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Use of benzimidazole agar plates to assess fall armyworm (Lepidoptera: Noctuidae) feeding on excised maize and sorghum leaves

Karen Harris-Shultz^{*}, Xinzhi Ni¹, Hongliang Wang¹, Joseph E. Knoll¹ and William F. Anderson¹

By causing damage to the growing whorl and inflorescence, the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a major pest of maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and turfgrass in the southern United States. Grain yield losses due to fall armyworm attack on maize and sorghum can be as high as 34% (Lima et al., 2010) and 80% (Andrews 1988), respectively. Two strains of fall armyworm are known: the corn-strain that feeds on maize and sorghum and the rice-strain that feeds on rice (*Oryza sativa* L.) and turfgrass (Pashley 1985). Fall armyworms can be controlled by the use of insecticides, but that can be costly, because the insecticides have difficulty in penetrating the whorl and require frequent applications. Insecticides also reduce the abundance of natural enemies. Furthermore the repeated application of insecticides leads to the development of insecticide resistance in the fall armyworms. Insecticide resistance in fall armyworm populations has been reported for several different insecticide classes including organophosphates, carbamates, pyrethroids, and benzoylureas (Yu 1991, 1992; Diez-Rodriguez & Omo-to 2001; Yu et al. 2003). Fall armyworms can also be managed by the use of plant genetic modification where genes from the bacterium *Bacillus thuringiensis* (Bt), each of which encode an insecticidal toxin, are inserted into the genomes of crop plants. However, the cultivation of transgenic plants can be controversial and may require the prevention of pollen dispersal, particularly for sorghum, which may be able to cross pollinate with wild relatives such as Johnsongrass (*Sorghum halepense* L. Pers.) (Morrell et al. 2005). In addition, as with conventional chemical controls, Lepidoptera can develop resistance to Bt toxins (Niu et al. 2013). An alternative to the use of synthetic insecticides or transgenic plants to control fall armyworm is the identification and use of plants that have innate genetic resistance or tolerance to fall armyworm feeding.

Screening host plants for genetic resistance to fall armyworm has been accomplished by planting under natural infestation in the field (Crubelati-Mulati et al. 2014) or by placing larvae directly on plants in the field (Diawara et al. 1992) or greenhouse (Ni et al. 2008). Natural infestations can be unpredictable and spatially variable in the field, and infestation of plants with fall armyworms in the field or greenhouse can often lead to the movement of larvae or adult moths to other host plants in close proximity or the loss of worms due to insect cannibalism or natural predators. Several different laboratory-based assays have been used to overcome these problems. Cheng et al. (2013) placed larvae in 30-mL plastic cups with agar and added fresh leaves daily from greenhouse grown plants. Plant material that is grown in the field

can be lyophilized and mixed with media, and then fed to larvae in the laboratory (Williams et al. 2006), or fresh greenhouse grown tissue can be supplied daily to larvae in petri dishes with wet filter paper (Jessup et al. 2011) or in insect diet cups (Braman et al. 2000). Laboratory bioassays are best for insect confinement but as the number of accessions and the number of replicates increase, the use of insect diet cups or petri dishes becomes labor intensive because the leaf material must be replaced daily, water must be added, and containers must be cleaned. Nowierski et al. (1995) used agar plates supplemented with an anti-senescence agent, benzimidazole, to assess the effects of the Russian wheat aphid (*Diuraphis noxia* [Kurdjumov]) on barley (*Hordeum vulgare* L.). The addition of benzimidazole helps to slow down the decay of excised tissue and prevents fungal growth while the agar helps retain leaf moisture. This study sought to determine if benzimidazole agar plates could be utilized to identify differences in maize and sorghum cultivars to fall armyworm feeding using minimal labor.

Fall armyworm resistant maize cultivars 'Mp708' (Williams et al. 1990) and 'FAW1430' (Ni, unpublished data) were used as resistant controls, while 'AB24E' (Williams 1989) was used as the susceptible control for the trials. The 4 sorghum cultivars used in the trials were 'Honey Drip' (PI 641821), 'Collier' (PI 641862), 'AN109' (Gorz et al. 1990) and Entry 22 which has a pedigree (A 'Wheatland' × 'AF28')-6-2-2-2-B, and was developed by Dan Gorbett, University of Florida. Sorghum and maize plants were sown on Jul 24, 2014 for Trial 1 and on Sep 3, 2014 for Trial 2 with 3 seeds per pot for 5 replicates (or pots). The pots contained a mixture of 5 gallons of masonry sand (Double A Concrete, Tifton, Georgia), 5 gallons of PRO-MOSS TBK peat moss (Premier Tech Horticulture, Quakertown, Pennsylvania), 5 gallons of coarse perlite, and 1,300 g of dolomitic lime. All of the pots of plants for the trials were randomized, and maintained in a greenhouse set at 32 °C. Plants were fertilized weekly with 0.5 × Hoaglands (Harris 2007) and grown for 36 d (Trial 1) or 37 d (Trial 2) after planting before the whorl tissues of the plants were excised to start the leaf-feeding bioassay.

Agar plates were prepared following the protocol of Nowierski et al. (1995). When the plants were 36 (Trial 1) or 37 d old (Trial 2), the top 2 leaves (the whorl) of each plant were removed, cut into approximately 7.6 cm long pieces, and 0.4 g (Trial 1) or 0.5 g (Trial 2) of the youngest leaf tissue from each pot (3 plants per pot) were placed onto 3 agar plates. Leaf tissue was arranged so that a neonate larva could travel easily from piece to piece with minimal time spent on the agar. The same procedure was used to set up all 15 agar plates per entry. The excised leaves corresponded to leaf 5-6 of sorghum or leaf 8-9 of

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maize for Trial 1 and leaf 5-6 of sorghum or leaf 4-6 of maize for Trial 2. Neonate larvae for Trial 1 were obtained from the USDA-ARS Insectary, Crop Protection and Management Research Unit, Tifton, Georgia. For Trial 2 the USDA-ARS Insectary had been shut down and neonate fall armyworm eggs were obtained from the Insectary from the USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, Mississippi. For both insectaries, the fall armyworm strain has not been tested but the colonies were both established from insects collected on corn plants. One fall armyworm neonate larva was placed on a leaf piece per agar plate and the plate was sealed with Parafilm (Bemis North America, Neenah, Wisconsin). Plates were randomized within each replicate and maintained in a Percival Environmental Growth Chamber (Percival, Perry, Iowa) at 25 °C with a 12:12 h L:D cycle for 7 days. Each larva was removed from the plates and weighed on an analytical balance at the end of the trial (on the 7th d). Dead larvae that died at the neonate stage (no growth) were recorded as having a weight of zero.

Data were analyzed in SAS version v. 9.2 (SAS Institute, Cary, NC) using the GLIMMIX procedure. Cultivar, trial, and cultivar \times trial were included in the model as fixed effects. Replication, nested within trial, was included as the random effect. The SLICE and SLICEDIFF options were used to compare cultivars within each trial. Significant differences were determined at $P \leq 0.05$, adjusted for multiple comparisons using the TUKEY multiple range Test option.

The larval weight for Trials 1 and 2 were significantly different ($F = 228.93$; $df = 1, 28$; $P < 0.0001$). For Trial 2, larval weight was greater than Trial 1 (3.551 mg vs. 0.703 mg) and a cultivar by trial interaction existed ($F = 5.87$; $df = 6, 146$; $P < 0.0001$). Thus, cultivar differences were assessed for each trial separately. Larval weights were also significantly different among the cultivars tested ($F = 16.48$, $df = 6, 146$, $P < 0.0001$). For Trial 1, larvae fed susceptible maize line 'AB24E' had a significantly greater larval weight than those fed resistant lines 'Mp708', 'FAW1430', or the 4 sorghum lines (Fig. 1A). No significant differences were observed among the 4 sorghum lines (Fig. 1A). For Trial 2, the weights of the larvae maintained on 'AB24E' whorl leaf tissue were significantly greater than those fed on the resistant maize lines 'Mp708' or 'FAW1430' (Fig. 1B). For Trial 2, no significant differences in larval weight were observed for the sorghum lines except 'Collier', which had a similar larval weight as the larvae fed the susceptible maize line 'AB24E' (Fig. 1B). Furthermore, the benzimidazole agar plate protocol prevented leaf desiccation and prevented insect escape (Figs. 2A and 2B).

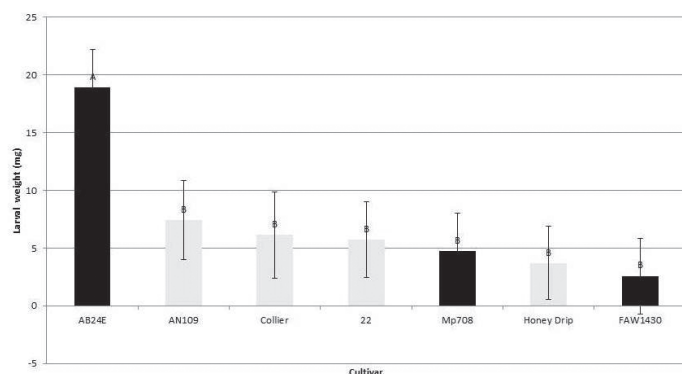


Fig. 1A. Fall armyworm weights for 7 sorghum (off white) and maize (black) cultivars for Trial 1. Among the maize lines 'AB24E' was known to be susceptible and 'Mp708' and 'FAW1430' were known to be resistant to fall armyworm feeding. 'AN109', 'Collier', Entry 22 and 'Honey Drip' are sorghum lines. In Trial 1, fall armyworm neonate larvae were taken from the Tifton, Georgia culture. Means with the same letter are not significantly different. Error bars represent one standard error of the mean.

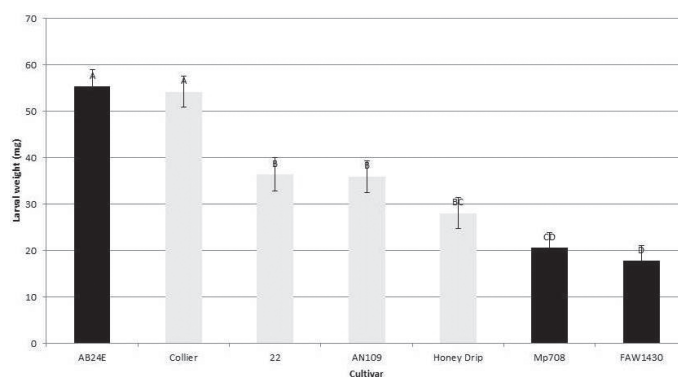


Fig. 1B. Fall armyworm weights for 7 sorghum (off white) and maize (black) cultivars for Trial 2. Among the maize lines 'AB24E' was known to be susceptible and 'Mp708' and 'FAW1430' were known to be resistant to fall armyworm feeding. 'AN109', 'Collier', Entry 22, 'AN109', and 'Honey Drip' are sorghum lines. In Trial 2, fall armyworm neonate larvae were taken from the Mississippi State, Mississippi culture. Means with the same letter are not significantly different. Error bars represent one standard error of the mean.

For both trials, the fall armyworm larvae fed susceptible line 'AB24E' had significantly greater weight than those fed resistant lines 'Mp708' or 'FAW1430'. Thus, the benzimidazole agar plate protocol accurately discriminated between known fall armyworm resistant and susceptible lines. Differences in larval weights between Trials 1 and 2 could be the result of many experimental factors. It is possible that the fall armyworms obtained from the Crop Protection and Management Research Unit for Trial 1 were not as vigorous as those obtained from Mississippi for Trial 2 as many of the larvae fed the resistant maize lines in Trial 1 died, whereas in Trial 2, larvae fed the resistant maize lines were alive on day 7 but smaller than the susceptible maize control 'AB24E'. Differences in fall armyworm feeding between the two populations may be due to genetic differences between the two populations. Nagoshi et al. (2012) showed that fall armyworms in southern Georgia are progeny of migrants from Florida whereas worms in Mississippi are from migrants that overwinter in Texas.

For both trials, no significant differences in larval weight were observed for larvae fed 'AN109' and Entry 22. The larval weights for larvae fed the sorghum lines 'Collier' and 'Honey Drip' were significantly different only in Trial 2. To determine if there is a significant difference

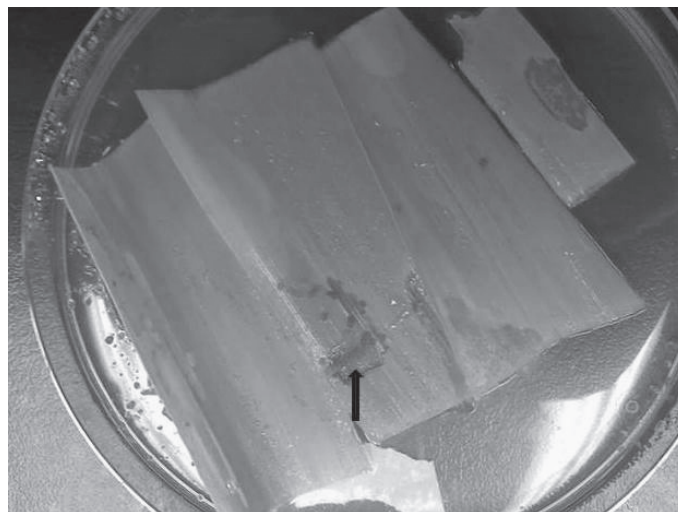


Fig. 2A. Seven day old fall armyworm larva (arrow) fed fall armyworm-resistant maize line 'Mp708' using the benzimidazole agar plate method.

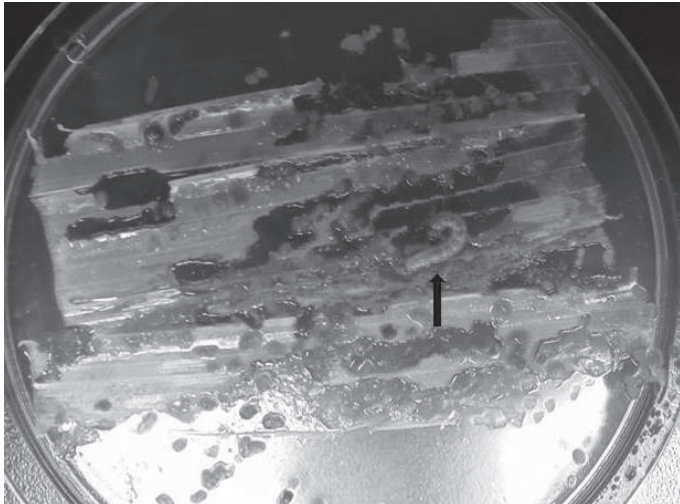


Fig. 2B. A 7 day old fall armyworm larva (arrow) maintained on fall armyworm-susceptible maize line 'AB24E' (susceptible control) using the benzimidazole agar plate method.

in larval weight between these 2 lines, further experiments should be conducted using the larvae from Mississippi.

Using the agar plates with the addition of benzimidazole, the excised maize and sorghum leaf tissues showed little leaf desiccation and low fungal contamination during the 7-d experimental period (Figs. 2A, 2B). Furthermore, this agar plate bioassay also eliminated the onerous daily replacement of fresh leaf tissues. In the previously reported methods, fresh leaf tissues or water had to be added daily (Braman et al. 2000; Jessup et al. 2011) or every other day (Cheng et al. 2013), which is laborious and larvae may be stressed or harmed during the process. Once the experiment was set up, there was no maintenance needed during the benzimidazole agar plate experiment until the termination of the trial at 7 days after its initiation. Thus, this protocol has greatly optimized the technique in conducting fall armyworm excised leaf-feeding bioassays, which could be a valuable alternative to the feeding bioassays using insect diet cups or Petri dish plates. Furthermore, this new method has the potential to be used with tissue from a myriad of plant species and insects.

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Summary

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is an economically significant pest of sorghum and maize. Laboratory bioassays are often conducted for convenience and for fall armyworm confinement but as the number of entries and replications increase, the replenishing of fresh tissue daily and the cleaning of insect diet cups or petri dishes becomes laborious. The current study was conducted to determine if agar plates, used to retain leaf moisture, supplemented with benzimidazole, a fungicide that delays leaf senescence and retards fungicide growth, can be used to assess fall armyworm feeding on fresh maize and sorghum leaf tissue with minimal labor. We conducted 2 trials consisting of 3 cultivars of maize with known resistance or susceptibility to fall armyworm feeding, and 4 cultivars of sorghum that are parents of existing mapping populations. The top 2 (whorl) leaves were removed from 36–37-day old plants, 7.6 cm long pieces of leaf were excised, and each piece was placed on a benzimidazole agar plate. One larva per plate was placed on the leaf tissue; the plate was sealed with Parafilm and placed in an incubator for 7 days. Average larval weights for Trial 1 were significantly different than Trial 2 after 7 days, and thus each trial was analyzed separately. For both trials, the larvae that were fed susceptible maize line 'AB24E' had weights that were significantly greater than larvae fed resistant maize lines 'Mp708' and 'FAW1430'. No significant differences in weight were observed for fall armyworm larvae fed on the 4 sorghum lines except for those larvae fed 'Collier' in Trial 2, which had weights significantly greater than larvae fed the other 3 sorghum cultivars (Entry 22, 'Honey Drip', 'AN109'). Thus, the benzimidazole agar plate method is an easy and effective method for assessing fall armyworm feeding on maize and sorghum, and thus can be used to identify maize and sorghum germplasm lines with resistance to fall armyworm.

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Key Words: *Spodoptera frugiperda*, *Zea mays*, *Sorghum bicolor*, whorl tissue, bioassay

Sumario

El gusano cogollero, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) es una plaga de importancia económica de sorgo y maíz. Los bioensayos de laboratorio se realizan a menudo por conveniencia y para el confinamiento del gusano cogollero pero con incremento en el número de entradas y repeticiones, el relleno diario de tejido fresco y la limpieza de los recipientes de dieta de insectos o placas de Petri se vuelve laborioso. El estudio actual fue para determinar si se puede utilizar placas de agar, que sirvan para retener la humedad de la hoja, suplementado con bencimidazol, un fungicida que retrasa la senescencia foliar y retarda el crecimiento fungicida, para evaluar la alimentación del gusano cogollero en maíz fresco y en tejido de las hojas de sorgo con un trabajo mínimo. Se realizaron 2 ensayos que consisten en 3 cultivares de maíz con resistencia conocida o susceptibilidad al gusano cogollero, y 4 cultivares de sorgo que son progenitores de las poblaciones de mapeo existentes. Se quitaron las 2 hojas (cogollo) superiores de las plantas de 36-37-días, se cortaron las hojas en piezas de 7.6 cm de largo, y se colocó cada pieza en una placa de agar con bencimidazol. Se puso una larva por placa en el tejido de la hoja; se selló la placa con Parafilm y fue puesta en una incubadora por 7 días. El promedio del peso de las larvas en el Ensayo 1 fue significativamente diferente del Ensayo 2 después de 7 días, y por lo tanto cada ensayo se analizaron por separado. Para ambos ensayos, las larvas que fueron alimentados con la línea de maíz susceptible 'AB24E' tenía un peso significativamente mayor que las larvas alimentadas con las líneas de maíz resistentes 'Mp708' y 'FAW1430'. No se observaron diferencias significativas en el peso de las larvas de gusano cogollero que se alimentaron sobre las 4 líneas de sorgo con excepción de las larvas alimentadas con la línea 'Collier' en el Ensayo 2, que tenía un peso significativamente mayor que las larvas alimentadas con los otros 3 cultivares de sorgo (Entry 22, 'Honey Drip' 'AN109'). Por lo tanto, el método de usar la placa de agar de bencimidazol es un método fácil y eficaz para evaluar la alimentación por gusano cogollero sobre el maíz y el sorgo, y por lo tanto puede ser utilizado para identificar líneas de maíz y sorgo de germoplasma con resistencia al gusano cogollero.

Palabras Clave: *Spodoptera frugiperda*, *Zea mays*, *Sorghum bicolor*, tejido del cogollo, bioensayo

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