Control of Contamination in Corn Callus Cultures Used for Insect Resistance Studies

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ABSTRACT Bioassays using corn, Zea mays L., callus have been used successfully to differentiate among genotypes that are resistant or susceptible to leaf feeding by fall armyworm, Spodoptera frugiperda (J. E. Smith) and southwestern corn borer, Diatraea grandiosella Dyar. The use of such bioassays has, however, been limited by bacterial and fungal contamination of callus cultures following their infestation with insect larvae. This investigation was undertaken to determine whether transferring callus from petri plates containing a Murashige and Skoog medium amended with sucrose, agar, 2,4-dichlorophenoxyacetic acid-(2,4-D), and zeatin to 30-ml plastic cups containing water agar amended with gentamicin and sorbic acid prior to infestation with fall armyworm or southwestern corn borer larvae would reduce contamination without diminishing our ability to differentiate among leaf feeding resistant and susceptible corn hybrids. We found that 62% of the callus cultures on Murashige and Skoog medium were contaminated after infestation with fall armyworm larvae while only 2% of the callus transferred to the plastic cups with water agar amended with gentamicin and sorbic acid were contaminated. Both fall armyworm and southwestern corn borer larvae fed on callus of leaf feeding resistant corn hybrids were significantly smaller than those fed on susceptible hybrids when the callus was placed in cups with water, agar, gentamicin, and sorbic acid. Transferring callus from Murashige and Skoog medium to cups with gentamicin and sorbic acid prior to infestation with insect larvae appears to satisfactorily reduce contamination without affecting the growth of larvae fed on callus.

KEY WORDS Corn, host plant resistance, fall armyworm, southwestern corn borer, Lepidoptera, Noctuidae, Pyralidae

Several years ago, we reported that southwestern corn borer, Diatraea grandiosella Dyar, larvae could be reared on corn, Zea mays L., callus (Williams et al. 1983). We found that when larvae were fed on callus of corn genotypes that exhibited resistance to leaf feeding in the field, the larvae weighed less than those

1 Accepted for publication 12 June 1994.
fed on callus of susceptible genotypes. Similar results were later demonstrated with other insects including fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Williams et al. 1985); corn earworm, *Helicoverpa zea* (Boddie) (Williams et al. 1987a); European corn earworm, *Ostrinia nubilalis* Hubner; and sugarcane borer, *Diatraea saccharalis* (F.) (Williams et al. 1987b). Croughan and Quisenberry (1989) also found that fall armyworm larvae fed on callus of Bermudagrass, *Cynodon dactylon* (L.) Pers., showed reduced growth when fed on resistant cultivars. Callus has also been useful in investigations of the mechanisms, inheritance, and chemical basis of insect resistance (Williams & Davis 1985; Isenhour & Wiseman 1988; Paiva 1988).

Most of our bioassays have involved placing neonate larvae on callus, allowing the larvae to feed for 7 d, and then weighing the larvae to determine differences in larval growth on callus of different genotypes (Williams et al. 1983, 1985, & 1987a, 1987b). Frequently, callus cultures have become contaminated with bacteria and fungi following infestation with insect larvae. The contamination affects larval survival and growth; therefore, we have routinely eliminated contaminated cultures before weighing. With high levels of contamination, entire experiments have sometimes been jeopardized or even lost.

Because contamination was a serious limitation to our evaluation of corn callus in laboratory bioassays for insect resistance, we modified our procedures to better control contamination. Experiments reported herein were undertaken to determine whether a modification in our procedures effectively reduced contamination without adversely affecting our ability to distinguish between leaf feeding resistant and susceptible corn genotypes.

**Materials and Methods**

For these studies, callus was initiated from two corn hybrids that are resistant to leaf feeding by southwestern corn borer and fall armyworm, Mp704 × Mp707 and Mp707 × Mp708, and two susceptible corn hybrids, Ab24E × Tx601 and SC229 × Tx601 (Williams & Davis 1982, 1984; Williams et al. 1989, 1990). We followed procedures similar to those described by Williams et al. (1983, 1987a, 1987b) to initiate callus from mature corn kernels. Kernels were swirled in a sterile beaker with 2 g laboratory detergent and 100 ml 5.25% sodium hypochlorite for 20 min. They were rinsed twice in sterile distilled water and then swirled for 5 min in a solution of 700 ml/liter ETOH. The seed were rinsed five times in sterile distilled water, soaked for 3 min in a solution of 100 mg gentamicin and 1.2 g sorbic (2,4-hexadienoic acid) per liter of sterile distilled water. The seed were maintained on germination paper moistened with the gentamicin, sorbic acid solution for 48 h at 25°C. Embryos were excised using forceps and a scalpel.

The embryos were placed in petri plates on Murashige & Skoog (1962) medium supplemented with 20 g/liter sucrose, 8 g/liter agar, 15 mg/liter 2,4 dichlorophenoxyacetic acid (2,4-D), and 0.15 mg/liter of zeatin (6-[4-hydroxy-3-(methylbut-2-enylamino)purine). The callus was maintained at 27°C with a photoperiod of 12:12 (L:D) and transferred to a maintenance medium after
4 wk. The callus was transferred to fresh medium at 4-wk intervals thereafter. The maintenance medium differed from the initiation medium in that 2,4-D was reduced to 5 mg/liter and zeatin solution was reduced to 0.1 mg/liter.

On 29 October 1993, approximately 500 mg of callus was placed on maintenance medium in petri plates, 15 plates per hybrid, and each plate was infested with one neonate fall armyworm larva. This was similar to the procedures used in earlier experiments (Williams et al. 1985). A mixture of 750 ml sterile distilled water, 7500 mg agar, 450 mg sorbic acid, and 45 mg gentamicin was prepared and heated to 82° C. The mixture was poured in 10-ml aliquots into 30-ml plastic cups and allowed to cool. We placed 500 mg callus and one neonate fall armyworm larva in each of 15 cups per hybrid. The petri plates and diet cups were arranged in a randomized complete block design with 15 replications and placed in a growth chamber at 29° C with a photoperiod of 12:12 (L:D). After 7d, we noted the presence or absence of fungal or bacterial contamination in each plate or cup and weighed the larvae from the uncontaminated plates and cups.

On 23 November 1993, an additional experiment was conducted using callus of three hybrids, Mp707 × Mp708, Ab24E × Tx601, and SC229 × Tx601. We placed 500 mg of callus in 14 cups per hybrid and infested each one with a neonate southwestern corn borer larvae following the same procedures as in the first experiment except that the larvae were allowed to feed 14 d before weighing.

Larval weights from the two experiments were analyzed using the method of least squares to fit linear models (SAS Institute Inc. 1987) because of the large number of missing values. In the first experiment, larval weights for petri plates and cups were analyzed separately. Significance of differences among means was determined by Fisher’s Protected LSD \( (P=0.05) \) (Steel & Torrie 1980).

**Results and Discussion**

When we completed the first experiment and the fall armyworm larvae were weighed after feeding for 7 d, 62% of the petri plates containing callus maintenance medium were contaminated while only 2% of the plastic cups containing the mixture of water, agar, gentamicin, and sorbic acid showed evidence of contamination. Larvae that fed on callus of the resistant hybrids, Mp704 × Mp707 and Mp707 × Mp708, and the susceptible hybrids differed significantly in weight whether the callus was placed in petri plates \( (F = 7.91; 2,12 \text{ df}; P < 0.05) \) or plastic cups \( (F = 19.65; 3,34 \text{ df}; P < 0.01) \). Larvae in this experiment were, however, heavier than those grown on callus in petri plates in an earlier experiment (Williams et al. 1985). Because all of the petri plates containing callus of SC229 × Tx601 were contaminated, none of the larvae for that treatment were weighed. On the other hand, none of the cups containing callus of SC229 × Tx601 were contaminated.

The second experiment was conducted to determine whether differences in weights of southwestern corn borer larvae fed on callus of leaf feeding resistant and susceptible hybrids would be expressed when the callus was
Table 1. Mean weights of fall armyworm larvae fed for 7 d on callus of four corn hybrids in petri plates with callus maintenance medium or in plastic cups containing agar amended with an antibiotic and fungicide.

<table>
<thead>
<tr>
<th>Corn hybrida</th>
<th>Plates</th>
<th>Cups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mp704 X Mp707</td>
<td>92 ± 69</td>
<td>97 ± 39</td>
</tr>
<tr>
<td>Mp707 X Mp708</td>
<td>94 ± 12</td>
<td>76 ± 33</td>
</tr>
<tr>
<td>Ab24E X Tx601</td>
<td>273 ± 68</td>
<td>181 ± 47</td>
</tr>
<tr>
<td>SC229 X Tx601</td>
<td>_b</td>
<td>156 ± 29</td>
</tr>
</tbody>
</table>

LSD (0.05) 103 30

a Mp704 X Mp707 and Mp707 X Mp708 are resistant to fall armyworm leaf feeding; Ab24E X Tx601 and SC229 X Tx601 are susceptible.
b All plates were contaminated and larval weights could not be determined.

Table 2. Mean weights of southwestern corn borer larvae fed for 14 d on callus of three corn hybrids in plastic cups containing water agar amended with an antibiotic and a fungicide.

<table>
<thead>
<tr>
<th>Corn hybrida</th>
<th>Larval weight (±SD), mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mp707 X Mp708</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>Ab24E X Tx601</td>
<td>30 ± 27</td>
</tr>
<tr>
<td>SC229 X Tx601</td>
<td>38 ± 18</td>
</tr>
</tbody>
</table>

LSD (0.05) 13

a Mp707 X Mp708 is resistant to southwestern corn borer leaf feeding; Ab24E X Tx601 and SC229 X Tx601 are susceptible.
placed in the 30-ml plastic cups with the water agar mixture containing gentamicin and sorbic acid. Because it had been found in previous experiments that southwestern corn borer larvae fed on callus grow more slowly than fall armyworm larvae (Williams et al. 1987a, 1987b), larvae were allowed to feed for 14 d rather than 7 d. After 14 d, it was found that 13% of the cups showed evidence of contamination. Again, differences in larval weights among hybrids were significant ($F = 6.42; 2,24$ df; $P < 0.01$), and the larvae that fed on callus of the resistant hybrid were significantly smaller than those fed on callus of the susceptible hybrids. Weights of larvae were higher than those reported in earlier experiments in which southwestern corn borer larvae were fed corn callus (Williams & Davis 1985, Williams et al. 1987b).

Although the use of callus for investigating insect resistance is a potentially useful technique, the high degree of contamination that we have frequently encountered after infesting the callus with insect larvae has been discouraging. Contamination has limited the use of this technique as a method of quantifying levels of resistance even though it offers a means of removing environmental bias when evaluating genotypes adapted to different areas. Bioassays can also be used in situations where appropriate procedures for field testing have not been developed. The modifications of our previous procedures appear to significantly reduce contamination without lessening our ability to distinguish between corn genotypes with different levels of fall armyworm and southwestern corn borer resistance. This modification will likely be useful to researchers working with other insects and other crops as well.

References Cited


Williams, W. P., P. M. Buckley & F. M. Davis. 1985. Larval growth and behavior of the fall armyworm (Lepidoptera: Noctuidae) on callus initiated from susceptible and resistant corn hybrids. J. Econ. Entomol. 78: 951-954.


