.

2. Materials and Methods

2.1. Necropsies and histological analysis

Due to unfavourable conditions (high external temperatures and the limited availability of veterinarians) necropsies were performed on few animals ($N=6$ in total) directly at farms. Heart, lung, brain, liver, kidney, spleen, reticulum, rumen, omasum, abomasum, and intestine samples were collected, fixed in 10% buffered formalin (4% formaldehyde), dehydrated and embedded in paraffin wax blocks. Each sample was then sectioned at 4-5 μm -thickness, mounted on glass slides and stained with haematoxylin and eosin to reveal histopathological alterations. Slides were examined by two independent veterinary pathologists.

2.2. Sorghum sample collection

To confirm the suspect of cyanogenic glycoside poisoning, samples of Sorghum cattle were exposed to were collected at each farm involved in the outbreak (Figure 3) and submitted for dhurrin determination (see below). It was also decided to collect and analyse additional specimens of both wild and cultivated Sorghum in order to measure dhurrin content in plants from different areas of the Piedmont region. In particular, the selection process was based on three main factors:

- Areas where strong drought conditions occurred [23];
- Requests of dhurrin analysis from a number of worried farmers;
- Financial resources.

In addition, in certain instances samples were collected from different plant portions and at diverse growth stages (Figure 3).

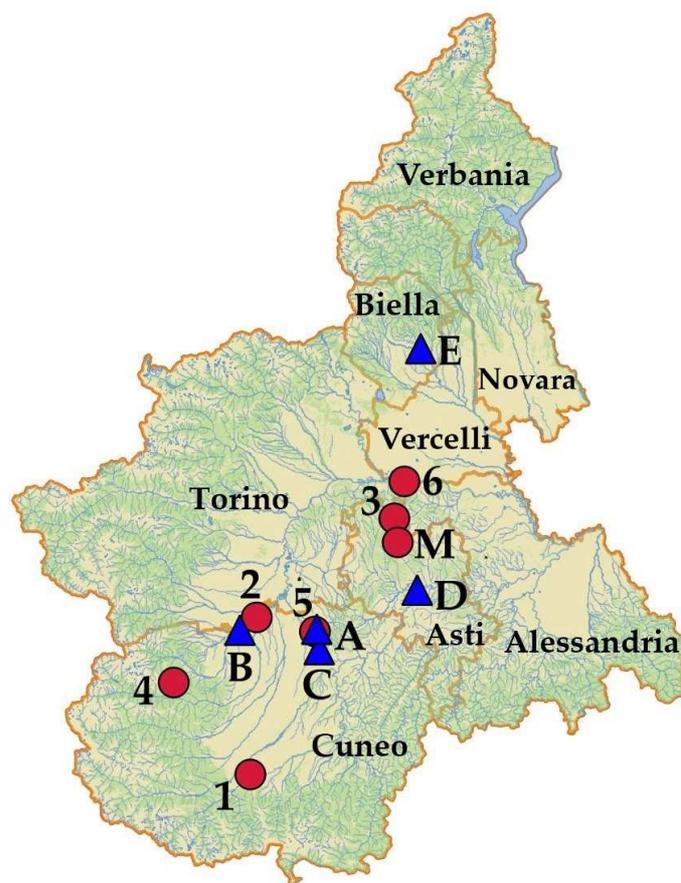


Figure 3. Map of the Piedmont region showing the location of the poisoning cases (blue triangles: A: Sommariva del Bosco, B-Moretta, C-Bra, D-Asti, E-Cossato), and the other farms selected for cultivated Sorghum sampling (red circle: M-Montechiaro d’Asti) or wild Sorghum sampling (red circles: 1-Cuneo, 2-Faule, 3-Montiglio Monferrato, 4-Sampeyre, 5-Sanfrè, 6-Verrua Savoia). All the samplings ($N=57$) were performed from August to November 2022. Orange lines indicate province borders.

As regards cultivated Sorghum, the *S. bicolor* × *S. sudanense* cultivar Suzy was involved in case A. Two cultivars, *S. bicolor* ssp. *drummondii* Piper and *S. bicolor* × *S. sudanense* Sudal [24], were then sampled in a farm in Montechiaro d’Asti (Asti), which was experiencing similar drought conditions as the farm of case A; Sorghum had not yet been harvested due to the severe outbreak occurred in Sommariva del Bosco.

The common Johnson grass, which is frequently used as fodder by Piedmontese farmers, was the cause of poisoning cases B, C, D and E. Further sites for *S. halepense* sampling were Verrua Savoia (Torino province), Montiglio Monferrato (Asti province), Cuneo, Faule, Sampeyre and Sanfrè (Cuneo province).

One pooled sample composed of a minimum of 500 g of fresh plant materials was collected randomly from different areas inside pasture fields or directly taken from the green forage offered to animals in the stalls. In one case (A) also the rumen content was collected from a dead cow. The whole sampling activities were accomplished from August to November 2022.

2.3 Dhurrin determination

Samples were analysed using an in-house liquid chromatography-tandem mass spectrometry (LC-MS/MS) method at the National Reference Laboratory for Plant Toxins, Food Chemical Department of *Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna (IZSLER)*, located in Bologna. Samples were grounded into flour and 1 ± 0.1 g of each one was extracted with 6 mL of aqueous methanol (80%). The sample was shaken vigorously for 30 seconds and placed in an ultrasonic water bath for 15 minutes. The mixture was centrifugated for 5 min at $4000 \times g$ and the

supernatant was collected in another tube. This extraction was repeated twice, the supernatant was combined and made up to a volume of 20 mL with water. Thereafter, 1 mL of solution was evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 0.5 mL of 10% methanol aqueous solution, diluted and analysed by using LC-MS/MS.

The LC-MS/MS analysis was performed on a XEVO Tq-XS Acquity ultra-performance liquid chromatograph (UPLC) I Class Plus Waters (Milford, MA, USA). The chromatographic separation was achieved on an Acquity UPLC C8 BEH 100 mm × 2.1 mm, 1.7 µm column (Water Corporation, Milford, MA, USA). Data acquisition and processing were carried out by MassLynx software v. 4.2. SCN1012. The mobile phase A consisted of 0.1% formic acid in water/acetonitrile (95:5, v/v), and the mobile phase B consisted in 0.1% acid formic in acetonitrile. The following gradient was used: 0-0.5 min, isocratic 2% B; 0.5-4 min linear gradient 2-50% B, it returned to initial conditions in 0.5 min and holding for 1 min. The total run time was 6 min. The flow rate was 0.4 mL/min. The injection volume was set at 5 µL. The ESI source operated in positive ionization mode with the following instrumental parameters: capillary voltage of 0.5 kV, cone voltage of 40 V, source temperature of 120°C, and a desolvation temperature of 600°C. The conditions of ionization and PA fragmentation were identified by continuous infusion of the tuning solutions and gradual adjustment of the parameters. According to SANTE/12089/2016 [25], dhurrin was identified by the retention time, ion fragments and ion ratio. The retention time was within ± 0.2 min of the reference peaks. The peaks showed similar shapes and overlapped with each other. The ion ratio was within ± 30% of the average of the calibration standards from the same sequence. The peaks were within the linear range of the detector with an S/N ≥ 3 [26]. The LC-MS/MS method's selectivity was evaluated by acquiring the data in MRM mode and monitoring one precursor ion and two daughter ions for each molecule [25].

Multi-level calibration curve-concentration levels from lowest to highest (0.2-0.5-1-2.5-5-10-15 µg/mL) was prepared in 10% methanol aqueous solution. A correlation coefficient (R^2) ≥ 0.99 and a normal distribution of residuals lower than 20% were achieved into every analytical batches. The calibration curve, a representative chromatogram of dhurrin reference material (2.5 µg/mL) and a chromatogram of a Sorghum sample are shown in Figure S1, S2 and S3.

The limit of quantification (LOQ) of dhurrin in feed was 50 mg/kg, corresponding to 4.3 mg/kg hydrogen cyanide (HCN), i.e. cyanide. It has been evaluated under conditions of accuracy and precision, verifying signal-to-noise ratio to be at least equal to 10. The recovery % (70-120) of the quality control spiked at LOQ was in line with the guidance document on performance criteria of the European Union Reference Laboratory for Mycotoxin and Plant Toxins [26]. According to EFSA Journal [27], 1 g of dhurrin releases 86.7 mg of HCN potential, representing the total amount of HCN released under conditions of complete hydrolysis of the present dhurrin. For the sake of simplicity, in this paper the HCN potential is referred to as HCN/cyanide concentration.

2.4 Clinical picture

Poisoned bovines showed multiple symptoms, with variable distribution among individuals. Many cows were found in sternal or lateral recumbency, mainly on the right side. Respiratory distress was observed in most of the poisoned animals consisting in tachypnoea, dyspnoea, panting and gasping. Several cows also displayed astonishment, convulsions, and muscle twitching with vocalizations (mooring). Sialorrhoea was an additional common symptom among intoxicated bovines. Moreover, light to moderate tympanism was detected in a few individuals. Hyperthermia, nystagmus, mydriasis and wheezes were occasionally observed.

2.5 Therapeutical protocols

Table 1 depicts the treatment performed in each case and the relative success rate.

Table 1. Treatments given, n. of survived animals and therapeutical success rate in the described outbreak of Sorghum toxicosis. Case D is not included because cows were not subjected to any treatment.

Case	Type of treatment	Treated animals	Survived animals	Success rate
A	Rehydrating solutions + multivitamin complex	30	26	87%

B	Sodium thiosulfate	5	5	100%
C	Methylene blue and (later) sodium thiosulfate	30	28	93%
E	Sodium thiosulfate	40	40	100%

2.5.1. Case A

Although, as mentioned above, a cyanogenic glycoside poisoning was suspected, it was difficult to find the proper remedies also because this outbreak happened during the weekend. Thirty animals were treated intravenously with a mix of rehydrating solutions (Ringer's lactate, physiological and glucose solutions), coupled with 60 mL of the multivitamin Dobetin B1® (cyanocobalamin 1 mg/mL, thiamine hydrochloride 100 mg/mL). Considered the hot external temperature -over 38 °C-, the cows were also cooled down by spraying with water taken from the mobile drinking troughs. Twenty-six of the treated animals survived.

2.5.2. Case B

Owing to the similarity with the clinical picture described for the Sommariva del Bosco poisoning (case A) and based on the first analytical results revealing the massive presence of dhurrin in sorghum samples from that case, the antidotal therapy was immediately started. However, due to the limited availability of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), it was decided to treat only the most severely affected individuals ($n=5$), lying in sternal/lateral recumbency with panting and vocalizations. Antidote solution was prepared by dissolving 5 g $\text{Na}_2\text{S}_2\text{O}_3$ in 4 L of Ringer's lactate, which was slowly administered i.v. (Figure 4).



Figure 4. Moretta (case B), a poisoned cow receiving the antidote (sodium thiosulfate) i.v. Note the cherry red blood on the neck of the animal.

Further 15 g of $\text{Na}_2\text{S}_2\text{O}_3$ were dissolved in 10 L of cold water and then given orally through drench guns. After 10-15 min from antidote administration, breathing started to improve and vocalizations almost ceased; cows were again able to stand in about one hour.

2.5.3. Case C

As mentioned above, poisoning symptoms were noticed during the night and this implied difficulties in getting $\text{Na}_2\text{S}_2\text{O}_3$ in sufficient amounts to treat all the affected animals ($N=30$). It was

therefore decided to administer first methylene blue i.v. (10 g dissolved in 4 L of Ringer's lactate), which however was only partially effective in reducing the severity of the clinical signs. As soon as $\text{Na}_2\text{S}_2\text{O}_3$ was fully available (late in the morning), it was readily administered i.v. (5 g dissolved in 4 L rehydrating solution) to all previously treated cows. This led to a rapid improvement of the clinical picture as described for case B. Twenty-eight cows survived, while 2 died few days later.

2.5.4. Case D

No treatment was performed.

2.5.5. Case E

Due to the alert system set up on purpose to tackle the cyanogenic glycoside outbreaks, the antidote $\text{Na}_2\text{S}_2\text{O}_3$ was made readily available to veterinarians. Accordingly, all poisoned animals were treated with the antidote as soon as 1 hr after the onset of clinical signs and a rapid recovery ensued within 2 hr from therapeutic intervention. Treatment schedule was the one detailed for case B.

3. Results

3.1 Gross lesions

Necropsy was performed on 6 carcasses: three from case A, one from case B and two from case C.

On post-mortem examination an intense sweet smell of bitter almonds was reported. The blood was bright red and clotted poorly. The tracheas were congested, with oedema, petechiae and a variable amount of froth; severe pulmonary emphysema and oedema were also noticed (Figure 5a). Hydropericardium, focal haemorrhages and necrosis of myocardium were observed (Figure 5b). The rumens were filled with fresh green material and bloated; suffusion and petechiae were present on rumens, reticulum and omasum as well. Congestion and petechial haemorrhages were observed in the gastrointestinal tract. Finally, abomasitis (Figure 5c), severe splenomegaly, enlarged and congested livers were observed in most of the animals. In a carcass from case C belonging to a cow which underwent the antidotal therapy but died two days after treatment subcutaneous gelatinous necrosis was additionally detected.



(a)

(b)

(c)

Figure 5. Necropsy findings in Moretta case (B), revealing lung emphysema (a), myocardial haemorrhages (b) and abomasitis (c).

3.2. Histopathological lesions

In most of the animals, the pulmonary parenchyma revealed foci of alveolar oedema, emphysema, and congestion of capillaries. Histologically, type-I pneumocytes necrosis and hyperplasia of type-II pneumocytes with hyaline membranes were observed, along with thickening

of septa due to mononuclear cell infiltration. Three individuals presented hearts with large haemorrhagic areas in myocardial and pericardial adipose tissue. A focal fibrosis and a moderate multifocal non purulent myocarditis were also observed.

In one cow, died two days after treatment (case C), the abomasum revealed an acute abomasitis characterized by severe hyperaemia, haemorrhagic exudate red-brownish in colour adherent to the mucosa and neutrophilic infiltration with focal oedematous-haemorrhagic fluid in the submucosa. Thrombosis and epithelial regressive alterations were also observed in the mucosa.

3.3. Dhurrin determination

Sorghum samples were taken from the farms involved in poisoning outbreaks and from other selected farms and fields, as detailed in Materials and Methods.

Table 2 reports the results related to the five intoxication cases. In case D two samples from different areas of the pasture were collected. In case E three samples from distinct parts of the plants and maturity stages were tested. HCN concentrations are expressed in mg/kg; values over 200 mg/kg are generally considered dangerous [6,28]. In all but one case Sorghum HCN concentrations were higher than 200 mg/kg; only in case E no samples reached 200 mg/kg. Also, in one sample picked on the border of the pasture from case D, HCN concentration measured 9 mg/kg.

Table 2. Dhurrin and hydrogen cyanide (HCN) concentrations in *Sorghum* samples implied in the five outbreaks of cyanogenetic glycoside poisoning occurring in Piedmont in August 2022. When plant part is not specified, analysis has been performed on the whole plant.

Case	Date	Sorghum species	Site of collection	Dhurrin (mg/kg)	HCN (mg/kg)
A	6-Aug	Suzy ¹ (sprout, height 30-45 cm)	Pasture	10.717	929
B	16-Aug	<i>S. halepense</i>	Trough	5.627	487
C	16-Aug	<i>S. halepense</i>	Trough	7.961	690
D	16-Aug	<i>S. halepense</i>	Pasture	4.834	419
		<i>S. halepense</i>	Pasture border	104	9
E	25-Aug	<i>S. halepense</i> (young plants)	Pasture	335	29
		<i>S. halepense</i> (leaves mix)	Pasture	488	42
		<i>S. halepense</i> (inflorescence)	Pasture	105	9

¹ *S. bicolor* x *S. sudanense* cultivar called Suzy.

One sample of ruminal content from a dead cow (case A) was also tested for dhurrin content with a negative result.

Dhurrin and HCN concentrations relative to the two farms selected for cultivated forage Sorghum are reported in Table 3 (Case A - Sommariva del Bosco) and Table 4 (Montechiaro d'Asti). For both farms, HCN content remained high for the whole period of August, tended to decline toward tolerable levels in September and reached negligible levels only in fall.

Table 3. Time course of dhurrin and hydrogen cyanide (HCN) concentration in the *Sorghum bicolor* x *Sorghum sudanense* variety called Suzy from Sommariva del Bosco (case A). When plant part is not specified, analysis has been performed on the whole plant.

Date	Dhurrin (mg/kg)	HCN (mg/kg)	Notes
6-Aug	10.717	929	Sample relative to the outbreak (case A)
14-Aug	6.869	596	Average plants height 50 cm
16-Aug	14.246	1,235	Open-air dried
16-Aug	5.590	485	Fresh, leaves > 1 m
16-Aug	8.300	720	Fresh, leaves < 50 cm
17-Aug	< LOQ	0	Bundled; cut of 14 th of July
21-Aug	6.550	568	Average plants height 60 cm

27-Aug	7.661	664	Average plants height 68 cm
5-Sept	1.420	123	
12-Sept	1.798	155	
23-Sept	958	83	
27-Sept	974	84	Field "Paolorio"
27-Sept	1.354	117	Field "Luppiano"
27-Sept	2.707	235	Field "Valè"
6-Oct ¹	< LOQ	0	Fresh, chopped
23-Nov	< LOQ	0	Mature silo (45 days), from mixed fields

¹ The same result (0 mg/kg HCN) was measured in six samples from 5 different fields.

Taken together, no clear differences in HCN concentrations between different portions of the plants were noticed, with the exception for the culm. Indeed, in Montechiaro d'Asti two culm samples collected on the 30th of August revealed lower HCN concentrations (0 and 147 mg/kg, respectively) than leaves and inflorescences ($n=7$) displaying HCN values in the range 155-868 mg/kg. Also, no strong evidence of a higher HCN content was found in younger/lower individuals with respect to older/taller ones, even though two samples picked from ensiled bales on the 17th of November revealed a low HCN level (19 mg/kg) in a bale made of low immature plants (height < 50 cm), and no HCN at all in a bale made of mature individuals (height > 150 cm).

Table 4. Time course of dhurrin and hydrogen cyanide (HCN) concentration in *Sorghum* samples from a farm in the Asti province (Montechiaro d'Asti), located near case D. All the samples belong to mixed individuals grown from a seed mixture of two varieties: the *S. bicolor ssp. drummondii* Piper, and the *S. bicolor* × *S. sudanense* Sudal. When plant part is not specified, analysis has been performed on the whole plant.

Date	Dhurrin (mg/kg)	HCN (mg/kg)	Notes
16-Aug	9.770	847	Sowed at the beginning of June, never cut
16-Aug	2.840	246	Grown back plants
16-Aug	2.065	179	Sowed at the beginning of June, grazed in July
30-Aug	1.792	155	Field 1; leaves > 150 cm
30-Aug	1.919	166	Field 1; leaves ~ 50 cm
30-Aug	< LOQ	0	Field 1; culm
30-Aug	3.251	282	Field 1; inflorescence
30-Aug	1.865	162	Field 1; grown back plants, without roots
30-Aug	10.010	868	Field 2; leaves > 150 cm
30-Aug	6.701	581	Field 2; leaves ~ 50 cm
30-Aug	1.697	147	Field 2; culm
30-Aug	4.553	395	Field 2; inflorescence
26-Sept	3.967	344	Culm and leaves
26-Sept	205	18	Inflorescence
17-Nov	229	19	Immature plants (without grains) ~ 50 cm; from ensiled bale
17-Nov	< LOQ	0	Mature plants (with grains) > 150 cm; from ensiled bale

Concurrently, a set of samples of *S. halepense* were gathered from several farms and fields scattered across Cuneo, Asti and Torino provinces. Their dhurrin and HCN concentrations are listed in Table 5. Despite the close proximity of some of the sampling sites with farms experiencing poisoning cases from cyanogenic glycosides, only in one case the threshold of concern of 200 mg/kg HCN was reached, even in specimens collected in August. The frosted sample collected on the 23rd

of November in Sampeyre, in a mountain area, revealed dhurrin concentrations < LOQ. In addition, a negative result was found in a sample from case D made of mixed grasses.

Table 5. Dhurrin and hydrogen cyanide (HCN) concentrations in *Sorghum halepense* collected from different farms and fields in Piedmont during 2022. The analysis has been performed on the whole plants.

Date	Location	Province	Dhurrin (mg/kg)	HCN (mg/kg)	Notes
16-Aug	Asti	Asti	< LOQ	0	Mixed grasses
17-Aug	Faule	Cuneo	85	7	Cut for haymaking
17-Aug	Verrua Savoia	Torino	1.558	135	Field used for haymaking
17-Aug	Montiglio M.to	Asti	2.036	176	Field "Sant'Anna"
17-Aug	Montiglio M.to	Asti	2.693	233	Field "Acquedotto"
17-Aug	Montiglio M.to	Asti	1.917	166	Field "Vallone"
5-Sept	Bra	Cuneo	289	25	Field of case C; forage for silo
15-Sept	Cuneo	Cuneo	57	5	Plants > 50 cm
15-Sept	Cuneo	Cuneo	401	35	Plants < 50 cm
16-Sept	Sanfrè	Cuneo	< LOQ	0	Mature plants (with inflorescence)
23-Nov	Sampeyre	Cuneo	< LOQ	0	Frosted plants

Overall, no relevant differences in average HCN concentrations were noted among older/taller ($n=6$, 393 mg/kg) and younger/lower individuals ($n=10$, 356 mg/kg).

The seasonal trend of cyanide concentrations in all collected samples ($N=57$) of either cultivated or wild Sorghum is depicted in Figure 6. Overall, a clear decreasing trend was noted: in August 2022, 58% of samples were found to contain levels > 200 mg/kg, while from September to November such amounts were detected in just 8% of the specimens.

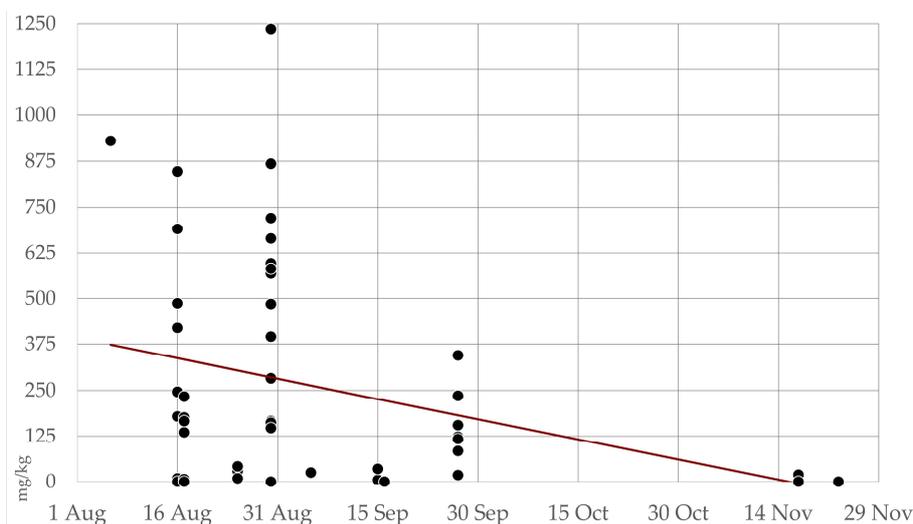


Figure 6. Seasonal trend of hydrogen cyanide (HCN) concentration in all cultivated and wild collected Sorghum samples. Figure shows aggregate data ($N=57$) of the whole sampling in Piedmont, including all areas from August to November 2022.

4. Discussion

The rapid onset of clinical signs in cows shortly after the ingestion of Sorghum, followed sometimes by sudden death, had immediately suggested cyanide poisoning. Respiratory distress, stupor, sternal or lateral recumbency, convulsions, muscle tremors, and sialorrhoea are typically

reported in cyanide poisoning in cattle [6,29]. In addition, the recorded intense sweet odour of “bitter almonds”, the bright cherry red colour of venous blood, lung congestion and emphysema as well as the presence of froth in trachea are consistently recorded in cyanogenic glycoside poisoned bovines [30]. The detected abomasitis, that featured oedematous-haemorrhagic and neutrophilic granulocyte infiltrations, has been also associated with cyanide poisoning [6]. Finally, myocardial haemorrhages further pointed to cyanide poisoning [31].

The gold-standard therapy for cyanide toxicosis [6,32] consists of supplying a chemical agent able to induce the formation of methaemoglobin (MetHb), i.e. oxidized (Fe^{+++}) haemoglobin, which is unable to bind O_2 and making it available to tissues. However, cyanide shows a higher affinity toward the Fe^{+++} central haem iron of MetHb than the Fe^{+++} of cytochrome oxidase. This causes the release of cyanide from the enzyme, the formation of cyanMetHb and the reactivation of cell respiration. MetHb formation in large animals may be primarily accomplished by administering sodium nitrite i.v. (10 to 20 mg/kg bw); this treatment should be repeated with great care, because of the danger of producing nitrite toxicosis, with further impairment of cellular respiration and severe hypotension [30]. Methylene blue at high dosages (1 to 3 g/~250 kg bw) has been recommended as an alternative to nitrites [31]. This treatment must be coupled with the sulphur donor $\text{Na}_2\text{S}_2\text{O}_3$, which, in the presence of rhodanese, reacts with HCN yielding thiocyanate (SCN^-); this metabolite lacks any detrimental effects on cellular respiration and is rapidly excreted via the kidneys. In the hereby reported cases, coupling methylene blue and $\text{Na}_2\text{S}_2\text{O}_3$ administration did not seemingly result in a visible improvement of the therapeutic efficacy; a significant and rapid relief of the clinical signs was indeed obtained only after $\text{Na}_2\text{S}_2\text{O}_3$ treatment, which was successfully used alone in cases B and E with 100% efficacy. It has been actually reported that in cattle there is no benefit in administering i.v. a MetHb-inducing agent over $\text{Na}_2\text{S}_2\text{O}_3$ alone [32]. In addition, the prompt oral dosing with $\text{Na}_2\text{S}_2\text{O}_3$ may help in detoxifying HCN released in the rumen even before the onset of clinical signs [33]. The overall good success of the antidotal treatment further confirmed the diagnosis of cyanide poisoning. It should be noted that treated cows from case A had a relatively high survival rate (87%) even though they did not receive specific antidotes, but only a palliative fluid therapy with a multivitamin complex. The prompt removal from the contaminated pasture, i.e. after the first sudden deaths, was likely the cause of the high recovery rate.

According to the European Directive 2002/32/EC [34], a threshold of 50 mg/kg cyanide has been established for animal feed and raw materials. Under field conditions, concentrations over 200 mg/kg are considered sufficient to induce overt toxicosis [6,28,31].

It is generally assumed that crop plants are less resistant to parasites and herbivores with respect to their wild counterparts due to the artificial genetic selection aiming at reducing the content of specific defence compounds (e.g., cyanogenic glycosides) that may prove harmful for humans and livestock [35]. However, this assumption cannot be generalized for Sorghum. Unexpectedly, broomcorn cultivars such as Suzy (a *S. bicolor* × *S. sudanense* variety, Sommariva del Bosco, case A) and the mixture of Piper and Sudal (Montechiaro d’Asti), revealed very high HCN concentrations in August 2022. Both cultivars are specifically marketed for animal feeding purposes; however, guidelines for use reported on seeds’ envelopes do recommend not to feed animals when plants are below 70/80 cm (70 cm for the mixture Piper and Sudal, and 80 cm for Suzy) and yet without any information on the potential related danger [36]. In case A, the farmer decided to allow his herd to graze on the field despite the sorghum plants were below the recommended height. As many other farmers during that summer, his farm was experiencing a shortage of forage due to its high cost and the scarce availability of green pastures. The increase in forage prices were a direct consequence of a lower offer on the market that, in turn, was caused by a diffuse drought particularly affecting the North-western of Italy. A parallel survey was conducted on cultivated hybrids (*S. bicolor* ssp. *Drummondii* Piper and *S. bicolor* × *S. sudanense* Sudal) from different fields surrounding a farm in the Asti province (Montechiaro d’Asti) near to poisoning case D; HCN concentrations > 200 mg/kg were detected in 50% of specimens collected in August 2022, with peaks of 847-868 mg/kg. Overall, our findings confirm that bovines should not be fed on young plants even of cultivated hybrids, including regrowth after cutting, because of the high risk of cyanide poisoning.

In the outbreak of cyanogenic glycoside poisoning in cows described herein, *S. halepense* was implied in 4 out 5 cases. Johnson grass is considered among the most invasive and dangerous weeds in Europe and extra European countries; beside the potential accumulation of toxic amounts of

cyanogenic glycosides, several potentially adverse effects have been reported, including displacement of natural flora, competition with other crops, synthesis of allelochemicals interfering with crop growth, and hosting of plant pathogens (for a review, see Peerzada et al. 2017 [37], and the numerous literature references therein). Despite that, the free growth of Johnson grass is rarely counteracted, and even, as reported in four cases (B to E), farmers traditionally employ Johnson grass as a fodder plant (hay or pasture) during periods of droughts. Similar to other Sorghum species, several factors, including soil chemical composition, plant age, use of nitrogen fertilizers, weather conditions as well as damage to plant tissues, are reported to affect dhurrin content and hence the potential HCN release in Johnson grass [34]. There is scant information on dhurrin and HCN content of *S. halepense*, particularly from European countries. In a study performed in India, calculated HCN concentrations (colorimetric method) of uncultivated Johnson grass from farm bunding averaged around 900 mg/kg at 30 days post weeding but fall to 120 mg/kg at 25% flowering stage [38]. Therefore, similar to cultivated Sorghum species, cattle should not be fed with Johnson grass at the early stage of crop. In the outbreaks reported here, 3 poisoning cases concerned with *S. halepense* revealed HCN concentrations in the range 419-690 mg/kg (cases B, C, D). The cause of the relatively low amount of HCN (below 50 mg/kg) detected in plant specimens from case E is probably attributable to uncorrected sampling procedures. For comparison, samples of *S. halepense* were collected in a more scattered way during August and September 2022 in fields from farms located in different areas of Piedmont, even near to poisoning cases (Sanfrè, Faule, Montiglio Monferrato); of note, only in one case HCN amounts > 200 mg/kg were detected in plant specimens, likely pointing to the occurrence of different pedo-climatic conditions not resulting in remarkable accumulation of dhurrin as it was reported for the areas of the outbreak.

As a matter of fact, in summer 2022 unfavourable weather conditions were registered all across Europe, and Northern Italy, particularly certain areas of Piedmont, resulted one of the driest regions [39]. According to the Piedmont Regional Agency for Environmental Protection (ARPA), summer 2022 was as one of the hottest and driest of the last 30 years in Piedmont [23]. Indeed, during that summer unprecedented temperatures were registered, occasionally reaching values at an all-time high (Figure S4). Also, the number of tropical nights ($T > 20^{\circ}\text{C}$) and days ($T > 30^{\circ}\text{C}$) were higher than in previous years (Table S1). Moreover, rainfalls were irregular, both in terms of quantity and regional distribution, with a decrease of 50-60% with respect to previous years especially in areas where cyanide poisoning outbreaks occurred (Figures S5-S6). Finally, hydric balance was in deficit since the previous winter (Figure S7), also due to limited snow reserves. These conditions were reasonably responsible for the excessive accumulation of dhurrin observed in most *Sorghum* specimens collected in the outbreak and surrounding areas.

5. Conclusions

The use of *Sorghum* plants for cattle feeding is a common practice in Northern Italy, where the most abundant species are the wild weed *S. halepense* and several cultivars of *S. bicolor*. Data from the described outbreaks in Piedmont indicate that not only Johnson grass but also different Sorghum cultivars may accumulate dhurrin concentrations which can trigger lethal poisoning of cows, particularly if animals are fed with young plants or sprouts and under adverse climatic conditions (drought, tropical temperatures). As expected, the decrease in diurnal and nocturnal temperatures together with the increase in the amount of precipitation occurring in October and November caused dhurrin levels to decline and thus HCN concentrations to fall well below the toxic threshold of 200 mg/kg.

An accurate management is needed when using Sorghum forages and specific instructions should be reported on every commercial *Sorghum* seeds' envelope. Moreover, both farmer unions and local authorities should disseminate technical information on how to avoid toxicoses when feeding animals with cultivated Sorghum, including instructions/good practices for a safe use of *S. halepense* as a fodder. Finally, our data further support the use of sodium thiosulfate alone in the treatment of cyanogenic glycoside poisonings in cattle, suggesting that this antidote should be made readily available for veterinary practitioners in order to ensure a rapid and efficacious intervention.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Numbers of tropical days ($T > 30^{\circ}\text{C}$) and nights ($T > 20^{\circ}\text{C}$) measured in

every province of Piedmont in 2022.; Figure S1: Calibration curve of dhurrin; Figure S2: Representative chromatograms of dhurrin reference material at 2.5 µg/mL.; Figure S3: Representative chromatograms of a Sorghum sample. Figure S4: Daily temperature anomaly in Piedmont during the summer 2022 compared to the period 1991-2020. Figure S5: Rainfall anomaly (%) in Piedmont basins during summer 2022 compared to the period 1991-2020; Figure S6: Rainfall anomaly (%) in Piedmont basins during September 2022 compared to the period 1991-2020; Figure S7: Daily hydro-climatic balance (i.e. the difference between rainfall and evapotranspiration expressed in mm) in Piedmont in 2022 compared to the period 1959-2022. Data in Table S2 and Figures S4-S7 were extracted from the 2022 drought report, published by the Piedmont Regional Agency for Environmental Protection (ARPA) [], openly available at www.arpa.piemonte.it/publicazioni-2/relazioni-tecniche/analisi-eventi/eventi-2022/rapporto-siccita-in-piemonte-2022.

Author Contributions: Formal analysis: A.F.; funding acquisition: F.B.; investigation: S.G., A.F., V.M., E.B., E.C., and G.F.; resources: S.G., A.F. and F.B; supervision: C.N.; visualization A.F. and F.G.; writing-original draft preparation: A.F., V.M., E.C., E.B., G.F. and S.G.; writing-review and editing: A.F., F.G. and C.N. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported from *Fondazione CRT*, a private non-profit organization (Turin, Italy; grant number 2022.1784 “Il pascolo e la tossicità delle piante erbacee: prevenzione e trattamento”, 7/12/2022).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The Authors would like to thank, for their availability during sampling and data collection, the farmers G. Olivero (Sommariva del Bosco, case A), E. Bollati (Moretta, case B), M. Cravero (Bra, case C), D. Viarengo (Asti, case D) and M. Bandiera (Montechiaro d’Asti); we thank the veterinarians that treated the cows (Dr G. Topi – freelance; Drs F. Barberis and F. Acciardi of ASL CN 2 – Veterinary Services Cuneo province).

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. FAOSTAT. Available online: www.fao.org/faostat/en/#data/QCL (accessed on 22 November 2023).
2. ISTAT. Available online: dati.istat.it/Index.aspx?DataSetCode=DCSP_COLTIVAZIONI (accessed on 21 December 2023)
3. Gunes, E.; Uludag, A.; Uremis, I. Economic impact of johnsongrass (*Sorghum halepense* [L.] Pers.) in cotton production in Turkey. *J. Plant Dis. Prot.* **2008**, *21*, 515–520.
4. Yadav, M.; Singh, I.K.; Singh, A. Dhurrin: A naturally occurring phytochemical as a weapon against insect herbivores. *Phytochemistry* **2023**, *205*, 113483.
5. Gleadow, R.M., Møller, B.L. Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. *Annu. Rev. Plant Biol.* **2014**, *65*, 155–185..
6. Panter, K. E. Cyanogenic Glycoside-Containing Plants. In *Veterinary Toxicology: basic and clinical principles*, 3rd ed.; Gupta, R.C., Eds; Elsevier - Academic Press: Cambridge, USA, 2018; pp. 935–940.
7. Bhat, B. V. Breeding Forage Sorghum. In *Breeding Sorghum for Diverse End Uses*, 1st ed.; Aruna C., Visarada, K.B.R.S., Venkatesh Bhat, B., Vilas Tonapi, A., Eds.; Woodhead Publishing: Cambridge, UK, 2018; pp. 175–191.
8. Mandrone, M.; Chiocchio, I.; Barbanti, L.; Tomasi, P.; Tacchini, M.; Poli, F. Metabolomic Study of Sorghum (*Sorghum bicolor*) to Interpret Plant Behavior under Variable Field Conditions in View of Smart Agriculture Applications. *J. Agric. Food Chem.* **2021**, *69*, 1132–1145.
9. Nahrstedt, A. Cyanogenic compounds as protecting agent for organisms. *Plant Syst. Evol.* **1985**, *150*, 35–47.
10. Lilian, A.; Ouma, Erick K. Cheruiyot, Joshua O. Ogendo, Biosynthesis and Role of Dhurrin in Forage Sorghum, *Reviews in Agricultural Science*, 2023, Volume 11, Pages 259-270
11. Bertero, A.; Davanzo, F.; Rivolta, M.; Cortinovia, C.; Vasquez, A.; Le Mura, A.; Masuelli, A.; Caloni, F. Plants and zootoxins: Toxic-epidemiological investigation in domestic animals. *Toxicon* **2021**, *196*, 25–31.
12. Nagy, A.-L.; Ardelean, S.; Chapuis, R. J. J.; Bouillon, J.; Pivariu, D.; Dreanca, A. I.; Caloni, F. Emerging Plant Intoxications in Domestic Animals: A European Perspective. *Toxins* **2023**, *15*, 442.
13. García-Arroyo, R.; Quiles Sotillo, A.; Hevia Méndez, M. L.; Míguez Santiyán, M. P. Agentes causales de intoxicaciones mortales en ganado bovino en España. *Informacion Tecnica Economica Agraria* **2017**, *113*, 228–243
14. Google. Available online: www.google.com (accessed on 22 September 2023). The keywords used were: “cattle sorghum poisoning”, “intoxication sorgho bovins”, “intoxicação sorgo bovinos” and “intoxicación sorgo bovino”. Each search was run twice, with a second one adding “2023” in the string.

15. da Nóbrega Jr, J. E.; Riet-Correa, F.; Medeiros, R. M. T.; Dantas, A. F. M. Intoxicação por Sorghum halepense (Poaceae) em bovinos no semi-árido. *Pesquisa Veterinária Brasileira* **2006**, *26*, 201–204.
16. Juffo, G. D.; Pavarini, S. P.; Wouters, F.; Oliveira, L. G. S.; Antoniassi, N. A. B.; Cruz, C. E. F.; Driemeier, D. Intoxicação espontânea por Sorghum sudanense em bovinos leiteiros no Rio Grande do Sul. *Pesquisa Veterinária Brasileira* **2012**, *32*, 217–220.
17. Riet-Correa, F.; Machado, M.; Micheloud, J. F. Plants causing poisoning outbreaks of livestock in South America: A review. *Toxicon: X*, **2023**, *17*, 100150.
18. Muwel, N.; Verma, R.; Nigwal, D.; Waskel, L.; Gurjar, R., Ingole, M. Sorghum poisoning in buffaloes and its treatment *J. Pharmacogn. Phytochem.* **2018**, *7*, 3737–3739.
19. Sinha, R. K.; Kumari, B.; Kumar, A.; Kumar, A.; Azad, C. S. Sorghum Poisoning in Cattle and its Therapeutic Management. *JAS* **2019**, *6*, 108–109.
20. Rajasokkappan, S.; Rajan, T.; Raghavendran, V.B. Sorghum poisoning in a cow and it's successful management. *Pharma Innovation* **2020**, *9*, 164–165.
21. OECD (Suzy cultivar). Available online: www.niab.com/oecd/v2/variety/details/100558/suzy/0/0/vs/ALL/ALL/0 (accessed on 22 December 2023).
22. Giantin, S.; Franzin, A.; Topi, G.; Fedrizzi, G.; Nebbia, C. Outbreaks of lethal cyanogenic glycosides poisonings of cattle after ingestion of Sorghum ssp. Grown under drought conditions in August 2022 in Piedmont (North-Western Italy). *LAR*, **2023**, *29*, 171–175 .
23. ARPA Piemonte. Rapporto Siccità in Piemonte 2022 - A cura del Dipartimento Rischi Naturali e Ambientali, Torino **2022**. Available online: www.arpa.piemonte.it/pubblicazioni-2/relazioni-tecniche/analisi-eventi/eventi-2022/rapporto-siccita-in-piemonte-2022 (accessed on 21 November 2023)
24. OECD (Piper cultivar). Available online: www.niab.com/oecd/v2/variety/details/2691/piper/1/1/vs/ALL/ALL/0 (Accessed on 22 December 2023).
25. European Commission. *Health and Food Safety Directorate-General (DG SANTE). Guidance Document on Identification of Mycotoxin in Food and Feed. SANTE/12089/2016*; European Commission DG SANTE: Bruxelles, Belgium, 2016.
26. European Union Reference Laboratory (EURL) for Mycotoxins and Plant Toxins. *EURL MP-Background Doc_003 Guidance Document Performance Criteria v1.1 Draft*; Wageningen Food and Safety Research: Wageningen, The Netherlands, 2021.
27. EFSA Panel on Contaminants in the Food Chain (Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, LeblancJ-C, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Vlemingx C, Wallace H, Benford D, Brimer L, Mancini FR, Metzler M, Viviani B, Altieri A, Arcella D, Steinkellner H and Schwerdtle T). Scientific opinion on the evaluation of the health risks related to the presence of cyanogenic glycosides in food sother than raw apricot kernels. *EFSA J.* **2019**, *17*, e05662.
28. Gensa, U. Review on Cyanide Poisoning in Ruminants. *Journal of Biology, Agriculture and Healthcare* **2019**, *9*, 1.
29. Vetter, J. Plant cyanogenic glycosides. *Toxicon* **2000**, *38*, 11–36.
30. Hatch, R.G. Poisons causing respiratory insufficiency. In *Veterinary Pharmacology and Therapeutics*, 5th ed.; Booth, N.H., McDonald, L.E., Eds; The Iowa State University Press: Ames, Iowa, USA, 1982; pp. 933–975.
31. Pickrell, J.A.; Oehme, F. Cyanogenic glycosides. In *Clinical Veterinary Toxicology*, 1st ed.; Plumlee, K.H., Ed; Mosby: St.Louis, Missouri, USA, 2004; pp. 391–392.
32. Roder, J.D. *Veterinary Toxicology*, 1st ed.; Butterworth-Heinemann: Boston, USA, 2001; pp. 138–142.
33. Nicholson, S.S. Southeastern Plants toxic to ruminants. *Vet Clin Food Anim* **2011**, *27*, 447–458.
34. EC Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed, 7 May 2002, pp. 1–27. *Off. J. Eur. Union L* **2002**, *140*, 10–21.
35. Al-Sultan, S.I. Sorghum halepensis and its cyanide content. *Pakistan Journal of Nutrition* **2003**, *2*, 123–124.
36. Zandrea sementi. Available online: zanandreasementi.it/prodotti/suzy-sorgo-foraggero-multi-sfalcio-da-fasciato-trinciato/ (accessed on 22 December 2023).
37. Peerzada, A.M.; Ali, H.H.; Hanif, Z. et al. Eco-biology, impact, and management of Sorghum halepense (L.) Pers.. *Biol Invasions* **2023**, *25*, 955–973.
38. Jadav, C.N.; Makwana, R.B.; Parikh, S.S. Assessment of Hydrocyanic Acid (HCN) Content during Different Stages of Growth in Johnson Grass (Sorghum halepensis). *Agric. Rev.* **2022**, *43*, 394–397.
39. Toreti, A.; Bavera, D.; Acosta Navarro, J. et al. Drought in Europe August 2022, EUR 31192 EN, Publications Office of the European Union, Luxembourg, 2022.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.