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Authors

Peacock, David Williams, Brian Christensen, Per

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The Search for a Toxicant in Native *Gastrolobium* Seed Historically Reported to Make Australian Native Fauna Toxic to the Introduced Cat and Dog

David Peacock and Brian Williams

Faculty of Sciences, The University of Adelaide, Glen Osmond, South Australia **Per Christensen** Ellendale Farm, Balingup, Western Australia.

Abstract: The decline and extinction of Australian mammals since the arrival of Europeans has been catastrophic. Attempts to reintroduce threatened species of native mammals to their former ranges have failed due to predation by the introduced red fox (*Vulpes vulpes*) and feral cat (*Felis silvestris catus*). Baiting with poisoned meat baits will control foxes, but feral cats are generally unwilling to consume a bait. This has seen recent reintroduction attempts fail due to cat predation, possibly involving only a few individual animals. Historical anecdotes from Western Australia record the death of cats and dogs after feeding on a number of the native animal species, with the bronzewing pigeons, *Phaps chalcoptera* and *P. elegans*, most frequently mentioned. The toxicity of the pigeons has been attributed to the birds' feeding on seeds from plants of the *Gastrolobium* genus. Such a mechanism has been suggested to have aided the survival of 35- to 5,500-g mammals in southwest Western Australia when such species have disappeared elsewhere. Analysis of the seeds of members of the genus *Gastrolobium* has found extremely high levels of organically bound fluorine. Previous studies report this fluorine to be stored in the seeds as monofluoroacetate (MFA – synonymous with Compound 1080). However, a number of the poisoning anecdotes suggest toxicity of the pigeon skeleton, and report times to death which appear too rapid for the known action of MFA or "1080."

The objective of this research is to examine the authenticity of the historical anecdotes and the feasibility of using a skeletally retained natural plant toxicant to aid the reintroduction of native fauna. Those individual cats and foxes effecting the predation may be removed at the first predatory event if the reintroduced fauna were toxic to the predating animal. This is anticipated to greatly improve the success rate of future reintroduction programs. Exhaustive extraction of *Gastrolobium* seeds with a variety of solvents and subsequent analysis utilizing a fluoride ion-specific electrode, ¹⁹F nuclear magnetic resonance, and other techniques has established the presence of a number of fluorinated and alkaloid compounds. Determination of the identity and toxicity of these compounds is expected to identify a compound(s) which may explain many of the historical poisoning anecdotes and could also provide a method for the control of feral cats in native mammal reintroduction programs.

Key Words: *Gastrolobium*, Fabaceae, Mirbelieae, seeds, retained toxicants, fluorine, alkaloids, bone retention, predation, cat, *Felis silvestris catus*, fox, *Vulpes vulpes*, bronzewing pigeons, *Phaps chalcoptera*, *Phaps elegans*, vertebrate pest control, reintroduced fauna, feral animals, introduced species

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INTRODUCTION

Colonists in Western Australian quickly discovered that toxic plants were present in the environment and were the direct cause of substantial stock deaths and the indirect cause of domestic cat and dog deaths. The first colonial botanist, Mr. James Drummond, was involved in numerous discussions, experiments, and expeditions with the aim of trying to determine which plants were the cause of these deaths (Drummond 1840).

The 3 species that appear to have caused most of the animal deaths were York Road poison (*Gastrolobium calycinum*), box poison (*G. parviflorum*), and heart-leaf poison (*G. bilobum*).

Drummond (1840) may have been the first to record the death of a cat or dog from the remains of a native animal, in this case the "...guts..." of a bronzewing pigeon (*Phaps* spp.) causing the death of a dog. He notes the commonly held view that the pigeon's toxicity was related to their feeding on York Road Poison (*Gastrolobium calycinum*), with the dog's symptoms similar to that observed in drenching experiments.

The death of domestic cats and dogs was an issue of major concern to the settlers, occurring when native animals were obtained as a food source either for the people or their animals. A newspaper of this period, The Western Mail, recorded at least 28 letters discussing the issue of toxicity of the native animals, from 1906-1922. For example, in The Western Mail on July 7, 1921 a person records their inability over a number of years to keep a cat, with their cats annually dying from eating bronzewing pigeons in late summer when the seeds of the poison plants were ripe (Anon. 1921). Another example from a different newspaper is the letter of the taxidermist Mr. W. Webb (1885) written to the editor of the Albany Mail and King George's Sound Advertiser. In this case, the bronzewing pigeon was cooked and eaten by Mr. Webb, with his dog eating the breastbone, exhibiting symptoms of poisoning in 10 minutes, and being dead

within 25 minutes. The pigeon's toxicity to the dog was attributed to *G. bilobum*, the seeds of this plant stated to be the primary food for the birds in that area.

In southwest Western Australia, the toxicity to cats and dogs of animals which have either directly or indirectly consumed *Gastrolobium* plant material has been suggested to have greatly aided the survival of native mammals ranging in weight from 35 - 5,500 g when such species have disappeared elsewhere. Christensen (1980) hypothesized that the survival of 35to 5,500-g mammals in the *Gastrolobium*-inhabited woodlands of southwest Western Australia may have been aided by the secondary poisoning of predating foxes and cats.

Gastrolobium Toxicity

The presence of historical anecdotes provides the foundation for the research currently being undertaken, focusing primarily on *G. bilobum*, the species identified in the Webb letter, and seeking to identify any skeletally retained toxins present in these seeds.

Early chemical analyses of the leaves of the 2 principal toxic species, York Road poison (G. calycinum) and box poison (G. parviflorum), described 2 new alkaloids, respectively cygnine and lobine (Mann 1905, 1906). In testing on guinea pigs (mean weight 458 g), cygnic acid caused death in a mean of 15 minutes (n = 2; SD = 12). In guinea pigs with a mean weight of 276 g, lobic acid caused death in a mean of 4 minutes (n = 2; SD = 1.2). Additional testing of cygnic acid on 2 sheep caused death in 12 minutes. Thus the original explanation for the toxicity of Gastrolobium species was the presence of these, or similar, alkaloids. This research proposed permanganate of potash as a remedy for stock poisoning, and the reported success of this remedy (e.g., Anon. 1920) supported the toxic agent being an organic compound. Subsequent research has detected significant concentrations of alkaloids in leaves of wallflower poison (G. grandiflorum) (Webb 1949) and identified 4 alkaloids in rock poison (G. callistachys) leaves (Cannon and Williams 1982).

Research on *Gastrolobium* toxicity has rarely examined seed material. The dry matter of the seeds of Leguminosae and Fabacae plants are known to accumulate up to 10% alkaloids (Stobiecki et al. 1993). This suggests that with 4 alkaloids extracted from the fresh leaves of *G. callistachys*, these or others are likely to be at a much higher concentration in the seeds. They may also be stored in another form. Chang and Hartmann (1998) examined the seeds of *Crotalaria scassellatii* (Fabaceae) and found that this plant stores pyrrolizidine alkaloids as "lipophilic tertiary amines" in its seeds and as *N*-oxides in its vegetative tissues.

The discovery of MFA (monofluoroacetic acid or monofluoroacetate) in the seeds of *Acacia georginae* (Oelrichs and McEwan 1962) and the leaves of G.

grandiflorum (McEwan 1964) served to focus the subsequent analysis of *Gastrolobium* toxicity on the presence of MFA. Thirty-three members of the genus *Gastrolobium* which are considered toxic are restricted to the southwestern corner of Western Australia. Of these, 23 species have been shown to contain quantifiable concentrations of MFA, and 10 have yet to be tested or provided only a "positive result" (Aplin 1971a,b; Gardner 1964; Gardner and Bennetts 1956). In almost all cases, the plant tissue examined for toxicity was leaf tissue and analysis was specifically directed at qualifying and quantification, often at extremely high concentrations, was used to explain the toxicity of these plants.

The present study has found a number of fluorinated compounds and the evidence for at least 2 alkaloids in the seeds of species of *Gastrolobium*.

Organic Fluorine and Alkaloids in *Gastrolobium* Seed

The seeds of twelve toxic species of Gastrolobium have been analyzed for their total fluorine by fusion with sodium hydroxide and subsequent analysis utilizing a fluoride ion-specific electrode. Some of these results, with additional inter-provenance replicates, are presented in Figure 1. Extreme variation exists between and within species, with species such as G. spinosum containing trace levels and G. tetragonophyllum containing an extremely high concentration. Almost all of this fluorine is stored as organic fluorine rather than free fluoride. For example, G. calycinum from Boddington, Western Australia had a total fluorine concentration of 816.0 \pm 79.5 ppm (n=3), with only 8.3 ± 7.2 ppm (n=3) free Additionally, most of this fluorine is stored fluoride. within the seed's cotyledons rather than the testa and aril. For the above G. calvcinum seed this was 89% in the cotyledons and 11% in the testa and aril.

Extraction of the seeds with solvents of increasing polarity has separated a number of different organic fluorine compounds. Analysis of these solvent extracts utilizing ¹⁹F nuclear magnetic resonance (¹⁹F NMR) has found evidence for 6 fluorine resonances in the non-polar extract, suggesting possibly 6 new fluorinated compounds, but not likely to be the omega-fluoro fatty acids of *Dichapetalum toxicarium* (Hamilton and Harper 1997). The majority of the fluorine is extracted in the final methanol extract. ¹⁹F NMR indicates a triplet characteristic of the CH₂F- moiety, and being almost coincident with the triplet of the sodium monofluoro-acetate standard is most likely to be the same compound.

Analysis of the seed of *G. bilobum* from Quindanning, Western Australia for alkaloids has been undertaken with thin layer chromatography (TLC). This has identified at least 2 compounds, and possibly 4, which are reactive to the alkaloid sensitive Dragendorff's reagent. These compounds are yet to be extracted and analyzed.

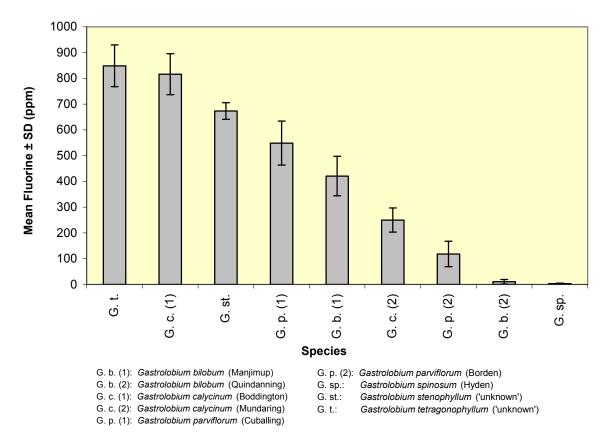


Figure 1. Flourine in Gastrolobium seed.

Poisoning with Gastrolobium vs. MFA

Many of the historical reports of toxic bone material and deaths of cats and dogs from eating native animal remains appear much too rapid for MFA, which has toxic effects known to take hours. There are a number of reports of bronzewing pigeon bones killing cats or dogs (e.g., Webb 1885). Deaths of cats and dogs associated with the secondary ingestion of *Gastrolobium* material, but too rapid for MFA, include:

- 3 cats "...died within an hour of eating the inside of the birds [bronzewing pigeons]" (Knight 1912, p. 5)
- retriever dog dead within 20 minutes after eating bronzewing pigeon intestines full of the seeds of York Road poison (Le Souëf 1907)
- "Ten minutes after eating it [bronzewing pigeon breast bone] he [dog] was quite mad... I caught him and poured castor oil down his throat, which caused him to vomit... this however did not ease him as in a quarter of an hour the poor brute died (Webb 1885, p. 3).

Direct administration of "1080" confirms a longer time to death. Symptoms of fluoroacetate poisoning in the dog are stated by Rammell and Fleming (1978) to arise 4-5 hours after ingestion. Bosakowski and Levin (1986) found dogs (adults 7.3 - 10.6 kg) did not exhibit symptoms until 1 - 2 hours after dosing with 8 - 32 mg/kg fluorocitrate, the toxic byproduct of fluoroacetate metabolism, with death occurring at approximately 4 hours. Even if a massive dose of fluoroacetate was ingested by the dog, this has not been found to produce an immediate effect but to reduce the latent period (Quin and Clark 1947). At the highest dose of 1.6mg "1080," Eason and Frampton (1991) recorded one 3.5-kg male cat dying in approximately 7 hrs. The mean for the 8 cats dosed at this level was, however, 16 hrs. It therefore seems very unlikely that the rapid deaths outlined above were due to MFA ingestion. Their rapid nature does, however, fit more with the *Gastrolobium* alkaloid induced deaths of Mann (1905, 1906).

Alkaloids – A Skeletally Retained Toxin?

The presence of alkaloids in species of *Gastrolobium* therefore provides a possible explanation for the deaths of cats and dogs from bronzewing pigeon bones. Other alkaloid research supports this hypothesis with, for example, major concentrations of the alkaloid colchicine detected in sheep bone marrow after a single oral dose (Panariti 1996). In addition, New Guinea birds of the genera *Pitohui* (5 of 6 species) and *Ifrita* (1 of 1 species) have been found to retain batrachotoxin alkaloids, primarily in their feathers (Dumbacher et al. 2000). These are the same alkaloids sequestered in the

skin of the poison-dart frogs (Daly 1995) and which can still be detected at least 2 years after ingestion (J. Daly, pers. comm.).

Chemical Defense in Reintroduced Fauna?

Aerial or ground baiting with "1080" baits is an effective broad-scale method for the control of red foxes (e.g., Christensen and Burrows 1995). Feral cats are, however, a different proposition, with the difficulty of their control increasing with their "wildness" or lack of reliance on humans (Risbey et al. 1997). Also, in the absence of foxes, feral cats have effected significant predation on numerous reintroduced, or relict, populations of animals. In many instances this predation has been attributed to individual cats (Christensen and Burrows 1995, Copley et al. 1999, Gibson et al. 1994, Powlesland et al. 1995, Spencer 1991). Despite recognition of the problem, an inability to remove the predating cat(s) with current control methods before loss of all, or many, of the reintroduced animals often occurs. Burrows and Christensen (1995, p. 40) describe their situation:

"Eleven of the 40 boodies [Bettongia lesueur] were recorded killed and eaten by cats. As far as we were able to tell it was the work of only two, possibly three, cats. Despite our best efforts, we were unable to trap, poison or kill the culprits."

Sinclair et al. (1998) examined 4 failed reintroduction programs and state a 90% reduction in predation as necessary to enable population growth. For 2 of the reintroductions, this may have been possible through removal of only a few individual predating cats.

Skeletally-retained toxicants are proposed as a strategy to significantly improve the survival of reintroduced CWR mammals. Skeletal retention, as a result of dietary intake, is intended to replicate the pigeon bone anecdotes and to provide a more long-term and physiologically stable site of toxicant retention. This strategy is targeted primarily at feral cats.

The following factors will affect choice of reintroduced fauna using the proposed strategy. The toxic species should:

1. be a prey size that is likely to be fully consumed by a cat. A skeletally retained toxicant should provide the most lengthy period of toxicant retention, but it does require some degree of skeletal ingestion and digestion by the predator. Species likely to be fully consumed include rodents (Veitch 1985) and from the absence of any locatable remains, greater sticknest rats (Copley et al. 1999) and golden bandicoots (Christensen and Burrows 1995).

2. be large enough to retain a lethal dose of toxicant.

3. have a high fecundity in order to absorb the predatory impact.

Possession of a skeletally retained toxicant should remove the predating cat(s) and halt any continuation or escalation of their predatory impact.

DISCUSSION

The object of this research is to determine if the seeds of species of *Gastrolobium* contain a toxicant that is bone retentive and toxic to predating cats and foxes. It is reported that such a phenomenon occurred in southwest Western Australia during agricultural and pastoral expansion. A skeletally retained toxicant is anticipated to improve the success of reintroduction programs by removing those predating cats and foxes which select the reintroduced fauna as prey. Numerous reintroductions have failed or been severely impacted by predation by only one, or a few cats. These animals are unwilling to eat a toxic bait but are willing to eat the reintroduced fauna. Consumption of a non-lethal dose could result in "prey-shyness," a similar result to death of the fox or cat in terms of cessation of their predatory impact.

It is expected that native fauna will possess a tolerance to this toxicant significantly greater than the cat and fox, the toxicant, like MFA, having long been a part of the natural environment. The strategy proposes skeletal retention of a plant derived toxicant to provide a long-term chemical defense and as such, a prey size ensuring skeletal ingestion and digestion is required.

The undescribed fluorinated and alkaloidal compounds may be unique chemical toxicants. The emphasis of further work is to establish the structure of at least some of these compounds and determine their toxicity in target species. If they are likely to accumulate in bone, or cause rapid disruption to biochemical pathways, especially those of the nervous system, then they may provide the basis for "chemical defense" of fauna vulnerable to catastrophic cat and fox predation.

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