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Acetonitrile (methyl cyanide) emitted by the African spider plant (*Gynandropsis gynandra* L. (Briq)): Bioactivity against spider mite (*Tetranychus urticae* Koch) on roses

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ABSTRACT

The African spider plant (*Gynandropsis gynandra* L. (Briq)) has repellent properties against spider mite on roses (*Rosa hybrida* L.) when grown as a companion plant. Gas chromatography–mass spectrometry (GC–MS) has been used to identify potential volatile compounds in the foliar emissions of this plant that may contribute to the anti-mite properties. Acetonitrile (methyl cyanide) was the most abundant compound emitted by entire plants and detached leaves of five lines of both glasshouse- and field-grown *G. gynandra*, with only trace levels emitted by roses grown under similar conditions. A toxicity bioassay was conducted to evaluate the bioactivity of acetonitrile against spider mite, and ≥2.5 μL/L of air rendered 100% of the mites inactive. Removal of the compound after an initial exposure of up to 5.0 μL/L of acetonitrile allowed some 10% of the mites to recover mobility, but above this level no mobility was observed. This study provides a very strong indication that foliar emissions of acetonitrile by *G gynandra* are responsible, to a significant degree, for the spider mite repellent activity of the plant when used as an intercrop with roses.

1. Introduction

The African spider plant (*Gynandropsis gynandra* L. (Briq)) is an important indigenous vegetable in the East African region and is recorded as having broad activity in traditional medicine (Maundu, 1999; Jalalpure et al., 2007), particularly antibacterial action (Ajaiyeoba et al., 2001). Previous studies have also shown that that *G. gynandra* effectively repels livestock ticks (Malonza et al., 1992; Lwande et al., 1999), limits thrips damage in snap beans when grown as an intercrop (Waiganjo et al., 2007) and, from our own study, significantly reduces spider mite infestation when used as a companion plant with roses (Nyalala and Grout, 2007). Companion planting to reduce infestation may have commercial potential in East African rose nurseries as part of IPM strategies, and there may also be a role for the use of foliar extracts.

The five *G. gynandra* lines included in this investigation are from distinct geographic regions in Kenya and Tanzania and are equally effective in reducing spider mite infestation when grown as glasshouse companion plants with roses. They provide at least 75% reduction in active mites per plant ($P < 0.05$) after 5 days of co-growth as potted plants between the roses (Nyalala et al., 2010). The present study was carried out to identify any volatile compounds emitted by these lines and not by roses (or in significantly lower quantities) as these compounds will be the likely candidates responsible for the reduction in mite infestation. Variation in volatile emissions between lines is also of interest as plants from the same seed accession of *G. gynandra* typically display wide phenotypic variability, with those from different ecological zones tending to show different growth habits (Chweya and Mnzava, 1997).

As plants constantly release volatile compounds from leaves, flowers, fruits and even roots to the surrounding environment as survival and self-defence mechanisms (Dudareva et al., 2006) then *G. gynandra* plants might well generate their activity against ticks and mites by emission of biologically active, volatile compounds into the surrounding environment. Lwande et al. (1999) attributed the tick repellent property of *G. gynandra* to essential oils, which might also be responsible for the bioactivity against thrips and spider mites. They employed hydrodistillation and gas chromatography–mass spectrometry (GC–MS) to isolate those essential oil constituents of *G. gynandra* that were repellent to ticks in a laboratory assay, but may have missed water-soluble components and highly volatile compounds that are easily lost during hydrodistillation (Mastelić et al., 2008; Huang et al., 2009). Consequently, the portfolio of volatile compounds detectable by hydrodistillation may not contain significant compounds emitted by the plants in a natural environment.

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As previous studies (Nyalala and Grout, 2007; Nyalala et al., 2010) have indicated that foliar volatiles from *G. gynandra* are responsible for its activity against spider mites, this study has taken whole plants, or detached leaves, and used GC–MS to assess the population of potentially active compounds. Those compounds emitted by both *G. gynandra* and roses, in broadly comparable amounts, and those at comparable levels to atmospheric contaminants were eliminated from consideration. A preliminary study of bioactivity of the most important of these compounds against spider mites has also been undertaken.

2. Materials and methods

The lines of *G. gynandra* used in the study were: "Simlaw" (Kenya Seed Company, Nakuru, Kenya); "Egerton" (volunteer from a polytunnel, Egerton University, Kenya 2008), "Taastrup" ("Simlaw" repeatedly glasshouse grown in Taastrup, Denmark); "PS" and "IP8" (AVRDC, Arusha, Tanzania).

2.1. Greenhouse-grown plants

Seeds were planted in June 2009, in 13 cm plastic pots with standard compost (Pindstrup no. 1) and maintained in a glasshouse at 18–25 °C, 65% RH with 12 h supplementary light (University of Copenhagen, Taastrup, Denmark). The pots were flood-irrigated for 10 min every morning with water containing standard nutrients. At day 14 (5 true leaves) the terminal shoot was pinched out to encourage lateral growth and, at day 21, plants were selected for analysis using only those with a minimum of 5 side shoots and several leaves at each of the leaf axils. A small population of plants of each of the *G. gynandra* lines was reserved for seed collection, isolated to prevent cross-pollination. Non-flowering pot roses (Forever® Portus Cale™), maintained in the glasshouse under the same conditions as the *G. gynandra* plants, provided control material.

2.1.1. Whole plant volatile sampling

Pots containing a single *G. gynandra* or rose plant were tightly wrapped in aluminium foil paper to prevent emission of volatiles from the pot or the contained compost and then sealed into 5 L cylindrical, gas-tight glass containers (4 replicates per treatment). These were held at room temperature for 24 h, without supplemental light, before a sample of the atmospheric volatiles was taken over 20 min using an extraction pump (100 ± 1 mL min^{-1} flow rate; PBI Dansensor, Handy Check-8000) connected to a collection trap (89 mm stainless steel tube, 6.3 mm outer diameter) packed with 250 mg TENAX-TA (60/80 mesh, 0.37 g/mL density from Buchem BV, Apelddorn, The Netherlands). The traps were capped and stored at 4 °C for subsequent GC–MS analysis. To assess the background levels of volatile compounds, the laboratory air and the atmosphere from closed, empty vessels were similarly sampled.

2.1.2. Detached leaves

Four seeds of each *G. gynandra* line were planted in 2 L plastic pots with standard compost (Pindstrup Peat moss substrate no. 1) and maintained in the greenhouse (March–June 2009) at the University of Copenhagen as described above. The plants were thinned to leave the strongest plant in each pot and the nutrients provided when the seedlings had developed three true leaves. Non-flowering pot roses (Parade®Apollo) were maintained in the glasshouse under the same conditions as the *G. gynandra* plants to, provide control material.

2.1.3. Detached leaf volatile sampling

Entire leaves (lamina and petiole) were randomly selected from 16 potted plants at flowering and mixed. A sub-sample of 15 g of leaf was placed in a 5 L cylindrical glass container as for the entire plants (4 replicates per treatment) and the volatile emissions sampled using the same conditions and procedures as described for whole plants (above). The same weight of fully-expanded rose leaves was used as the control.

2.2. Field-grown plants

2.2.1. Detached leaves

Seeds from the original seed lots of the five lines of *G. gynandra* were drilled into 2 m × 2 m plots at Egerton University, Njoro, Kenya in mid-November 2009, using a completely randomised design (5 rows per plot and 3 replicated plots). The seedlings were thinned to 8–10 cm within the rows 3 weeks after emergence and the crop maintained conventionally. Additionally, seeds of the five lines harvested in the Danish glasshouse in 2009 were planted in a randomised design in 2 m × 1 m plots (3 rows per plot and 3 replicated plots) in mid-December 2009 and maintained as described above. Leaves of the cut flower roses "Marie Clare" and "Aqua" provided control material (Sian Agriflora Kenya Ltd.).

2.2.2. Detached leaf volatile sampling

Fully expanded, entire leaves were detached from field grown *G. gynandra* lines and 30 g weighed and sealed in 5 L cylindrical glass containers (3 replicates per treatment). These were maintained for 24 h at 18–20 °C without supplemental light. Sampling of volatiles was carried out as described above and the traps transported by courier to the Faculty of Life Sciences, University of Copenhagen for GC–MS analysis.

2.3. GC–MS analysis

Trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, USA). Primary desorption was carried out by heating the traps to 250 °C with a flow of carrier gas (He) for 15.0 min. The stripped volatiles were trapped in a Tenax TA cold trap (30 mg held at 5 °C), which was subsequently heated at 300 °C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to a gas chromatograph–mass spectrometer (GC–MS, G1800A GCD System, Hewlett-Packard, Palo Alto, CA, USA) through a heated (225 °C) transfer line.

Separation of volatiles was carried out on a DB-Wax capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The column flow rate was 1.0 mL min^{-1} using helium as a carrier gas. The column temperature programme was: 10 min at 45 °C, 45–240 °C at 6 °C min^{-1}, and finally 10 min at 240 °C. The GC was equipped with a mass spectrometric detector operating in the electron ionisation mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were tentatively identified by matching their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A) and confirmed by running authentic standards of synthetic, pure compounds (Sigma–Aldrich, Denmark). The software programme, GCD Plus ChemStation G1074B (Version A.01.00, Hewlett Packard, Palo Alto, California) was used for data analysis.

2.4. Bioactivity of acetonitrile

2.4.1. Spider mites

Colonies of spider mite (*T. urticae*) were greenhouse-raised on pot roses (*Rosa hybrida* L. "Mercedes") at 18–25 °C and 45–65% RH at Taastrup, University of Copenhagen, Denmark. Infested rose leaflets (petiole + lamina) with at least 20 spider mites were used for the experiments.