LABORATORY SPRAY METHOD FOR DETECTING THE BXN® TRAIT IN COTTON SEEDLINGS

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Abstract

BXN® cotton is resistant to the herbicide bromoxynil which effectively controls many broadleaf weeds following field application of (BUCTRIL® 4EC) in cotton. BXN® seed lots must be tested prior to sale to insure that seed is indeed resistant to the herbicide to prevent crop injury. Delinted cottonseed is planted with equidistant spacing in nursery flats and grown at 7,500 lumens m^{-2} at 30 – 32°C with a 24-hr photoperiod. Spraying a 3,642 ppm bromoxynil solution over-the-top until runoff, to actively growing seedlings at the cotyledon to one-leaf stage, and then re-applying the herbicide two days later in the laboratory is a valid and cost-effective means to determine percent purity of cottonseed samples. Susceptible seedlings appear desiccated or burned at two days after herbicide application, followed by complete necrosis.

Introduction

Because young cotton seedlings grow slowly early in the season and are not very competitive, the removal of early weed pressure is extremely important in cotton production (ABGIOS, 2002). The herbicide bromoxynil (marketed as BUCTRIL® 4EC) is applied post-emergence to kill broadleaf weeds in cotton including annual morningglory species (*Ipomoea spp.*) and cocklebur (*Xanthium strumarium*) (Lee and Ball, 1999). This herbicide acts by blocking electron flow during the light reaction of photosynthesis. *Bromoxynil nitrilase* (BXN®), a gene from the bacterium *Klebsiella pneumoniae*, detoxifies bromoxynil. The BXN® cotton line was genetically engineered to express resistance to bromoxynil and allows farmers to use this herbicide for weed management in cotton (US FDA, 1994).

Polymerase Chain Reaction (PCR) is expensive, time-consuming, and is not conducive to testing large numbers of single-seed samples, Currently, there are no reliable immunoassays available commercially to test for bromoxynil resistance in cotton (Anklam et al., 2002). Because light is required for good herbicidal activity, an herbicide spray assay is used to identify resistance/non-resistance in the laboratory (AOSA, 2003). Within one day of herbicide application to actively growing seedlings, non-resistant seedlings show herbicide damage. After a second application the effect is obvious and percent BXN® purity may be determined.

Materials and Methods

Delinted seeds treated with a broad-spectrum fungicide are planted approximately 1.25 cm deep in a moist potting soil (such as 85% composted wood, 10% sand, 5% perlite) in 25 cm x 50 cm x 6 cm nursery flats. One-hundred seeds per flat are planted to achieve equidistant spacing in the sample area (42 cm x 25 cm), and seeds of susceptible and tolerant control samples are planted in the control area (8 cm x 25 cm).

Flats are placed in a 30-32°C growth chamber with 24-hour photoperiod and a light intensity of approximately 7,500 lumens m^{-2}. Flats are watered as needed to keep potting soil moist.

Following a 7-10 day growth period when seedlings reach the cotyledon to one leaf stage, flats are removed from the growth chamber and sprayed with a 3,642 ppm bromoxynil solution (add 10 ml BUCTRIL® 4EC to 1,500 ml water) until runoff. Two days following initial bromoxynil application, flats are sprayed again with the same bromoxynil solution. Note: Bromoxynil is a contact herbicide that destroys cell membranes in the presence of light by inhibiting photosystem II, therefore plants must be actively growing to have good herbicidal activity.

Two days after the second herbicide application, seedlings are evaluated as BXN® tolerant or BXN® susceptible. Tolerant seedlings have no damage and are unaffected by bromoxynil treatment, as bromoxynil is metabolized into non-toxic compounds. Susceptible seedlings appear desiccated or burned at two days after herbicide application, followed by complete necrosis (Fig. 1).

Percent BXN® purity is calculated by dividing the total number of tolerant seedlings by the number of seedlings that emerged, then multiplying by 100. For example, if an analyst determines that there are 337 tolerant seedlings out of 340 seedlings that emerged, then the formula 337/340 x 100 would be used to calculate BXN® purity of 99.1%.

References

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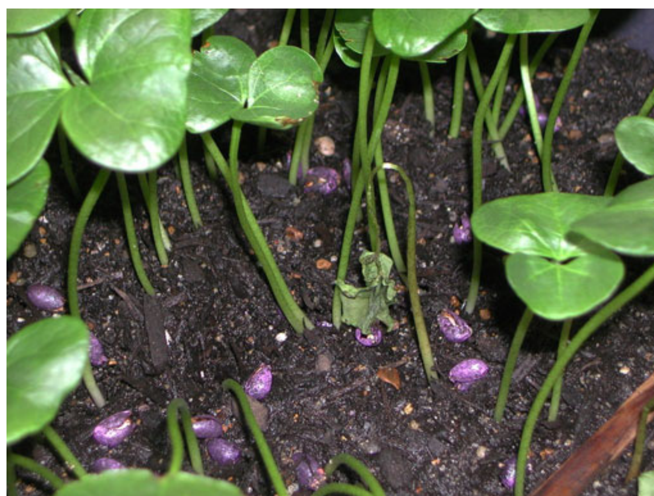

Figure 1. A single BXN® susceptible seedling is easily identifiable two days post bromoxynil application.