

Ingestion Of *Viburnum x rhytidophylloides* 'Alleghany' Potentially Lethal To White-Mantled Black Colobus (*Colobus guereza*)

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In December of 2000, three white-mantled black colobus (*Colobus guereza*) housed at the Denver Zoological Gardens began showing indications of pain, and as a result, were removed from their facility and hospitalized. One animal died of gastric ulcers, however the others recovered with treatment. Light microscopic evaluation of gastric biopsies from one of the monkeys, showed inflammation of the mucosal layer of the stomach with ulceration. A small spicule of plant material was embedded in the inflammatory exudate associated with the stomach lining. Management records indicated that the animals had been moved to a new exhibit in September upon which time they devoured a small shrub *Viburnum x rhytidophylloides* 'Alleghany' (found in the new exhibit) in its entirety (leaves, buds, flowers and stems). This viburnum has dark, green, leathery foliage with small yellowish-white flowers and red fruit. A commonly used landscaping plant, the viburnum tends to be semi-evergreen in nature, holding its leaves well into spring. This plant is one not readily consumed by most animal species, but some cultivars of viburnum are on preferred browse lists for colobus. Lumina imaging showed tops of mature viburnum leaves (collected in January) to contain few "spicule-like" structures that could potentially become embedded in the gastric lining of an animal. However, leaf bottoms were an "intense" mat of spicules. Scanning electron microscopy (SEM) showed that the spicules were bottle-brush in form, but not hooked in shape. Initial SEM observations of buds (collected in January) indicated some hooked spines, possibly those that are more immature. Initial results of Energy Dispersive X-ray Spectroscopy (EDS) indicated that the spicules were calcium-based. Zoological institutions need to be aware of potential health concerns if animals consume this viburnum cultivar.

Key words: browse; primates; spicules

INTRODUCTION

The importance role of browse (leaves and twigs from shrubs, trees and herbaceous plants) in certain zoological species is becoming more evident in the zoo community. Browse is commonly being supplemented not only as a form of enrichment, but in some cases as an important nutritional supplement to an animal's regular diet. This is most important to those animal species classified as folivores. To date, little research has been done to substantiate safety of browse species with individual institutions having their "accepted" lists. In 1992, Association of Zoological Horticulture (AZH) began a survey of zoological institutions and the use of browse. That survey was completed in 1998. Five of the responding institutions, including the Denver Zoo, reported utilizing viburnum species for browse supplementation of various animal species - including hoofstock, primates (specifically the colobus from one institution) and insects. The Toxic Plant Survey (1992) published by AZH and the American Association of Zoo Veterinarians (AAZV) lists all plant species reported to the survey by zoological institutions, to have caused harm to an animal in a zoological collection. None of the viburnum species were listed as of being a concern. Based on this information, browse species were planted in several primate exhibits throughout Primate Panorama – the primate exhibit at the Denver Zoological Gardens. Among those browse species planted throughout the institution were several viburnums.

Viburnums are a common landscaping plant used in the United States. Of particular appeal, other than attractive foliage, flowers and berries is the ability of several of the hybrids to maintain leaves even in the winter. This ability of some of the viburnum species has lead to them being called "evergreens" among the deciduous plant species. One hybrid that has become very popular is *Viburnum x rhytidophylloides* 'Alleghany' (Alleghany), a hybrid produced from crossing the two species *V. lantana* and *V. rhytidophyllum*. Alleghany has dark, green, leathery foliage with small yellowish-white flowers and red fruit and is commonly used in landscaping. It is one of the characteristic "evergreen" hybrids, retaining its leaves well into spring and summer. This plant is one not readily consumed by most animal species when planted within an animal exhibit, thus allowing zoo personal to create a more "natural" habitat appearance.

Colobus guereza kikuyuensis, is commonly referred to as the black and white colobus monkey and is found in areas ranging from eastern Nigeria to Ethiopia and Tanzania (Nowak, 1991). This colobus is part of the Cercopithecidae family, consisting of 37 species in six genera, found in south and southeast Asia and equatorial Africa (Macdonald, 1989). This animal species is characteristically black with a white beard and white mantle from the shoulders to the lower back and has a large white tuft on the end of the tail (Nowak, 1991). A solitary male is commonly found in groups of 3-15 animals. At the Denver Zoological Gardens (DZG), *C. guereza* primates consume the majority of browse presented to them. The colobus is classified as a folivore and is a forestomach fermentor (Robbins, 1993). Maintenance of vegetation in the *C. guereza* exhibit is challenging as they consume all vegetation within reach.

It is imperative that zoological animal collections be provided with a safe source of browse. This proceeding illustrates the concern when animals inadvertently consume a harmful plant, even though safety measures are in place. A further evaluation of six viburnum species currently raised at the Denver Zoological Gardens that are being used for browse has been implemented. This evaluation focuses on the Alleghany hybrid as well as 5 other viburnum species, in an attempt to determine their safety when being fed to captive wild animals.

MATERIALS

Animal – Plant Consumption

In mid-September 2000, a group of colobus (*C. guereza*) monkeys were moved from their original exhibit to a larger exhibit recently inhabited by another primate species. *Viburnum x rhytidophylloides* 'Alleghany' was growing in that exhibit. The previous primates were not interested in the foliage of the plant, but the colobus devoured the shrub in its entirety (leaves, buds, flowers and stems) within a very brief time.

On October 21, 2000, the male colobus from the group was removed from the enclosure and hospitalized at the zoo hospital after indications of pain, lethargy and being unable to climb. He was treated for parasites and was given IV fluids. The animal continued to worsen and ultimately became anemic with a packed cell volume (PCV) down to 14% of normal after 4 days. After the initial low PCV reading he was re-bled within 45 minutes, the results indicated it had lowered 12% of normal. This was an indication to the veterinary support staff that the animal was bleeding out internally, and the animal was then euthanized. A necropsy was performed substantiating that the animal had died of gastric ulcers that had ruptured a blood vessel. A diagnostic lab (Idexx Veterinary Services, Denver CO) used a light microscope to evaluate the gastric biopsies from the male colobus, showed inflammation of the mucosal layer of the stomach with ulceration. A small spicule of plant material was embedded in the inflammatory exudate associated with the stomach lining.

On December 8 and then the 9th, two of the colobus females began to distance themselves from the family group, acted lethargic and eating little. Both began to vomit and had diarrhea. They were treated for internal parasites, anti-yeast drugs, and ultimately IV fluids were administered. Rectal swabs were obtained for enteric pathogen cultures, as the animals were eructing a fetid gas. An oral gastric tube was used to collect stomach contents for culture, the stomach contents having a similar fecal odor as the diarrhea. Because of supportive treatments, both animals improved and were released back into the exhibit on January 17, 2001.

Animal – Plant Evaluation

Based upon the plant spicule found during necropsy, leaf samples from another Alleghany shrub were collected in January. The mature leaves collected were in good condition. Top and bottom leaf portions of the leaves were fixed for SEM evaluation by using a 2.5% glutaraldehyde and 2.0% paraformaldehyde in a 0.1 M Sorenson's sodium phosphate buffer (SPV) at a pH of 7.0 with 2 drops (10 mL) of Tween 20. There were fixed for 124.3 h at 4°C. Samples were then washed in the 0.1 M SPV twice for 10 min, followed by post-fixation in 2.0% OsO₄ in 0.1 M SPV for 1 h. There were then washed twice in SPV buffer for 10 min each washing, followed by 2 washes in distilled water for 10 min each. Samples were dehydrated in a graded series of ethanol and then critical point dried. After drying, the samples were coated with 15 nm gold on a Hummer VII sputter coater (Anatech Ltd.; Alexandria VA) and viewed on a Philips 505 scanning electron microscope (FEI Company; Hillsboro OR).

Lumina imaging at 15 kV showed tops of mature viburnum leaves to contain few "spicule-like" structures that could potentially become embedded in the gastric lining of an animal. Leaf bottoms were an "intense" mat of spicules. Scanning electron microscopy (SEM) showed that the spicules were bottle-brush in form, but not hooked in shape. Buds were also collected from Alleghany in January and initial SEM observations of buds indicated some hooked spines, possibly those that are more immature. Initial results of Energy Dispersive X-ray Spectroscopy (EDS) indicated that the spicules were calcium-based.

Plant – Evaluation of other viburnums

When the original SEM work on the Alleghany viburnum was initiated, samples available were mature, retained leaves. Concern about other viburnum species that had been planted for ornamental reasons and future browse harvests was now in question. In order to determine if there are differences between species and to determine if maturity was the reason for the rigid spicules, other harvests (late summer, late fall and winter) were planned. Immature growth of 6 viburnum species including the Alleghany were harvested in June 2001 with future harvests to follow in August, November 2001 and again in January 2002. The six viburnum cultivars harvested were: 1) *Viburnum trilobum* (also called American cranberry viburnum); 2) *Viburnum bodanantense* 'Dawn'; 3) *Viburnum x burkwoodii*; 4) *Viburnum prunifolium* (also called blackhaw viburnum); 5) *Viburnum rhytidophyllum*; and 6) *Viburnum x rhytidophylloides* 'Alleghany.' All of the viburnums were selected for evaluation based on those with sufficient numbers "designating" them as potential browse sources. Visual observation of the six viburnum leaf samples showed major differences in size and shape.

Top and bottom leaf portions of the six viburnum species were fixed for SEM evaluation as indicated earlier for the mature leaves. Samples were again coated with 15 nm gold on the Hummer VII sputter coater and viewed on the Philips 505 SEM. Lumina imaging of the prepared leaf samples at 35.8 times

magnification showed a great diversity among the six different viburnum species (Table 1). There was no indication of spicules on *V. prunifolium* at the 35.8 times magnification, or even at 143 times magnification. Samples of *V. bodanantense* 'Dawn' had only a few single-spined spicules per 1 mm on the top and bottom sides of the leaf. Both *V. trilobum* and *V. burkwoodii* had a single spicule / 1 mm on leaf tops, but then all similarities changed. *Viburnum trilobium* had only single-spined spicules on the tops of their leaves, but had 5 spicules / 1 mm on the bottom. *Viburnum burkwoodii*'s single spicules on the top leaves were bottle brushed-shaped, while the bottom was a solid mat of bottle brushed shaped spicules. The Alleghany samples had 2 bottle brush-shaped spicules / 1 mm on the top side of the leaf samples, but the bottom side was a mat of spicules. *Viburnum rhytidophyllum* had 3 spicules / 1 mm on the leaf tops, but they were primarily single spicules with some double spines. The bottom of this cultivar's leaves was also a mat of single and double spicules.

CONCLUSIONS

Safety of browse for animal collections found in zoological institutions is essential. Even though safety measures have been implemented, extrapolation of these measures can result in the death of an animal. Concerns from browse can be physical as indicated in this text, or metabolic from secondary compounds. Further certification of plant safety needs to be implemented for both physical and metabolic means. Utilization of the Scanning Electron Microscope is one way to determine the physical safety of a browse species.

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TABLE 1. Number of spicules and their shapes found by scanning electron microscope¹ on the top and bottom leaves of six viburnum species spring harvested at the Denver Zoological Gardens in 2001.

Viburnum Species/ Cultivar	Magnification	Top Number ²	Spicule Shape	Bottom Number ³	Spicule Shape
<i>V. x rhytidophylloides</i> cv. Alleghany	35.8	2	Bottle Brush	Mat	Bottle Brush
<i>V. trilobum</i>	35.8	1	Single	5	Single
<i>V. x bodanantense</i> cv. Dawn	35.8	0.5	Single	0.5	Single
<i>V. x burkwoodii</i>	35.8	1	Bottle Brush	Mat	Bottle Brush
<i>V. prunifolium</i>	35.8	0	NA	0	NA
<i>V. prunifolium</i>	143.0	0	NA	0	NA
<i>V. rhytidophyllum</i>	35.8	3	Single/ Double	Mat	Single/ Double

¹Philips 505 Scanning Electron Microscope (FEI Company, Hillsboro OR)

²Number of spicules measured on the top sides of leaves in 1mm at 15 kV.

³Number of spicules measured on the bottom sides of leaves in 1mm at 15 kV.