

1 **Insects visit *Fusarium xyrophilum* pseudoflowers on the host *Xyris surinamensis* (Xyridaceae) and**  
2 **carry fungal DNA on their bodies**

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27 **Abstract** – The fungus *Fusarium xyrophilum* produces flower-like structures (i.e., pseudoflowers) that  
28 were recently discovered on yellow-eyed grasses (*Xyris* spp.) in Guyana. It is unknown whether these  
29 pseudoflowers, which are composed entirely of fungal tissue, are true mimics that attract insects as a  
30 means of fungal dispersal. We evaluated the potential of *F. xyrophilum* to affect insect visitation patterns  
31 to flowers and pseudoflowers by 1) documenting insect visitation to *X. surinamensis* in Guyana, 2)  
32 measuring the presence of *F. xyrophilum* DNA on insects, and 3) evaluating fluorescence and volatile  
33 production on flowers and pseudoflowers. We report for the first time Vespidae, Formicidae, Salticidae,  
34 Acrididae, and Tetrigidae visiting *Xyris*. Diverse insects, including Conocephalini spp. (meadow  
35 katydids; Tettigoniidae), *Camponotus* spp. (carpenter ants; Formicidae), and a Geometridae sp. (geometer  
36 moths) were found to visit flowers and pseudoflowers. *Fusarium xyrophilum* DNA was detected on 3/12  
37 (25%) of captured insect bodies using conventional and quantitative PCR. Volatiles produced in the field  
38 by pseudoflowers and flowers were similar, except for the presence of a sesquiterpene, putatively  
39 identified here as  $\alpha$ -gurjunene, which was detected both in *F. xyrophilum* pure cultures and field-collected  
40 pseudoflower samples, but not from flowers. The production of this sesquiterpene by *F. xyrophilum* and  
41 the fluorescence of *X. surinamensis* peduncles represent potential signals involved in insect attraction for  
42 this system. These observations, along with the overlap in insect visitors of flowers and pseudoflowers  
43 and the detection of *F. xyrophilum* DNA on insect bodies, are consistent with insect visitors being vectors  
44 of *Xyris* pollen and *F. xyrophilum* propagules between host plants.

45  
46 **Key Words** – fungus-insect interactions, mimicry, Nectriaceae, sesquiterpenes, yellow-eyed grasses.

47 INTRODUCTION

48

49 *Fusarium* (Nectriaceae) is a ubiquitous genus of filamentous ascomycetes. The genus includes  
50 many plant pathogens of agronomic importance, opportunistic human pathogens, and mycotoxin  
51 producers (O'Donnell et al. 2013; Ma et al. 2013). Approximately 80% of all cultivated plants have at  
52 least one disease attributed to a *Fusarium* species; including head blights/ear rots on cereals and vascular  
53 wilts and root rots on vegetables and several other crops (Kvas et al. 2009). Exploration of new  
54 ecosystems continues to reveal novel *Fusarium* diversity with varied arrays of ecological functions and  
55 potential applications.

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57 A recently described species, *F. xyrophilum*, produces flower-like structures (i.e., pseudoflowers)  
58 on species of *Xyris* (yellow-eyed grass; Poales: Xyridaceae). *Fusarium xyrophilum* is a member of the *F.*  
59 *fujikuroi* species complex (FFSC), one of the most studied *Fusarium* species complexes that comprises  
60 diverse mycotoxigenic and plant pathogenic species (Kvas et al. 2009). Some other FFSC species induce  
61 morphological changes to plant inflorescences (Marasas et al. 2006; Freeman et al. 2014), but *F.*  
62 *xyrophilum* is the only *Fusarium* species reported to form pseudoflowers (Laraba et al. 2020a, 2020b).  
63 The pseudoflowers are yellow-orange discoid/lobulate masses that emerge at the tip of the inflorescence.  
64 These pseudoflowers can last for several days (Laraba et al. 2020a), unlike the bright yellow *Xyris*  
65 flowers, which emerge at a rate of one or two per day and open for only a few hours from mid-morning to  
66 mid-afternoon before shriveling (Kral 1988). The first report of pseudoflowers on *Xyris* dates to 1988,  
67 when orange masses noted on spikes of *X. subglabrata* and *X. setigera* were attributed to a “smut fungus”  
68 infecting the flowers. Despite these reports, no further research on these structures was published until  
69 2020, when pseudoflowers were noted on *X. surinamensis*, *X. setigera* and *X. bicephala* (Laraba et al.  
70 2020b). Furthermore, a survey of specimens in three United States herbaria revealed the presence of  
71 pseudoflowers preserved on accessions of *X. surinamensis*, *X. setigera*, and *X. subglabrata*, including  
72 some specimens archived since 1919 (Laraba et al. 2020b). The close visual resemblance of *F.*  
73 *xyrophilum* pseudoflowers to *Xyris* flowers suggests a newly discovered fungal-plant mimicry system  
74 (Laraba et al. 2020a, b).

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76 Pseudoflowers are thought to increase insect visitation to plants through color and scents (Roy  
77 1994; Naef et al. 2002; McArt et al. 2016) to facilitate diverse ecological functions like fertilization of  
78 fungi during sexual reproduction, dissemination of spores, or facilitating entry of fungi into the host without  
79 eliciting a defense response (Ngugi and Scherm 2006). The attraction of insects to plant hosts by  
80 pseudoflower-inducing fungi has been studied in a handful of mimicry systems. For example, in mummy

81 berry disease caused by *Monilinia vaccinii-corymbosi* (*Mvc*), infected blueberry leaves reflect ultraviolet  
82 (UV) light, providing a visual signal to pollinators that is similar to that of healthy flowers (Batra and  
83 Batra 1985). Infected leaves also mimic the floral scent of blueberry due to the presence of the bee-  
84 attracting volatiles cinnamyl alcohol and cinnamic aldehyde in the leaves (McArt et al. 2016). Several  
85 insects, including bees and flies, are attracted to the infected flowers and leaves and carry *M. vaccinii-*  
86 *corymbosi* conidia on their bodies likely acting as fungal vectors. However, not all fungus-induced  
87 pseudoflowers visually mimic uninfected flowers of their plant hosts. Pseudoflowers induced by *Puccinia*  
88 *monoica* on *Boechera stricta* (rockcress) mimic other plants in the vicinity that bloom at the same time,  
89 such as *Ranunculus inamoenus* (buttercups). These pseudoflowers emit a fragrance consisting mostly of  
90 aromatic alcohols, aldehydes, and esters, while *B. stricta* flower scent is a blend of terpenoids and  
91 aliphatic green leaf volatiles (Raguso and Roy 1998). A similar trend is observed for *P. arrhenatheri*  
92 pseudoflowers and *Berberis vulgaris* flowers, which share two volatiles (Naef et al. 2002). In these cases,  
93 the pseudoflowers do not chemically mimic floral scents, but mimic them functionally, as both attract  
94 pollinators. When comparing single-species plots to mixed plots of buttercups and infected rockcress,  
95 both buttercups and pseudoflowers receive more visits when they are both present in the same plot than  
96 when alone (Roy 1994). The difference in the production of volatiles between pseudoflowers and host  
97 flowers may account for this increased pattern of insect visitation in mixed plots because the emission of  
98 a diverse array of compounds attracts more diverse insects (Raguso and Roy 1998).

99  
100 Most knowledge of insect visitation to *Xyris* has been carried out in temperate regions, where *Xyris*  
101 species were hypothesized to be wind-pollinated due to the lack of nectaries, and ‘infrequent’ visitation  
102 by pollen-collecting andrenid bees (Kral 1983). However, *X. tennesseensis* is visited during anthesis  
103 mostly by halictid bees (Hymenoptera: Halictidae) and pollen-consuming syrphid flies (Diptera:  
104 Syrphidae) (Boyd et al. 2011; Moffett and Boyd 2013). *Lasioglossum zephyrus* (Hymenoptera:  
105 Halictidae) manipulates *X. tennesseensis* flowers to open prematurely, ensuring first access to floral  
106 rewards (Wall et al. 2002). Observations of Halictidae and Syrphidae are also reported for *X. asperulla*  
107 and *X. tortulla* (Freitas and Sazima 2006). Seed heads of *X. iridifolia* are presumed to be larval food for  
108 the moth *Coleophora xyridella* (Lepidoptera: Coleophoridae), which produces clusters of cigar-shaped,  
109 tan-colored coleophorid cases that attach to seed heads (Landry 2005). A recent study provided the first  
110 documentation of arthropods in the orders Araneae, Coleoptera, and Orthoptera on *Xyris* spp. in Guyana  
111 that are likely to carry pollen on their bodies and feed on pollen and petals, suggesting that arthropods  
112 could play a role in *Xyris* pollination (Torres-Cruz et al. 2024).

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114 Limited information is available on the extent to which the presence of *F. xyrophilum*  
115 pseudoflowers affect the interactions with *Xyris* insect visitors and the participation of insects in  
116 dispersing this fungus. Therefore, our objectives for this study were to **1)** assess insect visitation to *X.*  
117 *surinamensis* flowers and pseudoflowers in Guyana; **2)** determine if *F. xyrophilum* is detected  
118 molecularly on insects; and **3)** compare fluorescence and emission of volatile organic compounds (VOCs)  
119 produced by pseudoflowers and flowers of *X. surinamensis* in the field. Our study provides the first  
120 studies of insect visitation and VOCs production on *X. surinamensis* bearing true flowers and *F.*  
121 *xyrophilum* pseudoflowers. Along with the discovery of *F. xyrophilum* DNA on insect bodies confirming  
122 the hypothesis that insects vector *F. xyrophilum*, we have largely expanded the current knowledge of this  
123 recently discovered mimicry system.

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## METHODS AND MATERIALS

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*Study Area.* Observations and sample collection were conducted in Guyana between 15  
December 2021 and 2 January 2022. Two sites (1 and 2) were studied in the Demerara-Mahaica Region.  
The vegetation at both sites was comprised of short flowering trees (e.g., *Clusia* sp., Malpighiaceae),  
sedges, rushes, and grasses, including a variety of *Xyris* species. (e.g., *X. surinamensis*, *X. involucrata*).  
The surface of the white sand soil between plants was fairly covered by a diversity of mosses (e.g.,  
*Sphagnum*), as well as *Drosera kaeiteurensis*, *D. intermedia*, and Eriocaulaceae. Diverse plants with  
purple flowers (e.g., *Burmannia bicolor*, *Chelonanthus purpurascens*, *Sauvagesia* sp., multiple species of  
Melastomataceae) and yellow flowers (e.g., *Xyris* spp., *Perama hirsuta*, *Utricularia juncea*,  
*Chamaecrista* sp.) are found in the area. During sampling days, Site 1 (6° 26' 40" N 58° 11' 27" W; ~65  
m.a.s.l.) showed temperatures ranging from 22–38° C; while in Site 2 (6° 19' 10" N 58° 12' 13" W; ~60  
m.a.s.l.) temperatures varied from 28–35° C. Photography of plant, insect, and fungal observations are  
available in iNaturalist under the project: “[Ecosystem profiles of Xyris Research Sites](#)”. The incidence of  
plants bearing pseudoflowers was recorded by counting the number of dead and living *Xyris* plants with  
and without pseudoflowers in five 1 x 30 m nonoverlapping transects at the two sites in Demerara-  
Mahaica region. Data collection took place on 1 January 2022 at Site 1 and 2 January 2022 at Site 2.

*Insect Collection from Xyris surinamensis.* Insects were captured at both collection sites (1 and 2)  
in Demerara-Mahaica Region using yellow pan traps to facilitate insect identification in areas with *X.*  
*surinamensis*. Twelve-ounce yellow plastic bowls (Party Solids, Kingston, PA) were filled with ~200 ml  
of water mixed with two drops of unscented biodegradable dish soap as a surfactant. Three bowls were  
placed in a random pattern at ground level within 0.5–1 m of *X. surinamensis* plants for approximately 6

148 hours each day (9:00-15:00) over a period of seven days, totaling six bowls at Site 2 (two days of  
149 collection) and 15 bowls at site 1 (five days of collection). Differences in sampling efforts (two vs five  
150 days of collection) depended on the availability of opened flowers at each collection site on certain dates.  
151 At the end of the day, insects collected in each bowl were transferred into vials filled with 90% ethanol.  
152 Arthropods were pinned and identified using morphology.

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154 *Arthropod Visitation to Xyris surinamensis.* Time-lapse video was used to assess arthropod visits  
155 to *X. surinamensis* plants bearing flowers in comparison to plants bearing *F. xyrophilum* pseudoflowers at  
156 sites 1 and 2 (Demerara-Mahaica Region). Video clips were recorded between 9:00 and 16:00, based on  
157 patterns of insect visitation. Time-lapse pictures with 5 s intervals were captured using two GoPro Hero7  
158 Black cameras (GoPro Inc., San Mateo, USA) attached to extended batteries (refuel RF-6H50; Mizco  
159 International, Avenel, New Jersey) to provide longer battery life throughout the day. At each site, one  
160 camera was focused on a spike bearing a pseudoflower while the other on a true flower-bearing spike.  
161 Cameras were positioned ~15 cm away from their respective targets, using tripods for stability, and were  
162 provided with a macro lens (52 mm, 10x magnification). Approximately 40 min of recording was  
163 obtained at each target before moving the camera to a new flower or pseudoflower at the same collection  
164 site. Approximately seven ~40 min recordings per target type (i.e., pseudoflower or flower) were taken  
165 per field day. A total of 78 hours 13 min and 25 s of captured video footage was obtained over 10 days of  
166 fieldwork. Video data were assessed individually in duplicate. Morphological identity of the insect (i.e.,  
167 order, family, or genus), contact with the target, and interaction time with the specific target were  
168 obtained for each observation. The number of insect visitors, type of *Xyris* tissue visited and types of  
169 insect visitors were compared between *Xyris* flowers and *F. xyrophilum* pseudoflowers. Video clips  
170 captured herein are available on YouTube as playlist "[Fusarium xyrophilum – Xyris surinamensis insect  
171 visitation study](#)" (playlist ID: PL19pSjmfC9cSceih6nlMnaBQj57ITfvq8).

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173 *Detection of F. xyrophilum on Arthropod Visitors.* Due to the size and fragility of *Xyris* flowers  
174 and the frequency of insect visitation observed, hand-netting was not feasible. Efforts were made to hand-  
175 collect insects using tweezers. Two researchers canvassed the survey area at Site 1 in Demerara-Mahaica  
176 Region for about an hour on each sampling date to hand-collect insects in contact with plants bearing  
177 pseudoflowers or true flowers. Insects collected (n=12) were preserved individually in 90% ethanol to  
178 prevent cross-contamination. Genomic DNA was extracted from the full body of insects using the  
179 DNeasy Plant MiniKit (Qiagen) following the manufacturer's instructions. To assess the presence of *F.*  
180 *xyrophilum* on insect bodies, we PCR-amplified a ~173-bp fragment of the ribosomal intergenic spacer  
181 region (IGS rDNA) using primers IGS-1f and IGS-1r (Laraba et al. 2020b). DNA extracted from pure *F.*



182 *xyrophilum* cultures was used as a positive control and sterile ultrapure water was used as negative control  
183 in the PCR reactions. Additionally, insects were identified by amplifying a ~636 bp portion of the  
184 mitochondrial cytochrome c oxidase subunit I (COI) using the primer pair LCO1490 and HC02198  
185 (Folmer et al. 1994). IGS rDNA and COI amplicons were Sanger sequenced at the Penn State Genomics  
186 Core Facility (University Park, PA). Sequences were submitted to GenBank under accession numbers  
187 OQ121925–27 and OQ152379–90, respectively.

188  
189 *qPCR Quantification of F. xyrophilum Using IGS rDNA.* To determine the amount of *F.*  
190 *xyrophilum* biomass on arthropods visiting the pseudoflowers, a qPCR was performed using the same IGS  
191 rDNA primers used for conventional PCR above. Each 20  $\mu$ L reaction contained 10  $\mu$ L of 2X  
192 QuantiNova SYBR Green Master Mix (Qiagen, Redwood City, CA, USA), 1.4  $\mu$ L of each primer (10  
193  $\mu$ M), 6.2  $\mu$ L of sterile nuclease-free water, and 1  $\mu$ L of template DNA. Negative controls contained 1  $\mu$ L  
194 of sterile nuclease-free water instead of template DNA and were included on every run. All qPCR  
195 reactions had three technical replicates and were carried out using a Bio-Rad C100 Touch Thermal Cycler  
196 and CFX96 Real-Time System machine (Bio-Rad, Hercules, CA, USA). All reactions were run using the  
197 manufacturer's recommended cycling conditions for the QuantiNova SYBR Green PCR kit. Melting  
198 curve analysis consisted of 5 s at every 0.5  $^{\circ}$ C interval from 65  $^{\circ}$ C to 95  $^{\circ}$ C. The DNA concentration of a  
199 cleaned amplicon of *F. xyrophilum* was measured using a Qubit 3 fluorometer and 1X dsDNA High  
200 Sensitivity Assay Kit (Invitrogen, Waltham, MA, USA) and the number of amplicon molecules per  $\mu$ L  
201 was calculated. This was then diluted to a stock solution of  $3.092 \times 10^6$  molecules per  $\mu$ L as the highest  
202 concentration standard (standard 1). Seven additional standards were prepared via 8-fold standard  
203 dilutions. Each standard was run in triplicate using the qPCR cycling conditions and reaction volumes  
204 detailed above. The slope of the resulting calibration curve of quantification cycle (Cq) values vs. log<sub>10</sub>  
205 of amplicon molecules initially present in the PCR tube was used to calculate assay efficiency according  
206 to the following equation: PCR efficiency =  $10^{-1/\text{slope}} - 1$  (Bustin et al., 2009). To determine if there was  
207 qPCR inhibition caused by the extracted DNA sample, we followed a method similar to (Kaminsky and  
208 Bell 2022). Five concentrations of insect DNA were prepared from the 12 insect extractions obtained in  
209 the previous step: undiluted, 5-fold, 10-fold, 50-fold, and 100-fold dilutions in sterile nuclease-free water.  
210 qPCR reactions were conducted, as described before, with template consisting of 1  $\mu$ L of diluted insect  
211 DNA plus 1  $\mu$ L of either standard 1 ( $3.092 \times 10^6$  molecules per  $\mu$ L), standard 3 ( $4.831 \times 10^4$  molecules  
212 per  $\mu$ L) or standard 5 ( $7.549 \times 10^2$  molecules per  $\mu$ L), and 1  $\mu$ L less of sterile water to maintain a 20  $\mu$ L  
213 reaction volume.

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215 The Cq values of each insect dilution-standard combination were compared to that of the  
216 corresponding standard run alone. An increase in Cq value in the presence of insect DNA was not  
217 observed, indicating no qPCR inhibition. Subsequently, the qPCR reactions in this experiment were  
218 conducted using undiluted insect DNA. Cq values and melting curve results were determined with the  
219 Bio-Rad CFX Maestro software using default settings. A subset of qPCR amplicons was sequenced at the  
220 Penn State Genomics Core Facility (University Park, PA), as previously detailed, to confirm their identity  
221 as *F. xyrophilum*.

222

### 223 *Volatile Organic Compounds Produced by X. surinamensis Bearing Flowers vs. Pseudoflowers.*

224 Volatile organic compounds (VOCs) were collected from the headspace of randomly selected *F.*  
225 *xyrophilum*-infected *X. surinamensis* plants bearing pseudoflowers and *X. surinamensis* plants bearing  
226 true flowers at Sites 1 and 2 in the Demerara-Mahaica Region. Volatiles were collected between 10:00 am  
227 and 4:00 pm on four different days by placing bouquets of seven flowers or seven pseudoflowers into 8-  
228 oz glass chambers placed in the field. Bouquets were prepared by excising inflorescences of *X.*  
229 *surinamensis* a few centimeters below the spike and wrapping the stems in wet paper towels covered with  
230 aluminum foil. Each bouquet was placed into a glass chamber and closed with a lid fitted with two  
231 SuperQ filters (20 mg; Alltech Associates, Deerfield, IL, USA), one to clean the air coming into the  
232 chamber and the other to trap VOCs released in the headspace. Air was pulled from chambers at ~1 L  
233 min<sup>-1</sup> using a 9V battery-powered vacuum pump for 1.5 – 3 hours. In addition to collecting VOCs emitted  
234 from flowers and pseudoflowers, at each run a control glass chamber was set up without any samples to  
235 account for background corrections. A total of seven collection runs were conducted for each sample  
236 type, including the control. Filters were transported to the lab, eluted with 100 µL of n-  
237 hexane/dichloromethane (1:1, v/v) containing 500 ng of nonyl acetate as an internal standard and  
238 analyzed by gas chromatography-mass spectrometry (GC-MS).

239

240 To gain better insight into the VOCs profile of *F. xyrophilum*, VOCs were collected from pure  
241 cultures of *F. xyrophilum* (NRRL 62721, FRC M-8921), *F. verticillioides* (NRRL 20956, FRC M-3125),  
242 and *F. thapsinum* (NRRL 22048, FRC M-6562). The two latter species, genetically closely related to *F.*  
243 *xyrophilum* as part of the African clade of FFSC, were used to determine olfactory signatures specific to  
244 *F. xyrophilum*. The strains were cultured in duplicate on Potato Dextrose Agar (PDA) in 16-oz glass  
245 chambers covered with aluminum foil and incubated at room temperature under 12h/12h light/dark. After  
246 three weeks, VOCs were collected in duplicate for 30 min using a 100 µm polydimethylsiloxane solid-  
247 phase micro extraction (SPME) fiber (Supelco, Bellefonte, PA) that was introduced inside the glass  
248 chamber and exposed to the headspace above the fungus. Sterile PDA jars were used as control. Samples



249 were analyzed on an Agilent 6890 series gas chromatograph (GC) coupled to an Agilent 5973 quadrupole  
250 mass selective detector (MS; interface temperature, 250 °C; quadrupole temperature, 150 °C; source  
251 temperature, 230 °C; electron energy, 70 eV). Samples were injected in splitless mode onto an HP-5MS  
252 column (30 m × 0.25 mm i.d. × 0.25 µm thickness; Agilent, Palo Alto, CA, USA) using helium as the  
253 carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>. For liquid samples, the oven temperature was held at 40  
254 °C for 2 min, then increased from 40 to 100 °C at 8 °C min<sup>-1</sup>, 100 to 160 °C at 5 °C min<sup>-1</sup>, 160 to 260 °C  
255 at 40 °C min<sup>-1</sup>, and held at 260 °C for 7 min. For SPME samples, the oven temperature was held at 40 °C  
256 for 2 min, then increased from 40 to 160 °C at 4 °C min<sup>-1</sup>, 160 to 280 °C at 30 °C min<sup>-1</sup>, and held at 280  
257 °C for 4 min. Compounds were identified by comparing the mass spectrum to those in the NIST14  
258 library.

259

260 Three previously generated *F. xyrophilum* genome sequences (GenBank accessions  
261 VYXA00000000, VYWZ00000000, and VYWY00000000; Laraba et al. 2019) were examined using two  
262 methods to identify putative terpene synthase genes potentially involved in production of terpene VOCs  
263 (see results). First, the genome sequences were subjected to antiSMASH version 4 analysis following the  
264 same approach detailed in Kim et al. (2020). Second, a database of predicted proteins from the three *F.*  
265 *xyrophilum* genomes were subjected to BLASTp analysis using query sequences consisting of 40 coding  
266 region sequences representing the breadth of phylogenetic diversity of a previously analyzed set of  
267 terpene synthase genes from filamentous fungi (Agger et al., 2009) and all terpene synthase genes  
268 described in the species *Fusarium fujikuroi* (Niehaus et al., 2016).

269

270 *UV Fluorescence.* *Xyris surinamensis* flowers and *F. xyrophilum* pseudoflowers from Site 1 in  
271 the Demerara-Mahaica region were exposed to ultraviolet light generated by a Convoy C8 365nm UV  
272 light (Yooperlite, Brimley, MI) to evaluate their UV reflection. Photographs of flowers were taken in the  
273 field using an Olympus Tough TG-6 camera. Additionally, three flowers and pseudoflowers were  
274 photographed in the dark approximately 6 hours after being collected from the field using a Sony a7riii  
275 with a Sigma 105mm 2.8 DG DN macro lens.

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## RESULTS

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279 *Arthropods in the vicinity of Xyris surinamensis.* A total of 204 insects representing six orders  
280 and at least 25 families were collected in yellow pan traps (**Table 1**), along with 3 arachnids from two  
281 sites of *X. surinamensis* populations (Site 1 and 2) in the Demerara-Mahaica Region of Guyana.  
282 Additionally, three of the captured insect specimens were not identifiable to order level (XYR006,

283 XYR056, XYR059). Diptera and Hymenoptera were the most collected insects at the collection sites,  
 284 with 111 and 65 individuals, respectively. The most represented families were Dolichopodidae (74  
 285 specimens) and Formicidae (53 specimens). A comprehensive list of the 204 arthropods found in the  
 286 vicinity of *X. surinamensis* populations at Demerara-Mahaica, Guyana with their genus and species can  
 287 be found in **Supplementary Material 1**.

288

289 **Table 1.** Arthropods in the vicinity of *Xyris surinamensis* in the Demerara-Mahaica region of Guyana

| Order            | Family                    | Subfamily        | Species                 | Individuals |
|------------------|---------------------------|------------------|-------------------------|-------------|
| Araneae          |                           |                  |                         | 3           |
| Coleoptera       | Undetermined              |                  |                         | 1           |
|                  | Chrysomelidae             |                  |                         | 2           |
| Diptera          | Chironomidae              |                  |                         | 25          |
|                  | Chloropidae               |                  |                         | 1           |
|                  | Dolipochopodidae          |                  |                         | 74          |
|                  | Limoniidae                |                  |                         | 1           |
|                  | Muscidae                  |                  |                         | 1           |
|                  | Phoridae                  |                  |                         | 1           |
|                  | Sarcophagidae             |                  |                         | 2           |
|                  | Tachinidae                |                  |                         | 1           |
|                  | Undetermined              |                  |                         | 4           |
| Entomobryomorpha |                           |                  |                         | 5           |
| Hemiptera        | Cicadellidae              |                  |                         | 6           |
|                  | Delphacidae               |                  |                         | 3           |
|                  | Miridae                   |                  |                         | 1           |
|                  | Undetermined              |                  |                         | 2           |
| Hymenoptera      | Chalcidoidea <sup>a</sup> |                  |                         | 5           |
|                  | Chrysididae               | Chrysidinae      | <i>Caenochrysis</i> sp. | 2           |
|                  | Crabronidae               |                  |                         | 2           |
|                  | Dryinidae                 |                  |                         | 1           |
|                  | Formicidae                | Ectatomminae     | <i>Ectatomma</i>        | 32          |
|                  |                           |                  | <i>brunneum</i>         |             |
|                  |                           | Formicinae       | <i>Camponotus</i> sp. 1 | 2           |
|                  |                           |                  | <i>Camponotus</i> sp. 2 | 10          |
|                  |                           |                  | <i>Camponotus</i> sp. 3 | 1           |
|                  | Myrmicinae                | <i>Wasmannia</i> | 3                       |             |
|                  | <i>auropunctata</i>       |                  |                         |             |
| Pseudomyrmecinae | <i>Pseudomyrmex</i> sp.   | 5                |                         |             |
| Mymaridae        |                           |                  | 1                       |             |
| Pompilidae       |                           |                  | 1                       |             |

|                            |                       |            |     |
|----------------------------|-----------------------|------------|-----|
| Orthoptera                 | Acrididae             |            | 2   |
|                            | Trigonidiidae         | Nemobiinae | 2   |
|                            | Ensifera <sup>b</sup> |            | 1   |
| Unidentifiable             |                       |            | 3   |
| Total number of arthropods |                       |            | 207 |

290 <sup>a</sup>Superfamily

291 <sup>b</sup>Suborder

292

293 *Arthropod visitation to Xyris surinamensis.* In 10 days of field observation, a total of 78 h 13 min  
 294 and 25 s of video footage were captured as timelapse to document insect visitation to *X. surinamensis*  
 295 plants. Of these, 34 h 46 min 30 s were recorded to study insects visiting *X. surinamensis* flowers and 43  
 296 h 26 min 55 s for pseudoflowers. Over 10 days at Site 1, we found that on average >10 flowers opened  
 297 per day, with only two collection dates where <10 flowers were open with an average of 1.53% incidence  
 298 of pseudoflowers (**Table 2**). In contrast, the number of opened flowers at Site 2 varied across collection  
 299 dates from zero opened flowers to <5 in a day. Thus, more video data were collected for pseudoflowers  
 300 than flowers at this site. The incidence of pseudoflowers at Site 2 was on average 4.75%. Even with this  
 301 data collection bias, fewer insect visitors were observed in contact with pseudoflowers (n = 4) than  
 302 flowers (n = 19; **Table 3**).

303

304 **Table 2.** Percent incidence of pseudoflowers on *Xyris surinamensis* in Demerara Mahaica, Guyana based  
 305 on transect counts

| Transect       | Site 1                             |                                 |             | Site 2                             |                                 |             |
|----------------|------------------------------------|---------------------------------|-------------|------------------------------------|---------------------------------|-------------|
|                | <i>Xyris</i> without pseudoflowers | <i>Xyris</i> with pseudoflowers | % incidence | <i>Xyris</i> without pseudoflowers | <i>Xyris</i> with pseudoflowers | % incidence |
| 1              | 267                                | 6                               | 2.20        | 1072                               | 56                              | 4.96        |
| 2              | 267                                | 14                              | 4.98        | 570                                | 29                              | 4.84        |
| 3              | 444                                | 0                               | 0.00        | 370                                | 8                               | 2.12        |
| 4              | 543                                | 7                               | 1.27        | 70                                 | 6                               | 7.89        |
| 5              | 606                                | 6                               | 0.98        | 103                                | 10                              | 8.85        |
| <b>Overall</b> | <b>2127</b>                        | <b>33</b>                       | <b>1.53</b> | <b>2185</b>                        | <b>109</b>                      | <b>4.75</b> |

306

307 The same insects were observed to be in contact with pseudoflowers and flowers (**Table 3**). This  
 308 includes two observations of Conocephalini spp. found chewing on flowers (*e.g.*, **Figs. 1a, n**) and two  
 309 observed feeding on pseudoflowers (*e.g.*, **Fig. 1f**); seven observations of *Camponotus* spp. on *Xyris* spikes  
 310 bearing a flower (*e.g.*, **Fig. 1d**), including one observed carrying a petal out of the frame (**Fig. 1b**), and  
 311 one *Camponotus* spp. on a pseudoflower (**Fig. 1g**); and one Geometridae species attached to  
 312 pseudoflower tissue (**Fig. 1h**) as well as below the spike bearing a flower (**Fig. 1m**). However, it is worth  
 313 nothing that some arthropods were exclusively in contact with flowers but not pseudoflowers (**Table 3**).

314 These include two Anthophila species (e.g., **Fig. 1c**), a Vespidae sp. (**Fig. 1e**), an Araneidae sp., and  
315 seven observations of Formicidae spp. (e.g., **Fig. 1d**). Additionally, one Salticidae sp. was found in  
316 contact with the spike of *X. surinamensis* below the pseudoflower structure (**Fig. 1k**) and on a couple of  
317 occasions spider webs were observed between *Xyris* tissues (e.g., scapes, spikes) and the pseudoflowers  
318 (**Fig. 1l**).



319 **FIG. 1** *Xyris surinamensis* flower and pseudoflower arthropod visitors. **a–e.** Insects in contact with flowers of *X.*  
320 *surinamensis*. **a.** Conocephalini sp. chewing on petals. **b.** *Camponotus* sp. carrying petal. **c.** Anthophila. **d.**  
321 Formicidae. **e.** Vespidae. **g–h.** Insects in contact with *Fusarium xyrophilum* pseudoflowers of *X. surinamensis*. **f.**  
322 Conocephalini sp. chewing on pseudoflower. **g.** *Camponotus* sp. **h.** Geometridae sp. **i.** Conocephalini sp. on *Xyris*  
323 leaf near a pseudoflower. **j.** *Camponotus* sp. on dead *Xyris* leaf near a pseudoflower. **k.** Salticidae sp. on *Xyris* spike  
324 below pseudoflower structure. **l.** Spider web in contact with pseudoflower. **m.** Geometridae sp. attached to stem  
325 below inflorescence. **n.** Conocephalini sp. chewing on petals. Pictures A–J are still images from timelapse videos  
326 recorded with GoPro Hero7 and K–N are photographs taken with an Olympus Tough TG-6 while in the field. White  
327 arrows point to small arthropods for easier visualization. Scale bar = 10 mm  
328



329 **Table 3.** Arthropod visitors of *Xyris surinamensis* flowers, *Fusarium xyrophilum* pseudoflowers, and  
 330 other tissues

| Site                       | Date        | Target tissue | Contact time (min:s) | Arthropod identification               | Video file                      |
|----------------------------|-------------|---------------|----------------------|--|---------------------------------|
| Flowers                    |             |               |                      |  |                                 |
| Site 1                     | 17 Dec 2021 | Flower        | 0:52                 | <i>Pseudomyrmex</i> – Formicidae       | GH010017                        |
| Site 1                     | 17 Dec 2021 | Flower        | 1:00                 | <i>Pseudomyrmex</i> – Formicidae       | GH010017                        |
| Site 1                     | 17 Dec 2021 | Flower        | 0:45                 | <i>Pseudomyrmex</i> – Formicidae       | GH010017 <sup>a</sup>           |
| Site 1                     | 19 Dec 2021 | Flower        | 8:00                 | Conocephalini – Tettigoniidae          | GH010076 <sup>b</sup>           |
| Site 1                     | 19 Dec 2021 | Flower        | 12:30                | <i>Camponotus</i> – Formicidae         | GH010068 <sup>c</sup>           |
| Site 1                     | 19 Dec 2021 | Flower        | 5:30                 | <i>Camponotus</i> – Formicidae         | GH010069                        |
| Site 1                     | 19 Dec 2021 | Flower        | 6:15                 | <i>Pseudomyrmex</i> – Formicidae       | GH010069                        |
| Site 1                     | 31 Dec 2021 | Flower        | 9:15                 | <i>Pseudomyrmex</i> – Formicidae       | GH010095                        |
| Site 1                     | 31 Dec 2021 | Flower        | 0:10                 | Unidentified arthropod                 | GH010095                        |
| Site 1                     | 31 Dec 2021 | Flower        | 0:30 / 4:00          | <i>Pseudomyrmex</i> – Formicidae       | GH010098 <sup>d</sup>           |
| Site 1                     | 31 Dec 2021 | Flower        | 1:00                 | <i>Camponotus</i> - Formicidae         | GH010098                        |
| Site 1                     | 31 Dec 2021 | Flower        | 0:09                 | <i>Polybia occidentalis</i> – Vespidae | GH010099 <sup>e</sup>           |
| Site 1                     | 01 Jan 2022 | Flower        | 0:16                 | <i>Camponotus</i> - Formicidae         | GH010100                        |
| Site 1                     | 01 Jan 2022 | Flower        | 0:04                 | <i>Camponotus</i> - Formicidae         | GH010103                        |
| Site 1                     | 07 Jan 2022 | Flower        | ---                  | Araneidae                              | GH010128 <sup>f</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 1:04                 | Anthophila                             | GH010015 <sup>g</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:02                 | Anthophila                             | GH010015                        |
| Site 2                     | Dec-18-2021 | Flower        | 0:25                 | <i>Camponotus</i> - Formicidae         | GH010061                        |
| Site 2                     | 02 Jan 2022 | Flower        | 2:30                 | <i>Camponotus</i> - Formicidae         | GH010116                        |
| Site 2                     | 02 Jan 2022 | Flower        | 49:00                | Conocephalini - Tettigoniidae          | GH010114 <sup>h</sup>           |
| Pseudoflowers              |             |               |                      |  |                                 |
| Site 1                     | 20 Dec 2021 | Pseudoflower  | 0:27                 | <i>Camponotus</i> - Formicidae         | GH016576 <sup>i</sup>           |
| Site 1                     | 31 Dec 2021 | Pseudoflower  | 0:30                 | Conocephalini - Tettigoniidae          | GH016584                        |
| Site 2                     | 01 May 2019 | Pseudoflower  | 5:02                 | Conocephalini - Tettigoniidae          | GH020061, GH030061 <sup>j</sup> |
| Site 2                     | 02 Jan 2022 | Pseudoflower  | 2:15                 | Geometridae                            | GH016604 <sup>k</sup>           |
| Other <i>Xyris</i> tissues |             |               |                      |  |                                 |
| Site 1                     | 17 Dec 2021 | Pseudoflower  | 0:18                 | Formicidae                             | GH016526 <sup>l</sup>           |
| Site 1                     | 17 Dec 2021 | Pseudoflower  | 0:15                 | <i>Camponotus</i> - Formicidae         | GH016526 <sup>m,n</sup>         |
| Site 1                     | 31 Dec 2021 | Flower        | 27:30                | Conocephalini - Tettigoniidae          | GH010097 <sup>o</sup>           |
| Site 2                     | 13 May 2019 | Pseudoflower  | 0:08                 | Conocephalini - Tettigoniidae          | GH016493 <sup>1,p</sup>         |
| Site 2                     | 15 May 2019 | Flower        | 0:16                 | Unidentified arthropod                 | GH010013 <sup>q</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:16                 | Acrididae                              | GH010014 <sup>r</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:47                 | <i>Camponotus</i> – Formicidae         | GH010014 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:35                 | <i>Camponotus</i> – Formicidae         | GH010014 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:35                 | Formicidae                             | GH010015 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Flower        | 0:16                 | Formicidae                             | GH010015 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Pseudoflower  | 0:03                 | <i>Pseudomyrmex</i> – Formicidae       | GH016500 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Pseudoflower  | 0:01                 | <i>Camponotus</i> – Formicidae         | GH016500 <sup>l</sup>           |
| Site 2                     | 15 May 2019 | Pseudoflower  | 0:02                 | <i>Camponotus</i> – Formicidae         | GH016500 <sup>s</sup>           |
| Site 2                     | 18 Dec 2021 | Flower        | 0:12                 | Conocephalini - Tettigoniidae          | GH010061 <sup>m</sup>           |

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 343

<sup>a</sup> See Fig. 1d.

<sup>b</sup> Katydid eats the *Xyris* petals (Fig. 1a).

<sup>c</sup> Ant chews on a petal and carries it out of the frame (Fig. 1b).

<sup>d</sup> Ant in contact with inflorescence in two occasions during the same video.

<sup>e</sup> See Fig. 1e.

<sup>f</sup> Spider building a web between *Xyris* tissues (leaves and stems).

<sup>g</sup> See Fig. 1c.

<sup>h</sup> Katydid eats whole flower.

<sup>i</sup> See Fig. 1g.

<sup>j</sup> Katydid chewing on pseudoflower (See Fig. 1f).

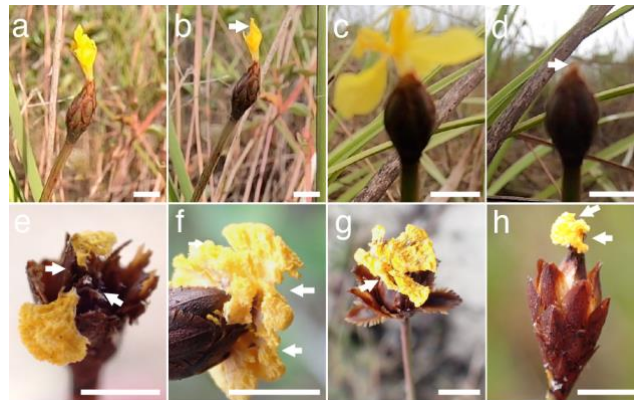
<sup>k</sup> See Fig. 1h.

<sup>l</sup> In contact with *Xyris* leaves.

<sup>m</sup> In contact with *Xyris* dead leaves.

344 <sup>n</sup> See Fig. 1j.  
345 <sup>o</sup> Katydid on *Xyris* spike in the background of the target tissue (inflorescence).  
346 <sup>p</sup> See Fig. 1i.  
347 <sup>q</sup> Arthropod on *Xyris* leaf in background of target tissue (inflorescence).  
348 <sup>r</sup> In contact with dead *Xyris* leaf in background.  
349 <sup>s</sup> *Xyris* stem below pseudoflower.  
350

351 Many of the arthropod visits to *Xyris* tissues lasted for less than a minute (**Table 3**). Yet, several  
352 Formicidae interactions with the flowers lasted between 1 min and up to 12 min and 30 s (**Table 3**). An  
353 Apidae sp. also remained in contact with a flower for 1 min and 4 s (**Fig. 1c**). Additionally, a  
354 Geometridae sp. (**Fig. 1h**) and a Conocephalini sp. (**Fig. 1f**) interacted with pseudoflowers for 2 min 15 s  
355 and 5 min 2 s, respectively. The longest interactions were captured between *X. surinamensis* and  
356 Conocephalini spp. One of these interactions lasted for 27 min 30 s and on the other one it took a katydid  
357 49 min to eat an entire flower (**Table 3**). Some insects also fed on pseudoflowers. Potential chewing  
358 damage was observed on pseudoflowers in the field (**Fig. 2e–h**), and our video footage captured a katydid  
359 chewing on a pseudoflower (**Table 3, Fig. 1f**), as well.  
360



361 **FIG. 2** Evidence of insect feeding on *Xyris surinamensis* in Demerara-Mahaica, Guyana. **a–d**. Still images from  
362 timelapse recordings where Conocephalidae spp. were observed chewing on flowers. **a**. Flower right before arrival  
363 of Conocephalini sp. at site 1. **b**. Same flower after 8 min of Conocephalini sp. chewing on it. **c**. Flower before  
364 arrival of Conocephalini sp. at site 2. **d**. Same flower after 49 min of Conocephalini sp. chewing on it and eating all  
365 the floral tissues. **e–h**. Potential evidence of insect feeding occurring on pseudoflower tissues. White arrows point to  
366 areas where chewing has occurred. Scale bar = 10 mm  
367

368  
369 Fourteen other instances of insects were recorded visiting *Xyris* tissues in the periphery of the  
370 intended flower or pseudoflower being observed. In most cases, these were insects previously observed in  
371 contact with flowers and/or pseudoflowers that were in the frame of the recording but not come in direct  
372 contact with these tissues, such as Conocephalini spp. (e.g., **Fig. 1i**) and several *Camponotus* spp. Other  
373 Formicidae and Acrididae sp. came in contact with *Xyris* leaves (e.g., **Fig. 1j**).



374 *Molecular detection of F. xyrophilum on arthropod visitors.* Eleven insects representing six  
375 families and one orb weaver spider (Araneidae), were hand-collected while in contact with *X.*  
376 *surinamensis* flowers or *F. xyrophilum* pseudoflowers in the field (**Fig. 3**). We collected one specimen  
377 from each of the families Chrysomelidae, Formicidae, and Geometridae. Eight of the specimens captured  
378 were orthopterans: five Conocephalini (Tettigoniidae), two Acrididae, and one Tetrigidae (**Table 3**). The  
379 12 arthropods collected were tested using IGS rDNA PCR primer pair targeting *F. xyrophilum*.  
380 Amplicons of expected size (i.e., ~173 bp) were observed for a Conocephalini (Tettigoniidae; **Fig. 3i**) and  
381 a Tetrigidae (**Fig. 3l**) collected while in contact with pseudoflowers, and a *Leptysm* sp. (Acrididae; **Fig.**  
382 **3k**) which was captured during a visit to a *Xyris* flower (**Table 4**). Sanger sequences of these three  
383 amplicons (OQ121925– OQ121927) confirmed their identity as *F. xyrophilum*.  
384

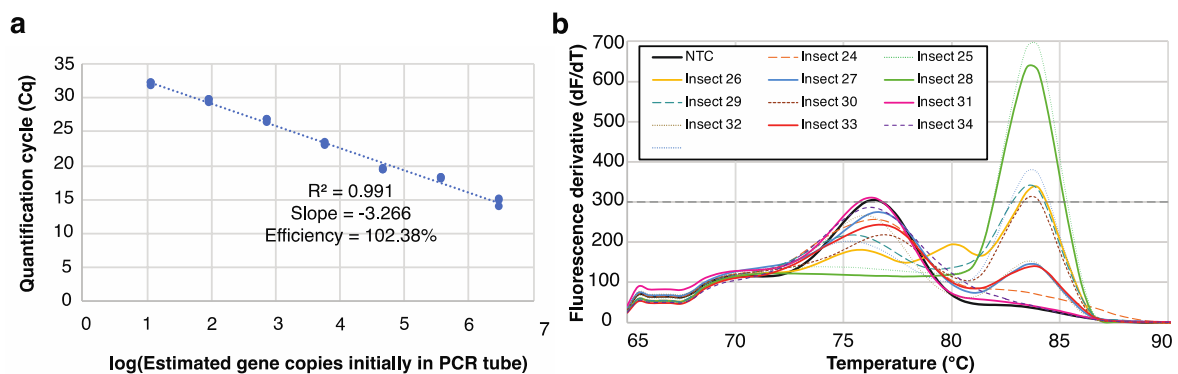


385  
386 **FIG. 3** Macroscopic pictures before DNA extraction of arthropods collected by hand while in contact with *Xyris*  
387 *surinamensis* flower or pseudoflower tissues at Site 1, Demerara-Mahaica, Guyana. **a–c.** Arthropods found in  
388 contact with flowers. **a.** Araneidae (insect 27). **b.** Chrysomelidae (insect 26). **c.** *Camponotus* sp. (insect 31). **d.**  
389 Geometridae found attached to the stem right below the spike (insect 35). **e–h.** Conocephalinae (Tettigoniidae)  
390 found in contact with flowers (insects 30, 32, 33, 34; respectively). **i.** Conocephalinae (Tettigoniidae) found in  
391 contact with a pseudoflower (insect 25). **j.** Acrididae found on flower (insect 24). **k.** *Leptysm* (Acrididae) found on

392 flower (insect 29). **I.** Tetrigidae collected while in contact with a pseudoflower (insect 28). **a–b.** Scale bar = 2.5 mm.  
393 **c–I.** Scale bar = 5 mm.

394

395 A qPCR of the 12 aforementioned arthropod samples using the IGS rDNA primers was also  
396 conducted to quantify the presence of *F. xyrophilum* on these insects. The serial dilutions of genomic *F.*  
397 *xyrophilum* DNA used for the curve of Cq vs. log number of amplicon molecules initially present  
398 demonstrated a reaction efficiency of 102.38% with an R<sup>2</sup> of 0.991 (**Fig. 4a**), which meets the guidelines  
399 set out in Bustin et al. (2009). We were able to reliably amplify the concentration of the lowest standard  
400 containing ~12 gene copies in the PCR tube at a Cq below 35. This sets the sensitivity of our assay  
401 towards *F. xyrophilum* IGS. Potential off-target amplification was observed in melting curves following  
402 qPCR reactions showing a peak at 76.5 °C (**Fig. 4b**), but this peak reached the cycle threshold (Ct value)  
403 in only one sample (insect 34) and the no template control (NTC). A subsequent gel electrophoresis  
404 revealed that for these samples with high 76.5 °C melting peaks only a band <50 bp was observed, that  
405 we suspect to represent primer dimer (data not shown), especially given the presence of this band in the  
406 NTC. Two samples (identifier 25 and 28; **Table 4**) presented especially high initial copy numbers,  
407 confirming the results from conventional PCR. However, several other insect samples (identifiers 26, 30,  
408 35; **Table 4**) had detectable amounts of *F. xyrophilum* DNA that were not detected with conventional  
409 PCR and gel imaging. Sanger sequencing of selected amplicons followed by BLAST analysis showed,  
410 respectively, a 122/122 bp (100%) and 121/121 bp (100%) match to isolates SEr (MT919914) and NRRL  
411 62710 (MT919908) of *F. xyrophilum*.



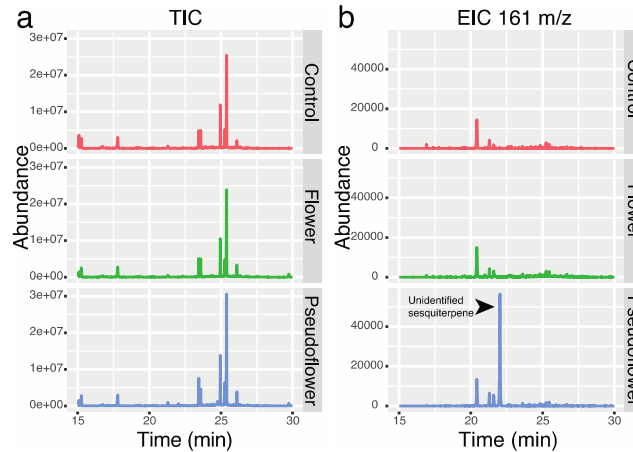
412

413 **FIG. 4** Calibration and melting curves for the IGS qPCR using primers IGS-1f and IGS-1r from Laraba et al. 2020.  
414 **a.** Calibration curve generated from a set of eight-fold standard dilutions of the IGS PCR product. **b.** Melt curves of  
415 qPCR products resulting from genomic DNA extracted from 12 insects that were hand collected from *Xyris*  
416 *surinamensis* tissues (flowers or pseudoflowers produced by *Fusarium xyrophilum*). Dotted line represents the cycle  
417 threshold (Ct value). NTC = negative control

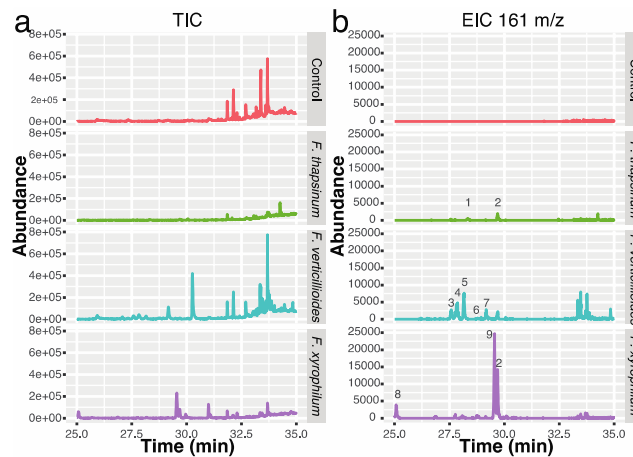
418 **Table 4.** Presence of *F. xyrophilum* and identification of arthropods collected while visiting flowers and  
 419 pseudoflowers on *Xyris surinamensis* at Site 1, Demerara-Mahaica, Guyana. Only results highlighted in  
 420 grey are considered to be the correct IGS amplicon for qPCR

| Sample ID | Collection date | Type of <i>Xyris</i> tissue       | Identification | COI                   | IGS      | <i>F. xyrophilum</i> IGS qPCR |             |                   |      |
|-----------|-----------------|-----------------------------------|----------------|-----------------------|----------|-------------------------------|-------------|-------------------|------|
|           |                 |                                   |                |                       |          | Cq                            | Copy number | Melting peak (°C) |      |
| 24        | Dec 20 2021     | Inflorescence                     | Orthoptera     | Acrididae sp.         | OQ152379 | 32.28                         | 10.79       | None              |      |
| 25        | Dec 20 2021     | Pseudoflower                      | Orthoptera     | Conocephalinae sp.    | OQ152380 | OQ121925                      | 27.14       | 397.50            | 84   |
| 26        | Dec 20 2021     | Inflorescence                     | Coleoptera     | Chrysomelidae sp.     | OQ152381 |                               | 32.17       | 11.79             | 84   |
| 27        | Dec 20 2021     | Inflorescence                     | Araneae        | Araneidae sp.         | OQ152382 |                               | 32.51       | 9.03              | 76.5 |
| 28        | Dec 20 2021     | Pseudoflower                      | Orthoptera     | Tetrigidae sp.        | OQ152383 | OQ121926                      | 26.45       | 651.67            | 83.5 |
| 29        | Dec 20 2021     | Inflorescence                     | Orthoptera     | <i>Leptysmia</i> sp.  | OQ152384 | OQ121927                      | 31.62       | 16.88             | 83.5 |
| 30        | Dec 31 2021     | Inflorescence                     | Orthoptera     | Conocephalinae sp.    | OQ152385 |                               | 31.53       | 18.62             | 84   |
| 31        | Dec 31 2021     | Inflorescence                     | Hymenoptera    | <i>Camponotus</i> sp. | OQ152386 |                               | 32.51       | 9.17              | 76.5 |
| 32        | Dec 31 2021     | Inflorescence                     | Orthoptera     | Conocephalinae sp.    | OQ152387 |                               | 32.12       | 11.91             | None |
| 33        | Dec 31 2021     | Inflorescence                     | Orthoptera     | Conocephalinae sp.    | OQ152388 |                               | 32.60       | 8.54              | None |
| 34        | Dec 31 2021     | Inflorescence                     | Orthoptera     | Conocephalinae sp.    | OQ152389 |                               | 32.43       | 9.58              | None |
| 35        | Dec 31 2021     | Stem below spike of inflorescence | Lepidoptera    | Geometridae sp.       | OQ152390 |                               | 31.39       | 20.06             | 84   |

421  
 422 *Volatile organic compounds emitted by X. surinamensis* flowers, and *Fusarium xyrophilum*  
 423 *pseudoflowers* and cultures. *Xyris surinamensis* flowers and *F. xyrophilum* pseudoflowers emitted (Z)-3-  
 424 hexen-1-ol and 1-hexanol. *Fusarium xyrophilum* pseudoflowers, but not *X. surinamensis* flowers, emitted  
 425 a sesquiterpene compound that eluted at 22.03 min, tentatively identified as  $\alpha$ -gurjunene using the  
 426 NIST14 library (**Fig. 5**). This sesquiterpene was also detected in the head space of *F. xyrophilum* PDA  
 427 cultures (**Fig. 6**), but eluted at 29.55 min, presumably because a different method (i.e., SPME fiber) was  
 428 used for volatile detection from fungal cultures. Interestingly,  $\alpha$ -gurjunene was not present in the blend  
 429 VOCs emitted by PDA cultures of *F. verticillioides* (NRRL 20956, FRC-M3125) and *F. thapsinum*  
 430 (NRRL 22048, FRC-M6562) used for comparison. The mass spectra of  $\alpha$ -gurjunene are provided in  
 431 **Supplementary Material 2**. It is noteworthy that  $\alpha$ -gurjunene was not detected in emissions from *X.*  
 432 *surinamensis* flowers (**Fig. 5**).



433  
434 **FIG. 5** GC-MS spectrum of volatiles emitted from flowers or pseudoflowers of *Xyris surinamensis* in Guyana. **a.**  
435 Total ion chromatogram (TIC) **b.** Extracted ion chromatogram (EIC) for 161 m/z. Arrowhead points to  
436 sesquiterpene peak identified potentially as  $\alpha$ -gurjunene  
437



438  
439 **FIG. 6** GC-MS spectrum of volatiles emitted from strains of *Fusarium thapsinum* (NRRL 22048, FRC-M6562), *F.*  
440 *verticillioides* (NRRL 20956, FRC-M3125), and *F. xyrophilum* (KOD596, NRRL 62721, FRC-M8921) grown on  
441 PDA. **a.** Total ion chromatogram (TIC) **b.** Extracted ion chromatogram (EIC) for 161 m/z. **1.** Dihydro- $\beta$ -ionone. **2.**  
442 Germacrene D. **3.** 4H-1,4a-Methanonaphthalene, 1,5,6,7,8,8a-hexahydro-2,5,5,8a-tetramethyl-,  
443 (1.alpha.,4a.alpha.,8a.beta.)-. **4.**  $\beta$ -Funebrene. **5.** Cedrene. **6.**  $\beta$ -Copaene. **7.** Acoradiene. **8.**  $\delta$ -Elemene. **9.**  $\alpha$ -  
444 Gurjunene  
445

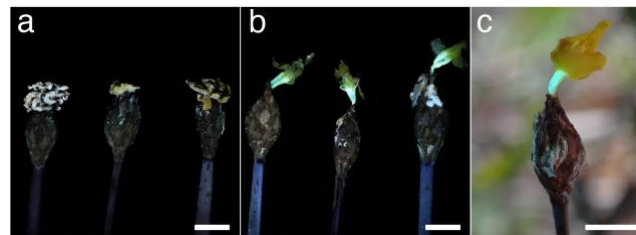
446 We conducted antiSMASH and BLAST analyses to determine if any of the predicted terpene  
447 synthase genes in the *F. xyrophilum* genomes (GCA\_008711575, GCA\_008711615, and  
448 GCA\_008711595) could be the  $\alpha$ -gurjunene synthase gene. A limitation of this analysis was that, as far as  
449 we are aware, the  $\alpha$ -gurjunene synthase gene has not yet been identified in any organism. Nevertheless,

450 the antiSMASH and BLAST analyses indicated that the *F. xyrophilum* genome has 12 terpene synthase  
451 genes. The high level of amino acid sequence identity (>83%, **Supplementary Material 3**) of 10 of the  
452 *F. xyrophilum* terpene synthase genes to orthologs in *F. fujikuroi* suggests the *F. xyrophilum* enzymes  
453 have the same metabolic functions as the *F. fujikuroi* enzymes. The functions in terpenoid biosynthesis of  
454 nine of these genes are known, but none of the functions indicate that the corresponding enzyme could  
455 catalyze synthesis of  $\alpha$ -gurjunene. We were unable to assign putative functions to three of the *F.*  
456 *xyrophilum* terpene synthase genes: genes KO596\_3141, KO596\_8005 and KO596\_9253 in strain NRRL  
457 62721 (**Supplementary Material 3**). KO596\_3141 has a closely related *F. fujikuroi* ortholog whose  
458 function in terpene synthesis has not been determined. KO596\_8005 and KO596\_9253 do not have  
459 closely related orthologs in *F. fujikuroi* and BLAST analysis did not provide clues as to terpenes the  
460 corresponding enzymes synthesize. Thus, KO596\_3141, KO596\_8005 and KO596\_9253 are candidate  $\alpha$ -  
461 gurjunene synthase genes.

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463 *Xyris surinamensis* flower and pseudoflower UV fluorescence. Imaging *F. xyrophilum*  
464 pseudoflowers irradiated with UV light at a wavelength of 365 nm did not cause any fluorescence of the  
465 structures (**Fig. 7a**). By contrast, at the same treatment of *X. surinamensis* plants caused bright green  
466 fluorescence of pedicels of both excised flowers (**Fig. 7b**) and intact flowers in the field (**Fig. 7c**).

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468

469 **FIG. 7** Fluorescence at 365 nm of *Xyris surinamensis* flowers and *Fusarium xyrophilum* pseudoflowers. **a–b.**  
470 Pictures taken approximately 6 h after removal from plant. **a.** Three *F. xyrophilum* pseudoflowers. **b.** Three *X.*  
471 *surinamensis* flowers. **c.** *Xyris surinamensis* flower in the field. Scale bar = 10 m

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473

## DISCUSSION

474

475 Despite the lack of nectaries and ‘infrequent’ insect visitation previously reported for *Xyris* (Kral  
476 1983), diverse arthropods were present in *Xyris surinamensis* populations in savanna areas of Guyana.  
477 Our study documents diverse insects in areas where *X. surinamensis* populations grow in Demerara-  
478 Mahaica Region of Guyana. Over 200 insects were captured with most individuals belonging to the orders  
479 Diptera and Hymenoptera. Dipterans from the families Syrphidae and Asilidae, as well as hymenopterans



480 from Halictidae and Apidae, have been previously reported on *X. tennesseensis* populations from Alabama  
481 and Georgia in the US (Boyd et al. 2011; Moffett and Boyd 2013), and *X. asperulla* and *X. tortulla* from  
482 Brazil (Freitas and Sazima 2006). However, herein, none of these families were observed on *X.*  
483 *surinamensis*. Dolichopodidae and Formicidae were the most common Dipteran and Hymenopteran  
484 families in our study, respectively. These differences may be due to bias in the collection methods used or  
485 perhaps Dolichopodidae and Formicidae may be more prevalent around *Xyris* populations in Guyana than  
486 those in temperate regions.

487

### 488 **Overlap in arthropods that visit *X. surinamensis* flowers and pseudoflowers**

489 We evaluated the possibility that insect visitation to *Xyris* could be affected by the presence of *F.*  
490 *xyrophilum* pseudoflowers. It had been proposed that few insects infrequently visit *Xyris* species (Kral  
491 1983). Follow-up studies showed a higher diversity of insect visitors, yet most of these studies were in  
492 North American *Xyris* species (Wall et al. 2002; Landry 2005; Freitas and Sazima 2006; Boyd et al. 2011;  
493 Moffett and Boyd 2013). First reports of arthropods in the orders Araneae, Coleoptera, Lepidoptera, and  
494 Orthoptera visiting *Xyris* spp. plants were recently made (Torres-Cruz et al. 2024); providing a more  
495 diverse view of the insects that visit these plants. Here we present the first assessment of insect visitors of  
496 *X. surinamensis* flowers and *F. xyrophilum* pseudoflowers.

497

498 Approximately 78 hours of video footage of insects visiting the flowers and pseudoflowers in the  
499 study area revealed a low number of insects in contact with *X. surinamensis* flowers (n = 19) and  
500 pseudoflower tissues (n = 4). This number of observations is much lower than those noted in other fungal  
501 mimicry systems. For instance, in *B. vulgaris* infected by *P. arrhenatheri*, between 27–30 insect visitors  
502 were observed in 80 min and 148 in 360 min (Naef et al. 2002). On the other hand, in the *Mvc*-blueberry  
503 mimicry system, between ~190–1450 bees and ~60–250 flies visited true flowers but lower visitation  
504 rates (3–24 per hour) to *Mvc* infected leaves were documented (McArt et al. 2016). Despite the low  
505 number of insect visitors in this system, we provide the first report of Vespidae, Formicidae, and  
506 Acrididae visiting *Xyris* flowers and Salticidae on a pseudoflower. Salticidae have previously been  
507 observed to visit *Puccinia arrhenatheri* pseudoflowers on *Berberis vulgaris* (Naef et al. 2002). By  
508 contrast, the presence of Conocephalini spp. (katydid) is specific to this putative *F. xyrophilum*-*Xyris*  
509 mimicry system, as they have not been observed to visit other known fungal mimicry systems.

510

511 *Xyris surinamensis* flowers and *F. xyrophilum* pseudoflowers were discovered to share certain  
512 visitors, including species of Conocephalini, *Camponotus*, and Geometridae, suggesting potential  
513 vectoring of *F. xyrophilum* conidia between uninfected and infected *Xyris* plants. While most arthropod



514 visits to *Xyris* tissues lasted for less than a minute, some lasted up to 49 min where insects (e.g., ants and  
515 katydids) fed on *Xyris* flowers or *F. xyrophilum* pseudoflowers. This is a well-known behavior for certain  
516 orthopterans that feed on pollen and petals as a supplementary source of nutrition (Tan et al. 2017) and  
517 has been reported in other *Xyris* species in Guyana (Torres-Cruz et al. 2024). *Xyris* can be a food source  
518 for insects, like syrphid flies that feed on the pollen of *X. tennesseensis* (Boyd et al. 2011), which is  
519 presumed food for *C. xyridella* larvae (Landry 2005). Here we expand florivory on *Xyris* to Formicidae  
520 spp. *Fusarium xyrophilum* produces aseptate microconidia on the pseudoflowers, but not the typical  
521 *Fusarium* multiseptated fusiform macroconidia (Laraba et al. 2020a). It is possible that these  
522 microconidia survive the insect gut and/or are carried on the insect's body. Hence, these field  
523 observations of insects chewing on the pseudoflowers suggest they might be tricking herbivores rather  
524 than pollinators for dispersal.

525

### 526 ***Fusarium xyrophilum* is detected on the bodies of arthropods**

527 Our detection of *F. xyrophilum* DNA on insect visitors of flowers and pseudoflowers supports  
528 their previous predicted role as vectors of *F. xyrophilum* propagules (Laraba et al. 2020b). Our field  
529 observations showed great diversity of insects in proximity of *X. surinamensis* plants. However, only a  
530 subset came in contact with *X. surinamensis* tissues. Therefore, we exclusively captured insects (n=11)  
531 and other arthropods (n=1) that visited host flowers or pseudoflowers right before they were captured.  
532 DNA of *F. xyrophilum* was detected on 25% of samples using a conventional IGS rDNA targeting PCR.  
533 Using qPCR, we also detected *F. xyrophilum* in three other insects (i.e., Chrysomelidae sp.,  
534 Conocephalinae sp., and Geometridae sp.), which did not test positive for *F. xyrophilum* in the  
535 conventional PCR. This is potentially due to higher sensitivity of the qPCR in comparison to the  
536 conventional PCR. These qPCR data increase our detection of *F. xyrophilum* DNA on insects to 50%. As  
537 they carry the fungal DNA on or in their bodies these insects are, therefore, likely vectors of *F.*  
538 *xyrophilum*. Despite the low number of insects tested in our study, our results are comparable to those  
539 from the *Mvc*-blueberry system where *Mvc* DNA was detected in ~ 33% of captured bees and flies, the  
540 potential vectors of *Mvc* spores (McArt et al. 2016).

### 541 **Emission of a sesquiterpene by *F. xyrophilum* pseudoflowers and cultures**

542 Pseudoflower-inducing fungi lure insects to infected plants through color but also using olfactory  
543 signals (Roy 1994; Naef et al. 2002; McArt et al. 2016). The volatiles produced by *F. xyrophilum*  
544 pseudoflowers were compared to those emitted by *X. surinamensis* flowers, *in situ* in Guyana to  
545 determine whether the pseudoflowers are mimicking the olfactory cues of the host flowers. *Xyris*  
546 *surinamensis* flowers emitted mainly two compounds, (Z)-3-hexen-1-ol and 1-hexanol, known green leaf  
547 volatiles likely formed after the flowers were excised (Ameye et al. 2018). This suggests that *X.*

548 *surinamensis* flowers produce few, if any, constitutive volatiles. Conducting a more controlled collection  
549 under laboratory conditions in the future would be beneficial to further explore these results. Interestingly,  
550 pseudoflowers and *F. xyrophilum* cultures emitted a sesquiterpene compound tentatively identified as  $\alpha$ -  
551 gurjunene. Our analysis of *F. xyrophilum* whole genome sequences detected three terpene synthase genes  
552 that are candidate  $\alpha$ -gurjunene synthase genes. That is, the genome sequences included 12 terpene  
553 synthase genes, and the functions of nine of these genes is known based on closely functional analyses of  
554 closely related orthologs in other *Fusarium* species, whereas the functions of closely related orthologs of  
555 the three candidate  $\alpha$ -gurjunene synthase genes have not been determined.  $\alpha$ -gurjunene was not detected  
556 from *X. surinamensis* flowers or cultures of *F. verticillioides* and *F. thapsinum*, which are close relatives  
557 of *F. xyrophilum*. Despite not assessing the volatile profile of all members of the FFSC, these data  
558 suggest that  $\alpha$ -gurjunene might be a *F. xyrophilum*-specific volatile with potential for involvement in  
559 insect attraction by pseudoflowers.

560

#### 561 **UV fluorescence of *Xyris surinamensis* flowers**

562 Insect pollinators are sensitive to the UV range of the electromagnetic light spectrum in addition  
563 to the visible spectrum (Briscoe and Chittka 2001). UV photoreceptors aid floral visitors in locating  
564 individual flowers that provide specific UV patterns, differing from other plants within the same  
565 community (Johnson and Andersson 2002). Our examination of *X. surinamensis* flowers and *F.*  
566 *xyrophilum* pseudoflowers revealed flower pedicels were UV fluorescent, but not the pseudoflowers. Blue  
567 UV- induced fluorescence emissions at 366 nm have been described on the floral parts, fruits, and seeds  
568 of several grasses (e.g., *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Sorghum bicolor*, *Ochlandra*  
569 *travancorica*, *Eleusine coracana*) and are suggested to be visual cues that attract pollinators to nectar  
570 (Thorpe et al. 1975), petals (Gandía-Herrero et al. 2005), and pollen (Mori et al. 2018). On the other hand,  
571 it has been proposed that the fluorescence quantum efficiency of floral pigments is low (~1%) and a  
572 fluorescence effect under natural conditions may be swamped by petal reflections (Iriel and Lagorio  
573 2010). Therefore, fluorescence is currently regarded as ‘unimportant’ for visual signaling for pollinators  
574 (Van Der Kooi et al. 2019). Nevertheless, it is important to highlight that this has been evaluated from the  
575 perspective of UV fluorescence on the petal surface, which is superimposed on to the light reflected by  
576 the organism (Iriel and Lagorio 2010). However, in our observations *X. surinamensis* fluorescence was  
577 located on the pedicel, at the base of the flower, and not on the petals. The bright fluorescence of pedicels  
578 on *X. surinamensis* flowers may or may not be involved in insect attraction, and it should be evaluated  
579 with respect to the photoreceptor responses in the insect visitors’ eyes. Additionally, much of the  
580 knowledge of floral reflectance is based on studies of bees. Our studies of insect visitation to *Xyris* sp. and  
581 *X. surinamensis* in Guyana indicate the frequency of visits by bees is limited (Torres-Cruz et al. 2024).

582 Nonetheless, it may be that these different cues act together to attract insect visitors to *Xyris* flowers and  
583 *F. xyrophilum* pseudoflowers. Whether or not, and to what extent, the UV reflectance and color of both  
584 flowers and pseudoflowers are visual signals involved in insect attraction remain to be determined.

585

586

## CONCLUSIONS

587

588 Here, we have expanded the list of *Xyris* insect visitors to include Vespidae, Formicidae,  
589 Salticidae, Acrididae, and Tetrigidae. Our results exemplify the need to study tropical regions, with an  
590 emphasis on remote and underexplored locations, to expand our knowledge of interactions in different  
591 ecosystems, often subject to sampling bias. Although we were unable to quantify differences in insect  
592 visitation to flowers and pseudoflowers, we have confirmed visitation to pseudoflowers by a diverse array  
593 of arthropods. Potential vectoring of *Xyris* pollen and *F. xyrophilum* conidia between plants is supported  
594 by 1) an overlap on the identity of insect visitors of *Xyris* flowers and *F. xyrophilum* pseudoflowers  
595 (Conocephalini, *Camponotus*, Geometridae), 2) observations of pollen on insects' antennae and other  
596 parts of their bodies (Torres-Cruz et al.2024), and 3) evidence of *F. xyrophilum* DNA on insect bodies  
597 confirmed by IGS rDNA targeting PCR. It is also important to highlight that the interaction of *Xyris* and  
598 *F. xyrophilum* with other organisms could affect insect visitation. It has been suggested that microbial  
599 effects on pollen could influence pollen-eating animals, as they could plausibly affect pollen scent,  
600 nutrition, or physiology. Nevertheless, this topic has received little experimental attention (Vannette  
601 2020). Additionally, we detected the emission of the sesquiterpene  $\alpha$ -gurjunene on pseudoflowers and *F.*  
602 *xyrophilum* pure cultures but not by *Xyris* flowers, which could function as an insect attractant.  
603 Pseudoflowers have been shown to possess two pigments with fluorescence emission maxima in light  
604 ranges to which trichromatic insects are sensitive (Laraba et al. 2020b), and we have determined that the  
605 peduncle of *X. surinamensis* flowers fluoresces under UV light. These two visual cues, from flowers and  
606 pseudoflowers, paired with the different volatile emissions of pseudoflowers may work together to attract  
607 the high and diverse pool of insects we have observed visiting *Xyris*. Our study has also provided  
608 fundamental knowledge of insect interactions with *Xyris* flowers and *F. xyrophilum* pseudoflowers,  
609 including the first evidence for insect visitations, volatile production and fluorescence, and confirmation  
610 that insects disperse *F. xyrophilum*. Despite these advances in knowledge, much remains to be determined  
611 in the interaction of *Xyris* and *F. xyrophilum*. Our hypotheses for insect vectoring of *F. xyrophilum* and  
612 the involvement of insects in this potential mimicry system will need to be evaluated under extensive field  
613 experimentation.

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725

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727 The authors have no relevant financial or non-financial interests to disclose.

728

729 **Author Contributions**

730 Terry Torres-Cruz developed the initial concept of the study, secured funding, led the fieldwork  
731 and laboratory analyses, analyzed the data, and wrote the first draft of the manuscript. Tristan M. Cofer  
732 advised on VOC experimental design, provided training in VOC sample collection, carried the VOC  
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744

745 **Conflicts of Interest**

746 The authors have no relevant financial or non-financial interests to disclose.

747

748 **Data availability**

749 All data supporting the findings of this study are available within the paper and its Supplementary  
750 Information files. Sequences were submitted to GenBank under accession numbers OQ121925–27 and  
751 OQ152379–90. Photography of plant, insect, and fungal observations at each collection site are available  
752 in iNaturalist under the project: “[Ecosystem profiles of Xyris Research Sites](#)”. Video clips of insect  
753 visitation observations are available on YouTube as playlist “[Fusarium xyrophilum – Xyris surinamensis](#)  
754 [insect visitation study](#)” (playlist ID: PL19pSjmfC9cSceih6nlMnaBQj57ITfvq8).

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756 **Disclaimers**

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