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Mass Spectrometry 2017: GC-MS analysis of semiochemicals produced by cowpea plant in response to herbivory attack

Jonathan Osei-Owusu

Kwame Nkrumah University of Science and Technology, Ghana

Plants respond to herbivore feeding through increased biosynthesis and emission of volatile compounds that act as attractants for natural enemies such as parasitoids or predators which serve as a means of indirect defense against herbivores. For emitted volatiles to effectively act as a signal for natural enemies, the emitted volatiles should be specific for prey and must be distinguishable from intact plants odor. Plants emit different blend of volatiles in respond to different herbivore attack. Chemical composition of the emitted volatiles are variable but are usually dominated by isoprenoids, lipoxygenase-derived volatiles and aromatics. Mass spectrometry to be continuously preferred technique for structure elucidation, due to the higher degree of sensitivity that can be achieved by the use of this technique. Cowpea is an important food legume in Africa because of its cheap source of protein. The plant however, is attacked from seedling to podding stage by insect pest causing yield losses up to 80%. Exploring the use of natural enemy could help protect the plant and increase yield. To investigate the volatiles been produced by cowpea plant in responds to different herbivory attack, the plants were challenged with Aphis craccivora, Myzus persicae and Latio vivida. The response signals were analyzed using a Gas Chromatography and coupled Gas Chromatography-Mass Spectrometer (GCMS) and chemical structure confirmed by co-eluting authentic compound with plant volatiles. The plant responded differently to the three modes of feeding. Compounds produced were dominated by terpenoids, green leaf volatiles (GLV) and an aromatic compound indole.

Methods and Materials

The seeds of the Ghanaian cowpea cultivar Padi-tuya were obtained from Savanna Agriculture Research, Tamale, Ghana. One seed per pot (9 × 9 × 10 cm) was grown in a greenhouse at Rothamsted Research, United Kingdom (27 ° C: 25 ° C and 16: 8 h L: D photoperiod, LED lighting) in the soil (pH 5.5–6.0; 75% medium quality peat, 12% sterilized silt, 3% medium quality vermiculite; 10% grain without lime 5 mm; grain size <2 mm after sieving). Seeds of S. cannabina have been supplied by the International Institute of Tropical Agriculture (IITA) in Benin for experimental use. One seed per pot (20 × 30 cm) was cultivated in a silty soil sterilized with heat at 36 ° C: 23 ° C and 12:12 h Photoperiod L: D in a greenhouse at the University of Sciences and Technologies of Kwame Nkrumah (KNUST), Kumasi, Ghana.

The colonies of Maruca vitrata were raised to KNUST at 26 ° C, with a photoperiod of 12:12 L: D and 76% relative humidity. Adult females mated for five days were transferred to transparent cylindrical plastic cups (3 cm in diameter × 3.5 cm in height) for laying for 48 h, and stored in a 10% honey solution on a piece of filter paper. Cups with eggs were then incubated in a large plastic container with sprouted cowpea grains as a feeding substrate for the newly hatched larvae, which were replaced with new grains every three days until pupation. The nymphs were removed from the diet after 15 days and placed inside a net cage under similar conditions of emergence. Fifth instar M. vitrata larvae were transported to Rothamsted Research, UK, where they were placed in open petri dishes with artificial diet, prepared by Jackai and Raulston (1988) and supplied by IITA , Nigeria [400 g cowpea flour, 127.2 g Wheat germ, 44.4 g Wesson salt mix, 25 g ascorbic acid, 3.9 g aureomycin, 60 g

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sugar, 3 , 6 g of methyl p-hydroxybenzoate, 6.8 g of sorbic acid, 2 L of water, 22 ml of potassium hydroxide (4 M aqueous solution), 29.6 ml of choline chloride (15 %), 50 ml of acetic acid (25%), 26 ml of formaldehyde (10%), 30 ml of vitamin suspension and 59.2 g of agar] and stored in a net cage at 25 ° C: 22 ° C and 12: 12 h L: photoperiod D. The emerging adults were fed with a 10% sugar solution. After five days, the mated females were isolated in groups of two in transparent plastic cups for two days for laying. Cups with eggs were placed under artificial larval diet.

A colony of endoparasitoid larvae Apanteles taragamae was created by obtaining cocoons from a basic crop maintained at IITA, Benin. Subsequent colonies were produced by exposing the caterpillars of first-stage M. vitrata (two days old) to parasitization by A. taragamae female of 3 days for 24 h. Parasitized caterpillars were reared on germinating cowpea grains until pupation. A cocoons. Taragamae were taken from the germination diet after seven days of parasitism and placed in a mesh cage for emergence. Emerging adults were fed a solution of striated honey inside the cage. Cocoons of the parasitoid egg Phanerotoma syleptae were also obtained from IITA, Benin. Emerging adults were fed a solution of striated honey inside the walls of the breeding cage. To allow mated wasps to parasitize hosts, eggs of M. vitrata in small cylindrical cups (3 cm in diameter × 3.5 cm in height) were offered to 2-day-old mated females of P. syleptae.

Collection of Cowpea Volatiles from Flowers

Two cowpea flowers were enclosed in a glass container (100 mm inside diameter × 60 mm high) attached to semicircular aluminum plates around the stem of the plant, and air entrainment lasted 24 hours. For the infestation experiment, two M. vitrata larvae were carefully placed on two cowpea flowers at night. The larvae then had 30 minutes to settle before entraining the flowers for 24 h.

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