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Repellency of dimethyl disulfide to *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae) under laboratory and field conditions

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ABSTRACT

The mirid bug, *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae), is a major pest of cotton, fruit trees, and many other crops in China. While previous trials have found relatively low infestation levels of *A. lucorum* in fields treated with the acaricide dimethyl disulfide (DMDS), its mode of action has not been determined. In this study, we assessed the insecticidal and repellent action of DMDS against *A. lucorum* under laboratory and field conditions. DMDS did not cause mortality of *A. lucorum* adults or nymphs at concentrations of 10.6 and 170.9 mg a.i./1. In Y-tube olfactometer tests, both male and female *A. lucorum* adults preferred clean air over DMDS odors. In choice and no-choice cage trials, feeding damage and the number of *A. lucorum* eggs were lower on mungbean plants treated with DMDS than on control plants. Under field conditions, adult *A. lucorum* density was lower in DMDS-treated mungbean and cotton fields than in untreated fields, and this effect lasted 6 d, but nymph populations were not affected. Under field conditions, adult *A. lucorum* were repelled at a distance of up to 6 m from DMDS-sprayed cotton plants for 6 d after application. This study demonstrates the non-lethal repellent action of DMDS against adult *A. lucorum* and suggests its potential inclusion in integrated pest management (IPM) schemes.

1. Introduction

The mirid bug, *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae), is one of the most damaging agricultural pests in China (Lu et al., 2008a, 2010a). During the last 10 years, *A. lucorum* populations have steadily increased in cotton, *Gossypium hirsutum* L. in China (Lu et al., 2010a). Additionally, the species has become a key pest of local Chinese dates, grapes and other crops (Lu and Wu, 2011; Li et al., 2012). Adults and nymphs feed on terminal buds, tender leaves, squares and flowers, bolls (i.e., fruits) and other tissues of various host plants. Feeding often causes tattered leaves, abscission of squares and small bolls, and deformation of flowers and bolls (Lu and Wu, 2008).

At present, pesticide applications are the only effective means for controlling *A. lucorum*. An average of 5 insecticide sprays is needed for effective population control per cropping season; however, accounts show that farmers occasionally use up to 10–15 applications per season (Lu and Wu, 2011). In other crops, such as stone fruits, insecticide use against *A. lucorum* is equally high (Lu and Wu, 2011). Insecticide over-use can negatively affect natural enemies and biodiversity in the broader agro-ecosystem and potentially lead to the evolution of insecticide resistance in *A. lucorum*. Hence, safe and environmentally sound alternatives for *A. lucorum* control are urgently needed.

Dimethyl disulfide (DMDS) is an organic compound with a high sulfur content that is widely used in the management of agricultural pests. It has low acute toxicity and short residual activity and is registered in China as an acaricide against pest mites on cotton, grain crops, fruit trees and vegetables (Chinese Pesticide Information Network, http://www.chinapesticide.gov.cn/). In 2010, while conducting cotton mite control trials, we noticed a reduced *A. lucorum* infestation level and damage in DMDS-treated field plots (Lu YH, unpublished data), leading us to suspect that DMDS has insecticidal and/or repellent effects on *A. lucorum*.

In this study, we investigated the mode of action of DMDS against *A. lucorum* populations. We first determined the susceptibility of different *A. lucorum* developmental stages to DMDS in the laboratory and then conducted repellency trials under laboratory and field conditions.

2. Materials and methods

2.1. Study insects and plants

Laboratory colonies of *A. lucorum* were started with approximately 2000 adults and nymphs that were collected from cotton

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fields in July and August of each study year at the Langfang Experimental Station, Chinese Academy of Agricultural Sciences (CAAS), Hebei Province, China (39.53°N, 116.70°E). Colonies were kept in 20 × 10 × 6 cm transparent plastic rearing containers at 29 ± 1 °C, 60 ± 5% RH, and a 14:10 (L:D) photoperiod in climate-controlled rearing chambers. In each container, we provided green bean (*Phaseolus vulgaris* L.) pods as a food source and oviposition material, and a cotton ball soaked with a 10% sucrose solution was provided for adult feeding (Lu et al., 2008b). Second- and third-generation individuals were used for experiments. Field plots were established using mungbean (*Vigna radiata* (L.) Wilczek; var. JILVDOU2) and cotton seeds (var. SGK321) provided by the Baoding Institute of Agricultural Sciences (Hebei Province) and the Biotechnology Research Institute of CAAS (Beijing), respectively. DMDS (99% purity) was purchased from International Laboratory, San Bruno, California, USA.

2.2. Toxicity bioassays

To test the insecticidal activity of DMDS on *A. lucorum*, we followed the procedures described by Liu et al. (2008). In this experiment, four different concentrations of DMDS, 170.9, 42.5, 10.6, and 5.3 mg a.i./l, were prepared in 99% pure acetone, and 99% pure acetone also served as a control. For laboratory testing, 0.5 ml aliquots of each concentration were added to 20-ml glass scintillation vials. The vials were then agitated to fully cover their inner walls, followed by evaporation of the acetone. Next, two *A. lucorum* individuals, either 2nd instar nymphs, 4th instar nymphs, or 5-day-old adults, were placed into each vial with a 2-cm-long section of green bean for food. The vials were sealed with a cotton ball and incubated upright for 72 h at 25 ± 1 °C, 60 ± 5% RH, and a 14:10 (L:D) photoperiod. At the end of this period, survival of the test insects was determined, with mortality defined as the failure to move when prodded. In each experiment, a total of 5 vials were set up for a particular mirid developmental stage or insecticide concentration. The experiment was repeated 3 times, resulting in a total of 15 vials (i.e., 30 individuals) for each mirid development stage and insecticide concentration.

2.3. Y-tube olfactometer trials

The behavioral responses of *A. lucorum* to DMDS odors were analyzed using a Y-tube olfactometer (Blackmer et al., 2004; Yu et al., 2010) consisting of 2-cm-diameter glass tubing with a 15 cm central tube and two 15 cm lateral arms at 75° angle. The olfactometer was placed within a 100 × 100 × 60 cm white observation chamber with two overhead 40 W fluorescent lamps (2000 lux). The chamber was maintained at 25 ± 1 °C. Air was pumped through activated charcoal and an Erlenmeyer flask filled with distilled water before entering each lateral arm of the apparatus at 300 ml/min. The flasks were connected with Teflon tubing between the air supply and each arm of the olfactometer and provided DMDS or control solution.

We applied 10 μl of the DMDS solution (10.6 mg a.i./l; mineral oil as solvent) onto a 5 × 0.5 cm filter paper and immediately placed it into one of the flasks, with the same volume of mineral oil placed in the opposite flask as an unscented control. Next, a 5-day-old *A. lucorum* adult was introduced at the base of the central arm of the olfactometer using a 10-ml glass vial. The insect was then observed for 5 min, with a 'choice' being registered when it stayed for >5 s at 1 cm beyond the Y-junction in one of the lateral olfactometer arms. If no choice was made after 5 min, it was removed and recorded as 'non-responsive'. Each *A. lucorum* individual was used only once.

The position of the arms containing treatment and control odors was reversed after testing every 5 *A. lucorum* adults to avoid position bias. The Y-tube olfactometer was also replaced with a clean one after testing 10 individuals. After each set of assays, the apparatus was washed and rinsed with acetone. All olfactometer assays were conducted between 0800 and 1800 h, and a total of 60 individuals of each gender were tested.

2.4. Cage trials

Paired choice and no-choice cage experiments were conducted to determine the effect of DMDS on feeding and oviposition preference of *A. lucorum* females. Because preliminary trials had shown poor performance of *A. lucorum* females on cotton seedlings (Dong et al., 2013), mungbean was chosen as a more suitable host (Geng et al., 2012). Four-week-old mungbean plants were established, with 2 seeds per pot, in 20-cm diameter plastic pots that were kept outdoors in screen houses under natural lighting and ambient temperature. Prior to use in experiments, all plants were checked to ensure they were free of insects. In the choice experiments, 2 pots of mungbean plants [two 20–25 cm high plants (with 8–10 leaves) per pot] were placed in opposite corners of a 1 m^3 screen cage. A cotton wick soaked in 5% honey water was placed at the center of the cage for adult feeding. Six 8-day-old, mated females were released into each cage. Plants in opposite corners were either treated with DMDS-water solution ('treated plants') or with water ('control plants'). In the no-choice experiments, only 1 pot of mungbean plants was placed in the center of each cage, and 3 mated females were released into each cage. This plant was either treated with a DMDS solution or with water. In all experiments, 'treated plants' were sprayed half an hour before insect release with 6–8 ml of a 10.6 mg a.i./l DMDS solution, while 'control plants' were treated with an equal amount of water. Both experiments were conducted at 25 ± 1 °C. Seventy-two hours following insect release, *A. lucorum* females were removed from the plants. Plants were subsequently held for 96 h before the number of *A. lucorum* feeding punctures was determined (see Lu and Wu, 2008). Afterward, whole plants were collected and dyed for 2 min with a 1% eosin solution (Beijing Chemical Work), which dyes *A. lucorum* egg caps red (Dong et al., 2013), and the number of eggs laid per plant was determined by counting under a binocular microscopy. Paired choice and non-choice experiments were repeated 8 and 12 times, respectively.

2.5. Open field trial #1: duration of repellent activity

Field trials were conducted at the CAAS Langfang Experimental Station in a mungbean field in 2011 and in both mungbean and cotton fields in 2012. In each test field, we established eight 10 × 10 m plots separated by 5 m plant-free strips. The plots were seeded uniformly (cotton in early May and mungbean in mid-June) and received identical fertilizer and pesticide-free irrigation treatments. Four plots were randomly selected for DMDS treatment, with the other 4 plots serving as controls. 'Treated plots' were sprayed with a 10.6 mg a.i./l DMDS solution at 750 l/ha (i.e., recommended dosage for field use), while the 'control plots' received the same amount of water. Each plot was surveyed for *A. lucorum* adults and nymphs immediately before DMDS treatment and 4 times following treatment, i.e., every 2 days until day 8. For each survey, four 1 m^2 subplots were randomly selected per mungbean plot, and the numbers of *A. lucorum* adults and nymphs were recorded using a standard white pan beating method (Lu et al., 2009). In cotton fields, sampling was performed by selecting 4 points per plot and randomly choosing 5 plants at each point. On those plants, the presence of *A. lucorum* adults and nymphs was determined by visual inspection (Lu et al., 2010a).