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Authors: Stuhl, Charles J., and Romero, Maritza

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Attraction of *Sitophilus zeamais* (Coleoptera: Curculionidae) to four host plants

Charles J. Stuhl[1,], and Maritza Romero[1]*

Abstract

The maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) is an important pest of stored grains, predominantly maize (*Zea mays* L.), wheat (*Triticum* L. spp.), rice (*Oryza* L. spp.), and sorghum (*Sorghum bicolor* [L.] Moench) (all Poaceae). Weevil infestations occur in the field and weevils are carried to the storage facility after harvest. Infestation also occurs in the grain storage facility. There is no effective field or storage trapping system for control. They are commonly parasitized by *Pteromalidae* Dalman (Hymenoptera: Chalcidoidea) spp. parasitoids in the Tropics; however, there is no biocontrol use of these Hymenoptera. Therefore, pesticide application and the development of resistant grain are the current control strategies; although, studies have indicated that odors associated with the grain upon which the weevils feed, mate, and oviposit are attractive to the weevil. Host plant produced volatiles and their associations with the maize weevil have not been investigated fully. To better understand the weevil's attraction to crop plants, a study was initiated to investigate the weevil's preference for immature host plants. Weevils that had been reared on 4 types of grain for several generations were presented immature plants of all 4 types of grains in choice tests to determine if they prefer grains of the host plant on which they had been reared. This research demonstrated that immature rice plants have the greatest attraction, and that previous feeding did not influence the weevil's host plant attraction. The isolation of key rice plant semiochemicals will be the focus in the development of an attractant for *S. zeamais* and may also prove effective for the control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).

Key Words: stored grain pests; pest management; insect behavior

Resumen

El gorgojo del maíz *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) es una plaga importante de los granos almacenados, predominantemente del maíz (*Zea mays* L.), trigo (*Triticum* L. spp.), arroz (*Oryza* L. spp.) y sorgo (*Sorghum bicolor* [L.] Moench) (todas Poaceae). Las infestaciones de gorgojos ocurren en el campo y luego se llevan los gorgojos a la instalación de almacenamiento después de la cosecha. Las infestaciones también ocurren en la instalaciones de almacenamiento de granos. No existe un sistema efectivo de captura de campo o almacenamiento para el control. Suelen ser parasitados por *Pteromalidae* Dalman (Hymenoptera: Chalcidoidea) spp. parasitoides en los trópicos; sin embargo, estos himenópteros no los usan como control biológico. Por tanto, la aplicación de plaguicidas y el desarrollo de grano resistente son las estrategias de control actuales; aunque los estudios han indicado que los olores asociados con el grano del que se alimentan, aparean y oviposita los gorgojos, son atractivos para el gorgojo. La planta hospedera produjo volátiles y sus asociaciones con el gorgojo del maíz no se han investigado completamente. Para comprender mejor la atracción del gorgojo por las plantas de cultivo, se inició un estudio para investigar la preferencia del gorgojo por las plantas inmaduras hospederas. A los gorgojos que se habían criado con 4 tipos de grano durante varias generaciones se les presentaron plantas inmaduras de los 4 tipos de granos en pruebas de elección para determinar si prefieren los granos de la planta hospedera en la que se habían criado. Esta investigación demostró que las plantas de arroz inmaduras tienen la mayor atracción y que la alimentación previa no influyó en la atracción de la planta hospedera del gorgojo. El aislamiento de semioquímicos clave de la planta de arroz será el foco en el desarrollo de un atrayente para *S. zeamais* y también este podria resultar eficaz para el control de *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae).

Palabras Clave: plagas de granos almacenados; manejo de plagas; comportamiento de los insectos

Specific blends of semiochemicals are used by insects for the detection of plants and other host organisms; control and monitoring of pest populations may be accomplished by isolating and using these specific odors (Norin 2007). Host plant volatiles and pheromones produced by male and female insects are used for mating, aggregation, defense, and host recognition, and have been isolated effectively for many insects (El-Sayed et al. 2006). Aggregation pheromones and host plant related volatiles have been reported in many species of insects and typically attract both sexes (Landolt 1997). The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is a serious pest of stored grains worldwide. Adults and larvae can survive on a wide variety of food substances but are known as primary insect pests of sorghum (*Sorghum bicolor* [L.] Moench), rice (*Oryza* L. spp.), wheat (*Triticum* L. spp.), and maize (*Zea mays* L.) (all Poaceae). *Sitophilus* spp. have a long history as a crop pest; Chinese, Greek, and Roman societies all have records of these weevils infesting wheat between ca. 2,500 to 2,200 BP (Obata et al 2011). The maize weevil can infest grains prior to harvest (Ni et al. 2011; Vyavhare & Pendleton 2011), and control is done by the application of insecticides when the grain is loaded into silos, or as a surface treatment after storage (Arthur & Throne 2003). Damage to the grain occurs when the female weevil pierces the grain and lays a single egg inside the kernel (Stuhl 2019). The hole is then sealed with a waxy secretion that hardens, protecting the egg. The larva hatches and consumes the pulp inside of the grain. Because the weevil imma-

[1]USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida 32608, USA; E-mail: charles.stuhl@usda.gov (C. J. S.), maritza.romero@usda.gov (M. R.)
*Corresponding author; E-mail: charles.stuhl@usda.gov

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ture stages develop within the grain, they are protected from pesticide exposure, and hence the chemical control of this pest is limited to the adult weevil (Walgenbach et al. 1983). Alternatives to chemical pesticides are necessary due to consumer preference, pesticide regulation, and insect resistance (Vyavhare et al. 2018).

The success of the maize weevil depends on its ability to locate and identify a suitable host plant, access plant fitness, and efficiently use the plant's resources. Most trees, grasses, and shrubs emit volatile compounds at varying times and levels (Conchou et al. 2019). These emissions may attract pollinators and seed dispersers, as well as regulate plant-to-plant and plant-to-animal interactions, and may function as abiotic stressors (Unsicker et al. 2009; Loreto & Schnitzler 2010). However, little is known about host plant produced volatiles that attract the maize and rice weevils, which is information that is necessary to be able to develop a semiochemical based attractant for control and management of these pests. In addition, host volatile attraction may be enhanced greatly by the synergistic effect of a sex or aggregation pheromone (Bartelt & Dowd 1991). Although male specific, both sexes may be captured in an aggregation pheromone baited trapping device (Burkholder 1990; Sato & Touhara 2008). This dynamic has been shown in many trapping systems for Coleoptera, such as the boll weevil (*Anthonomus grandis* Boheman; Coleoptera: Curculionidae).

Our research explores the behavioral responses of mature weevils in laboratory conditions when it encounters several possible host plants. A behavioral response to an olfactory message may be detected by the weevil's olfactory system where high sensitivity and selectivity allow a specialist phytophagous insect to feed selectively only on a limited number of plant species, as well as a lower selectivity for generalist phytophagous insects feeding on a diverse range of plants in many families (Bruce et al. 2005; Martin et al. 2011). It has been shown that previous host grain feeding does not make the weevil biased to a certain grain (Stuhl 2019). It is not known if weevils will be attracted to a host plant odor that it has never encountered after prior feeding on a certain host grain. It is also unknown if this naïvety is passed to future generations. A semiochemical-based monitoring and management system that employs specific isolated host plant odors may be the key to weevil management (Rodriguez-Saona & Stelinski 2009). The goal of this research was to identify a specific plant that may be used in the future for the isolation of attractive semiochemicals. However, multiple attractants would be needed to attract weevils feeding on a wide range of hosts if prior feeding changes their preference to an attractant. The heightening of an insect pheromone response by plant volatiles has been identified from numerous hosts of bark beetles and the boll weevil as synergists to the insect pheromone (Bedard et al. 1969). Therefore, the isolation of key semiochemicals when paired with the aggregation pheromone will have a synergistic effect that greatly enhances attraction (Philips et al 1985; Bartelt & Dowd 1991).

Materials and Methods

SOURCE OF WEEVILS

Sitophilus zeamais weevils used in this study were reared at the USDA-Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS, CMAVE) in Gainesville, Florida, USA. The source of the weevils was from dried intact ears of maize purchased from a commercial animal feed retailer in Gainesville, Florida. Positive taxonomy using a morphological key was used for identification by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville, Florida, USA. Before the onset of the assays, weevils were reared on 4 different cereal grains for 10 generations. To test for conditioning on host plant selection behavior, weevils were reared on: sorghum (Milo, Flint River Mills, Bainbridge, Georgia, USA); brown rice (Nishiki Premium Brown Rice, JFC International, Inc., Los Angeles, California, USA); barley (Rolled Barley, La Crosse Milling Co., Cochrane, Wisconsin, USA); and maize (Yellow Maize, Flint River Mills, Bainbridge, Georgia, USA). The rearing of the weevils was done in 950 mL glass canning jars (Jarden Home Brands, Daleville, Indiana, USA) containing 1 of the 4 grains. To allow for ventilation, a metal screw band held in place copper mesh that covered the mouth of the jar. Sex determination of *Sitophilus zeamais* was done by using di-morphic rostrum characteristics as stated in Halstead (1962). The rearing jars were placed in a temperature-controlled chamber at 23 ± 5 °C, 60% RH, and photoperiod of 12:12 h (L:D).

CHOICE ASSAY

To investigate the response of *S. zeamais* weevils to immature host plants, 200 unsexed weevils of the same age were transferred from the rearing containers and released in an escape-proof clear container with lid measuring 60 × 45 × 28 cm (Ziplock Weathertight Box, Pleasant Prairie, Wisconsin, USA). Four treatments containing immature plants grown from seed in a greenhouse were maize (Earlivee Hybrid, R.H. Shumway's, Randolph, Wisconsin, USA); oats (Streaker Hulless, Johnny Seeds, Winslow, Maine, USA); rice (Carolina Gold, Baker Creek Heirloom Seeds, Mansfield, Missouri, USA); and sorghum (Flint River Mills, Bainbridge, Georgia, USA), and were offered in 7.6 cm green plastic pots (T.O. Plastics, Clearwater, Minnesota, USA) that held the 4 treatments within the assay container. Plants were grown in a seedling starter mix soil (BWI, Apopka, Florida, USA). The oat plants were substituted for the barley plants due to difficulties growing the barley plants in our greenhouse. Due to the difference in growing times, all plants were used when they reached 20 cm. Each pot contained four 20 cm tall plants of either maize, oats, rice, or sorghum, respectively. At the end of a 24 h period, the lid was removed and the inside of the container was checked for insects that did not respond. The weevil's presence on the plant, on and below the soil, and on the roots were counted for each treatment. Insects collected from plants, soil, and roots from each pot were placed in a vial and labeled for later sexing. To account for positional bias within the chamber, assay containers were rotated to 4 different shelf locations within the environmental chamber; a single repetition consisted of a clockwise rotation of each treatment to all 4 sides within the container to account for positional bias. To provide for optimum conditions, the assay containers were placed in a temperature-controlled chamber at 23 ± 5 °C, 60% RH, and photoperiod of 12:12 h (L:D). There were 10 replications from each weevil rearing substrate performed with a total of 40 observations (n = 8,000 weevils). Data were checked for normal distribution using Shapiro-Wilk test, followed by Levine's Test for Homogeneity of Variances. Independent analyses were performed for each treatment, sex, and positional rotational combination. A Dunn's test of multiple comparisons was followed by a Kruskal-Wallis test control, the experiment wise error rate, and a Pairwise Mann-Whitney tests in R 3.5.0 (R Core Team 2020).

Results

CHOICE ASSAY

The number of insects were collected and counted from the plants, soil surrounding the plants, and those located on the roots of the plants.

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Plant leaf

When provided with a choice of host plants, barley-reared males and females indicated strong preference toward rice (F = 6.02; df = 3; P < 0.0001, and F = 9.95; df = 3; P < 0.0001, respectively) (Fig. 1a), followed by an equal distribution on oats and milo; the lowest number of weevils were found on maize. Maize-reared males (F = 10.62; df = 3; P < 0.0001) and females (F = 13.63; df = 3; P < 0.0001) were located on rice plants, followed by milo, oats, and maize, respectively. Milo-reared males (F = 9.98; df = 3; P < 0.0001) and females (F = 16.59; df = 3; P < 0.0001) indicated a preference for rice and milo plants. The male response to oats and maize were equal, whereas females preferred oats over maize. However, male (F = 22.11; df = 3; P < 0.0001) and female (F = 0.19; df = 3; P < 0.0001) weevils reared on rice found the rice plant to be the most attractive. Additionally, male and female rice-reared weevils selected milo, oats, and maize, respectively. There was no positional bias for the plants (F = 0.02; df = 3; P = 0.9991) in the assay containers.

Soil

Upon inspection of the soil surrounding the plants, there was equal distribution of barley-reared males (F = 5.15; df = 3; P = 0.0293) and females (F = 2.22; df = 3; P = 0.0749) amongst all of the plants (Fig. 1b). The maize-reared males (F = 2.25; df = 3; P = 0.05877) and females (F = 3.09; df = 3; P = 0.0288) were located equally amongst all the plants. Additionally, the rice-reared males (F = 2.27; df = 3; P = 0.0824) and females (F = 1.36; df = 3; P = 0.2567) along with the milo-reared males (F = 3.88; df = 3; P = 0.0104) and females (F 3.11; df = 3; P = 0.0283) and the rice showed no preference for the plant roots. There was no positional bias for soil (F = 1.11; df = 3; P = 0.3510) in the assay containers.

Roots

The plant roots showed no significance for any of the insects reared on a specific substrate (Fig. 1c). However, results indicated that milo-reared males (F = 45.90; df = 3; P = 0.0001) and females (F = 36.79; df = 3; P = 0.0001) showed a preference for the roots of milo. There was no positional bias for plant roots (F = 0.29; df = 3; P = 0.8825) in the assay containers.

Discussion

Volatile emission by host plants and their association with the maize weevil has not been investigated until now. Infestations are known to occur in the field, after which the weevils are transported the storage facility when maize is harvested. Assaying weevils reared on multiple substrates over multiple generations demonstrated that the rearing substrate of previous feeding over multiple generations has a clear but limited effect on host plant selection. These results indicate that the volatile emissions from the rice plant appear to be most attractive even in the presence of other potential host plants. We speculate that the maize weevil has an evolutionary history with maize, although this weevil is very closely related to the rice weevil (*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). The speciation of the 2 weevils likely

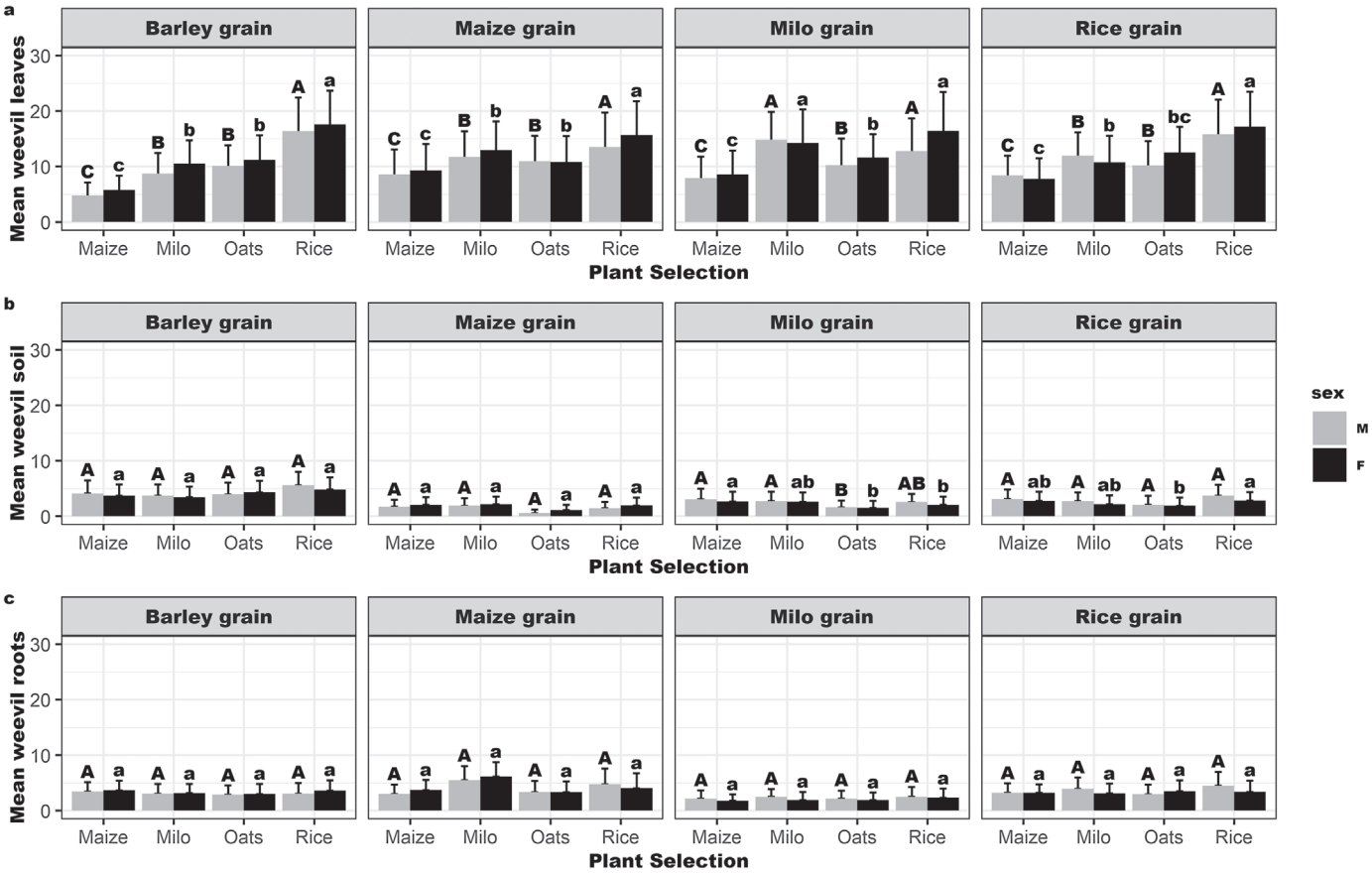


Fig. 1. Weevils reared on corn, barley, brown rice, and milo, and presented a choice of 4 host plants. (a) Mean (SE) number of *S. zeamais* located on maize, oat, rice, and milo plants; (b) mean (SE) number of *S. zeamais* located on the soil surrounding maize, oat, rice, and milo plants; (c) mean (SE) number of *S. zeamais* located on the roots of maize, oat, rice, and milo plants. Means with the same letter are not significantly different.

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occurred about 8.7 million years ago (Corrêa et al. 2017). The maize weevil is a stronger flier than the rice weevil counterpart; therefore, this may indicate that the maize weevil relies more on field infestations and the rice weevil heavily depends upon infesting stored grains (Hurley & Vinson 2018). Maize weevil attraction to the rice plant may be a residual behavior from speciation that began with the rice weevil. Current literature states that the maize weevil overwinters in leaf litter or buried in the soil (Hara 1971). This behavior may be reflected in the weevils being located on the plant roots, although it is not known if the weevils were feeding actively on the roots.

Our results indicated that a weevil that is associated with maize overwhelmingly selected the rice plants. Volatiles emitted by a plant in a monoculture or standing alone are the initial attractants for weevils. Once a potential host plant is located, the search for the grain for food, mating, and reproduction ensues. It is known that maize weevils are generalist feeders and thus will exploit a multitude of grains, although not without an innate preference. However, weevils that are not fortunate enough to encounter a preferred crop could possibly use wild plant seeds for feeding and reproduction.

It has been shown that the sex pheromone when combined with green leaf volatile enhanced pheromone trap captures for the boll weevil and extended the longevity of attractiveness, thus increasing trap captures (Dickens 1989). The synergistic effect of a sex pheromone combined with a host volatile significantly enhances attraction (Bartelt & Dowd 1991).

Future research should focus on the isolation of key rice plant semiochemicals. When paired with the commercially available aggregation pheromone, we anticipate an attractant that will trap male and female weevils better than the sex attractant alone. Since we found rice plant volatiles appeal to all maize weevils, independent of previous grain exposure, this eliminates the need for a multitude of attractants targeting weevils feeding on a specific host.

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