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Detection of monofluoroacetate in *Palicourea* and *Amorimia* species

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ABSTRACT

Numerous plant species worldwide including *Palicourea marcgravii* and *Tanaecium bila-biatum* in Brazil cause sudden death and are known to contain monofluoroacetate (MFA). Other species in Brazil including some species traditionally assigned to *Mascagnia* but now properly called *Amorimia* species and other *Palicourea* species are reported to cause sudden death in livestock and are suspected to contain MFA due to the similarity of clinical signs. In this study, an HPLC-APCI-MS method to detect and quantify MFA was developed and was used to investigate plant material from field collections and/or herbarium specimens of *Mascagnia*, *Amorimia*, and *Palicourea* species suspected of causing sudden death. MFA was detected in *Amorimia amazonica*, *Amorimia camporum*, *Amorimia exotropica*, *Amorimia pubiflora*, *Amorimia rigida*, and *Amorimia septentrionalis* as well as *Palicourea aeneofusca*. MFA concentrations differ greatly between *Palicourea* species and *Amorimia* species, which may explain the incidence of poisoning and the amount of plant material required to cause sudden death between these taxa.

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1. Introduction

Numerous plant species worldwide cause sudden death syndrome in livestock; a number of these species are suspected or known to contain the toxic organofluorine compound monofluoroacetate (MFA; Twigg et al., 1996). For example, Dichapetalum cymosum native to southern Africa (Marais, 1944); Acacia georginae, Oxylobium parviflorum, and Gastrolobium grandiflorum plants in Australia (Alpin et al., 1983); and Palicourea marcgravii (Oliveira, 1963; Moraes-Moreau et al., 1995) and Tanaecium bilabiatum (synonym Arrabidaea bilabiata; Krebs et al., 1994) in Brazil contain MFA and cause sudden death syndrome. Clinical signs associated with sudden death are loss of balance, ataxia, labored breathing, muscle tremors, and

recumbency leading to death. Numerous other plant species in Brazil including some species traditionally assigned to *Mascagnia*, *Pseudocalymma elegans*, *Fridericia japurensis* (synonym *Arrabidaea japurensis*) and other *Palicourea* and *Tanaecium* species are reported to cause sudden death in livestock and are suspected to contain MFA due to the similarity of clinical signs; however, the presence of MFA has not been verified in these species (Tokarnia et al., 1990, 2000, 2002; Vasconcelos et al., 2008a; Riet-Correa et al., 2009).

Since the mid-part of the 20th century, poisoning of livestock by species then assigned to *Mascagnia* or "tingui" was reported throughout the northeast and southeast regions of Brazil (Tokarnia et al., 1990, 2000, 2002). Five *Mascagnia* species, *Mascagnia elegans*, *Mascagnia exotropica*, *Mascagnia pubiflora*, *Mascagnia rigida*, and *Mascagnia aff. rigida*, are reported to cause sudden death in livestock (Tokarnia et al., 1990, 2000, 2002; Riet-Correa et al., 2009). *M. rigida* is one of the most important

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poisonous plants of Brazil because of its widespread distribution throughout the nine states of northeastern Brazil and the southeastern region in the states of Minas Gerais and Espírito Santo (Tokarnia et al., 2000). Recent taxonomic research using morphological and molecular studies of *Mascagnia* species led to the description of a new genus, *Amorimia*, to which four of the *Mascagnia* species (*exotropica*, *pubiflora*, *rigida*, and *aff. rigida*) suspected of causing sudden death syndrome have been assigned (Anderson, 2006; Davis and Anderson, 2010). *Amorimia* species are distinguished from *Mascagnia* species by leaf glands on the abaxial surface, abaxially hairy petals, large gland-bearing bracts, straight erect styles, and fruit (samara) morphology (Anderson, 2006).

The objective of this research was to develop a method to detect and quantify MFA in taxa suspected of causing sudden death in livestock. *P. marcgravii* was verified to contain MFA as previously reported. *Amorimia* species, *M. elegans* (under its earlier synonym, *Mascagnia divaricata*), and *Palicourea aeneofusca* suspected of causing sudden death syndrome were investigated for the presence of MFA. Additionally, MFA concentrations were compared between *P. marcgravii* and other species suspected of causing sudden death.

2. Materials and methods

2.1. Plant material

Collections of *P. marcgravii* were made in the states of Goiás (S16°28.82′ W49°21.40′; elevation 800 m) and São Paulo, Brazil (S21°57.12′ W47°27.80′; elevation 628 m). Mature leaves from 10 plants were collected from the Goiás location, while leaves from 10 plants were separated into mature leaves and immature developing leaves at the São Paulo location. *P. aeneofusca* was collected 90 km inland from the coastal area of Paraíba (S6°57.51′ W35°42.92′; elevation 589 m). Mature leaves from 10 plants were harvested. Identifications of *Palicourea* spp. were verified by local botanists. Voucher specimens were deposited at the USDA-ARS Poisonous Plant Research Laboratory herbarium (PPRL). Voucher numbers of *P. aeneofusca* are in Supplementary Table 1.

A number of samples from Malpighiaceae taxa were provided courtesy of the University of Michigan Herbarium (MICH): Amorimia amazonica (Mascagnia amazonica); Amorimia camporum, a new species described by Anderson (2006), specimens previously identified as M. pubiflora; Amorimia exotropica (M. exotropica); Amorimia kariniana, a new species described by Anderson (2006), specimens previously identified as M. amazonica; Amorimia maritima (Mascagnia maritima); Amorimia pubiflora (M. pubiflora); Amorimia rigida (M. rigida); Amorimia septentrionalis, a new species described by Anderson (2006), specimens previously identified as M. rigida; Amorimia velutina, a new species described by Anderson (2006), specimens previously identified as M. rigida; and M. divaricata (M. elegans). These specimens from the University of Michigan Herbarium were examined for the presence of MFA. All specimens were identified by William Anderson at the University of Michigan Herbarium. Vegetative and floral

Table 1Survey and detection of monofluoroacetate in herbarium specimens of *Amorimia* species and *Mascagnia divaricata*.

Taxa	Specimens sampled	MFA detected	MFA concentration (%)
Amorimia amazonica	8	1	< 0.0007
Amorimia camporum	5	1	< 0.0007
Amorimia exotropica	7	2	0.02
Amorimia kariniana	1	0	nf
Amorimia maritima	6	0	nf
Amorimia pubiflora	18	3	0.006
Amorimia rigida	8	2	0.002
Amorimia septentrionalis	6	1	0.002
Amorimia velutina	1	0	nf
Mascagnia divaricata	8	0	nf

tissues were sampled from the specimens. Numbers of specimens sampled for each taxon are shown in Table 1. A map showing the distribution of the *Amorimia* species is shown in Fig. 1. Voucher numbers of the surveyed taxa are in Supplementary Table 1.

Collections of *A. septentrionalis* were made in the state of Paraíba, Brazil at two locations (S7°09.47′ W37°19.06′; elevation 305 m; S7°12.24′ W37°15.11′; elevation 749 m). Ten plants per location were separated into leaves, stems, and floral parts and seeds if available. Specimens were verified as *A. septentrionalis* and voucher specimens were deposited at University of Michigan Herbarium and the USDA-ARS Poisonous Plant Research Laboratory Herbarium (PPRL). Voucher numbers of these taxa are in Supplementary Table 1. Further, one collection of *Amorimia* sp. (*M. aff. rigida*) was made in a cultivated garden in Seropédica, in the state of Rio de Janeiro, courtesy of Carlos Tokarnia; no voucher specimen is available for this collection.

2.2. Extraction

Extraction of plant material for monofluoroactetate (MFA) analysis was accomplished by weighing 100 mg of ground plant material into a 13 mL screw top test tube equipped with Teflon lined caps (Pierce, Rockford, IL, USA). Water (deionized, 5 mL) was added to each test tube and placed in a mechanical shaker for 30 min, then centrifuged to separate the plant residue and water extract. The water extract was transferred to a clean 13 mL test tube. The plant residue was extracted once more with 5 mL water for 30 min. The water extracts were combined for a total of 10 mL. A 1 mL aliquot of the water extracts was filtered through a filter made from a kimwipe and disposable pipette and transferred to a 1 mL autosample vial for analysis. P. marcgravii samples were initially diluted 1 to 5 and then transferred to a 1 mL autosample vial for analysis. Samples of Palicourea with MFA concentration values higher than the standard curve when diluted 1:5 v:v were further diluted 1:20 v:v and re-analyzed.

2.3. HPLC-APCI-MS

The HPLC-APCI-MS method used in this study was based on a previously reported method (Noonan et al.,

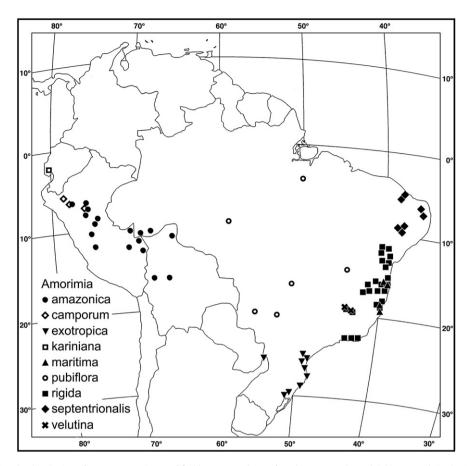


Fig. 1. Map showing the distribution of Amorimia species, modified by W. R. Anderson from his map on the Malpighiaceae website (Anderson et al., 2006).

2007). In this study, samples were injected (20 μ L) onto a Poroshell 120 EC-C18 reversed phase column ($50 \times 3 \text{ mm}$ i.d., 2.7 µm) (Agilent Technologies, Santa Clara, CA, USA) that was protected by a Betasil C-18 guard column (10 \times 2.1 mm i.d., 5 μ m) (Thermo Electron Corporation, Waltham, MA, USA). MFA was eluted from the column with an isocratic flow (0.300 mL/min) of 85:15 (5 mM formic acid, 5 mM tributylamine: MeOH) mobile phase. Flow from the column was connected directly to a Thermo Finnigan (San Jose, CA USA) LCQ ion trap mass spectrometer via an atmospheric pressure chemical ionization (APCI) source. Selected ion monitoring (SIM) at m/z 77 for the (M – H) ion of MFA was used. Sodium fluoroacetate (MFA) (Sigma-Aldrich, St. Louis, MO) was prepared in water to give an eight point standard curve over the range of 0.078 μg/mL-10.0 μg/mL by serial dilution. The total HPLC run time was 7.0 min with MFA eluting at 3.8 min (Fig. 2).

3. Results and discussion

There have been attempts to verify the presence of MFA in some Brazilian plants causing sudden death (Cunha, 2008; Peixoto et al., 2011), but these indirect methods were neither conclusive nor quantitative (Riet-Correa et al., 2009). In this study, an HPLC-APCI-MS method for the analysis of MFA in complex food matrices was modified for

the analysis of MFA in plant samples (Noonan et al., 2007). The new method was specific for MFA and was linear over 2 orders of magnitude (0.078 μg/mL–10.0 μg/mL MFA/H₂O). The presence of MFA has been verified in P. marcgravii (Oliveira, 1963; Krebs et al., 1994; Moraes-Moreau et al., 1995) and T. bilabiatum (Krebs et al., 1994). With the method reported herein, MFA was detected and quantified in the 10 plants of *P. marcgravii* collected at each location. Concentrations of MFA in mature leaves of P. marcgravii were determined to be 0.24 \pm 0.10% and 0.21 \pm 0.17% from the Goiás and São Paulo collections, respectively. The young, newly developing leaves from the São Paulo collections contained MFA at concentrations of $0.88 \pm 0.08\%$. Krebs et al. (1994) reported that *P. marcgravii* leaves contained 0.00054% MFA. P. aeneofusca from eastern Paraíba contained 0.09 \pm 0.05% MFA, thus verifying that MFA is the toxin in this species (Tokarnia et al., 2000; Vasconcelos et al., 2008a).

MFA was detected in the two populations of *A. septentrionalis* surveyed. However, MFA was only detected in 60% of the plants sampled from each population. MFA concentrations varied among plant parts with concentrations ranging from approximately $0.002 \pm 0.0009\%$ in leaves, $0.001\% \pm 0.0003$ in stems, 0.008 ± 0.004 in flowers, and $0.006 \pm 0.002\%$ in seeds. Further, MFA was found in low concentrations (0.005%) in *Amorimia* sp. (*rigida* complex;

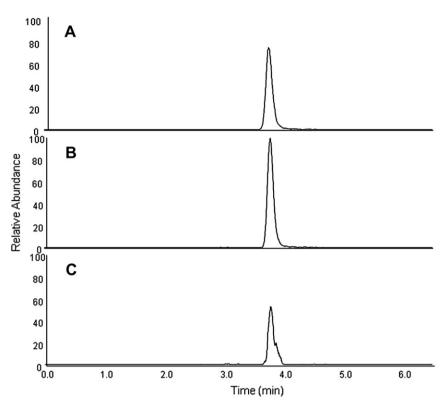


Fig. 2. Selected ion monitoring (SIM) at *m/z* 77 from (A) 2.5 μg/mL standard of sodium monofluoroacetate, (B) water extract of *Palicourea marcgravii* mature leaves diluted 1:5 v:v, and (C) water extract of *Amorimia pubiflora*.

M. aff. rigida) grown in a garden in the state of Rio de Janeiro.

Amorimia and Mascagnia taxa suspected of causing sudden death were screened for MFA using plant material from herbarium specimens. MFA was detected in specimen(s) of A. amazonica, A. camporum, A. exotropica, A. pubiflora, A. rigida, and A. septentrionalis (Table 1). Concentrations in the plant material representing herbarium specimens of Amorimia taxa were similar to that found in the field collections of A. septentrionalis. MFA was not detected in many of the herbarium specimens from MFA-positive taxa as was similarly observed in A. septentrionalis field collections. Lastly, MFA was not detected in A. kariniana, A. maritima, A. velutina, and M. divaricata. We suspect that this may be due to the limited number of specimens that were available for sampling in some taxa. Furthermore, these species have not been reported to be toxic (Tokarnia et al., 2000), and they may not contain MFA. A more extensive sampling scheme will be necessary to verify the lack of MFA in these Brazilian species.

The incidence of poisoning and the amount of plant material required to cause sudden death differs greatly between *P. marcgravii* and the *Amorimia* species previously identified as *Mascagnia* species. Rabbits dosed with *P. marcgravii* were fatally intoxicated at approximately 1 g/kg BW (Tokarnia et al., 1998) while in collections of *Amorimia* (formerly *Mascagnia*) species from southern Brazil tremendous variation in lethality of the plant was reported

in dosed rabbits. For example, some animals were fatally intoxicated with doses of 4-6 g/kg BW while others showed no clinical signs at doses up to 12 g/kg body weight, Further, Medeiros et al. (2002) similarly reported that some rabbits were fatally intoxicated with A. rigida (formerly M. rigida) doses of 2.5 and 5 g/kg BW, yet others given 10 or 20 g/kg BW were not affected. Gava et al. (1998) reported that doses of Amorimia spp. (formerly Mascagnia spp., later identified as A. exotropica) of 7-10 g/kg body weight were fatal in cattle. Vasconcelos et al. (2008b) found that some goats given A. rigida at 10 g/kg BW died, whereas others given 20 g/kg BW recovered. The results reported here provide quantitative evidence that explains the observed widespread variation in toxicity in Amorimia, particularly in comparison with MFA-containing Palicourea species (marcgravii and aeneofusca). In the genus Amorimia there is large variation in MFA concentration leading to differing clinical results when given to animals. The concentrations of MFA in Palicourea species are approximately 50-100-fold greater than the concentrations in the Amorimia species (Fig. 2); thus the amount of MFAcontaining *Palicourea* species required for a toxic dose is dramatically lower (Fig. 2).

In addition to the differences in lethality from concentrations of MFA, these plants are reputed to differ substantially in palatability to grazing livestock. *Palicourea* spp. are reported to be highly palatable to livestock (Tokarnia et al., 2000), whereas the *Amorimia* spp. formerly

assigned to *Mascagnia* are not preferred by grazing livestock and are typically eaten only when other forage is lacking (Pavarini et al., 2011). Furthermore, the time of grazing will influence toxicity as the relative concentration of MFA in the plant material may change as a function of plant phenology as has been shown for the toxic alkaloids in *Delphinium* and *Oxytropis* species (Ralphs and Gardner, 2003; Cook et al., 2012).

Recently, it was reported that *Mascagnia sepium* (also cited with the unpublished name *Amorimia sepium*) causes sudden death syndrome in Rondônia, a state of northern Brazil (Schons et al., 2011). *M. sepium* has not been reported in this region of Brazil; rather it is found in southeastern Brazil. Fortunately, a photo of the plant shown to cause sudden death was included in this report. The plant was identified as an *Amorimia* species by W. Anderson (University of Michigan) on the basis of the fruits (i.e., samaras) shown in the photo. Fruit morphology differs significantly between *Mascagnia* and *Amorimia* species (Anderson, 2006). On the basis of the location of the case report we suspect the plant responsible for causing sudden death in this report was likely *A. amazonica*; however, this cannot be verified without a voucher specimen.

M. divaricata (under the later synonym M. elegans) is reported to have caused sudden death syndrome in Pernambuco (Tokarnia et al., 1990); however, no herbarium collections of M. divaricata are known from any of the states of the semi-arid area of northeastern Brazil. It seems highly likely that the plant reported to have caused the sudden death syndrome was misidentified and was probably A. septentrionalis, not M. divaricata. This emphasizes the importance of voucher specimens in plant poisonings and the importance of finding experts to properly identify the plants.

We hypothesize that MFA is synthesized early in development and is diluted as the plant part matures, as evidenced by the five-fold greater concentrations in young, newly developing leaves compared to mature leaves of *P. marcgravii*. Other secondary compounds such as *Delphinium* norditerpenoid alkaloids have the greatest concentrations early in plant growth and development and are diluted as the plant matures (Ralphs and Gardner, 2003).

Herbarium specimens have been used to screen other plant taxa including *Delphinium* and *Lupinus* species for alkaloids responsible for their toxicity (Cook et al., 2009 a,b). Likewise, here we demonstrate that herbarium specimens are useful in screening for MFA in *Amorimia* species suspected of causing sudden death syndrome. The detection of MFA in herbarium specimens at concentrations similar to those found in recently collected plant material suggests that MFA is stable in plant material. Furthermore, these results demonstrate the utility of herbarium specimens for evaluating the presence of MFA in other taxa suspected of causing sudden death syndrome.

In summary, this is the first report of MFA in *Amorimia* species (*A. amazonica*, *A. camporum*, *A. exotropica*, *A. pubiflora*, *A. rigida*, and *A. septentrionalis*), previously identified as *Mascagnia* species. This is also the first report of MFA in *P. aeneofusca*. MFA concentrations differ significantly between *Palicourea* species and *Amorimia* species, which

may explain the incidence of poisoning and the different amounts of plant material required to cause sudden death in these taxa.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.toxicon.2012.05.029.

Conflict of interest

The authors declare that there are no conflicts of interest.

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