Crop Protection 94 (2017) 28–32

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Management of Ramularia leaf spot on cotton using integrated control with genotypes, a fungicide and *Trichoderma asperellum*

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form
21 November 2016
Accepted 4 December 2016

Keywords:
Cotton varieties
Gossypium hirsutum
Ramularia areola
Ramularia leaf spot
Biological control

ABSTRACT

Ramularia leaf spot, caused by *Ramularia areola*, is responsible for premature defoliation of cotton, resulting in potential reductions in yield. The objective of this study was to evaluate integrated control using genotypes, a fungicide and *Trichoderma asperellum* to manage Ramularia leaf spot on cotton. In the greenhouse, the resistance of eighteen genotypes was evaluated with two isolates of *R. areola* (IMA 244 and IMA 237). In field, the severity of Ramularia leaf spot was evaluated on cotton genotypes CNPA MT 2009-1381, CNPA GO 2007-419, BRS 293, BRS 372, CNPA GO 2008-1265 and FMT 701 treated or untreated with a fungicide in Primavera do Leste, MT, Correntina, BA, and Santa Helena de Goiás, GO, during the 2011–2012 season. The varieties BRS 293 and BRS 372 were sprayed with a fungicide or *Trichoderma* in Sapezal, MT, during the 2012–2013 season, and the disease severity and fiber yield were evaluated. Significant interactions were detected between *Ramularia* isolates and cotton genotypes; the lowest disease severity was observed with IMA CD 05–8276 and CNPA GO 2007-419 genotypes. In the field tests, the lowest disease severity was with variety BRS 372 and the highest was with BRS 293, when grown in two different regions. The chemical fungicide and *T. asperellum* both reduced the disease severity in cotton varieties BRS 372 and BRS 293; however, yields were not significantly affected. In conclusion, an integrated strategy with the management tools of resistant varieties, fungicides and biocontrol agents should be used to control Ramularia leaf spot on cotton.

1. Introduction

Ramularia leaf spot, caused by *Ramularia areola* G. F. Atk., teleomorph *Mycosphaerella areola* Ehrlich & F.A. Wolf, is the most important disease of cotton *(Gossypium hirsutum)* in Brazil (Suassuna and Coutinho, 2007). Symptoms typically are primarily on the lower parts of the plant and are characterized by white-blue lesions on the under surface of leaves from which the fungus develops white or yellow powdery sporulation (Bell, 1981). *Ramularia areola* infects cotton leaves causing premature defoliation and a reduction in photosynthetic capacity of the plants, with a consequent reduction in the yield potential of different varieties. Aquino et al. (2008a) found that the disease reduced fiber yield 49% in Brazil. According to Rathaiah (1976), Ramularia leaf spot must be managed, and varieties resistant to the pathogen are the cheapest and most efficient method for disease control. Cia et al. (2009) evaluated 18 cotton genotypes; 61% of the genotypes were moderately or highly susceptible and 29% were moderately resistant to *Ramularia*. In glasshouse conditions, Zandoná et al. (2012) found that a single dominant gene governed the resistance to *R. areola* strain 44 in the genotypes CNPA BA 2003–2059 and FMT 02102996. Additionally, Aurangabadkar et al. (1981), Mukewar and Mayee (2001) and Mukewar et al. (1995) demonstrated that some cotton genotypes have immunity to *R. areola.*

Currently, chemical fungicides are the primary method for control of Ramularia leaf spot in Brazil (Suassuna and Coutinho, 2007; Aquino et al., 2008a). Although Ramularia leaf spot is controlled efficiently with fungicides, disease control alternatives must be developed for integrated management. Because biopesticides are an alternative to sustain high production with low ecological impact, research in the development of biocontrol is currently expanding exponentially (Pérez et al., 2015), with *Trichoderma*-based products the most important biofungicides used

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http://dx.doi.org/10.1016/j.cropro.2016.12.006
0261-2194/

worldwide (Lorito et al., 2010). According to Bettiol et al. (2014), *Trichoderma* is the most important commercialized biocontrol agent and is it used for control of soilborne, foliar and postharvest diseases in several crops (Harman et al., 2004; Harman, 2006; Woo et al., 2006). This biocontrol agent protects plants by producing antibiotics and enzymes, promoting plant growth, and inducing plant defenses, in addition to increasing competition for nutrients (Hermosa et al., 2012). Biofungicides efficiently control plant diseases and are environmentally safe; however, although use is expanding annually, the adoption by growers remains limited (Bettiol, 2011). On cotton, the foliar application of *Trichoderma viride* reduces the severity of Ramularia leaf spot when sprayed alone or in combination with a fungicide (Duarte et al., 2007; Freitas et al., 2007).

The aim of this study was to evaluate the integrated management of Ramularia leaf spot on cotton with genotypes, a fungicide and *T. asperellum*.

2. Materials and methods

2.1. Inoculum of R. areola

Dr. Rafael Galbieri (Instituto Matogrossense do Algodão - IMA) provided isolates IMA 244 and IMA 237 of *R. areola*. The isolates were grown on 20 mL of malt extract-agar 2% in plates (polystyrene, 90 × 10 mm), which were incubated for 7 d at 25 ± 2 °C with 12 h of fluorescent light and 12 h of darkness. The conidia of *R. areola* were suspended in Tween 80 solution with sterile distilled water (0.01% v/v), and the suspensions were filtered to remove spore aggregates. Conidial concentrations were adjusted by hemocytometer, and dilutions were prepared with sterile Tween 80 solution (0.01% v/v) for inoculation of foliar tissues.

2.2. Assessment of cotton genotypes for Ramularia leaf spot management

Ten seeds of cotton genotypes LD CV03, BRS 336, IAC 08-2031, IMA CD 03-1661, IPR Jataí, DP 604 BG, Nuopal, FM 910, PRGOA 03-231-04, BRS 2080, IAC 08/90, LD CV 12, FM 993, FMT 709, IMA CD 05–8276, FMT 705, CNPA GO 2006-174 and CNPA GO 2007-419 were sown in 1 L pots containing soil (red dystroferric latosol–oxisol with clayey texture) and substrate (composted pine bark) (10:1). After emergence, two of the most vigorous seedlings were selected and maintained in a screen house (25% light reduction) with a sprinkler system. The seedlings were watered twice daily. Cotton plants were inoculated with a suspension of 1×10^6 conidia mL^{-1} of *R. areola* isolates IMA 244 and IMA 237 45 d after sowing; the suspensions were sprayed (1.5 L Pre-Compressed hand sprayer – Tramontina-78610150, Carlos Barbosa, RS) on leaves until runoff. Disease severity was evaluated 15, 20, 30 and 40 d after inoculation. The evaluation of disease severity was based on a scale of notes as follows: 0.05, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 67.20% of Ramularia leaf spot (Aquino et al., 2008b). The means per plant were used to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977; Madden et al., 2007). The experiment was a completely randomized design with three replicates that each consisted of 5 pots with two plants.

2.3. Integrated management of Ramularia leaf spot with cotton genotypes and a chemical fungicide

The genotypes CNPA MT 2009-1381, CNPA GO 2007-419, BRS 293, BRS 372, CNPA GO 2008-1265 and FMT 701 were sown in Primavera do Leste, Mato Grosso State, São Desiderio, Bahia State, and Santa Helena de Goiás, Goiás State, for the 2011–2012 crop season. The experiments were performed twice in Primavera do Leste, with seeds sown for both a first and a second season. The treatment plots were sprayed with the fungicide Tetraconazole (Emerald^R - FMC) at the rate of 0.5 L ha^{-1} and were compared with untreated plots. The plants were sprayed with the fungicide 25, 40, 55, 70, 90, 105 and 120 d after emergence (DAE). A CO_2 pressurized backpack sprayer was used for spray the fungicide, with a two and half meter long bar and a five spray nozzles type double fan TJ 110.02, spaced at 50 cm (Herbicat, Catanduva, SP). The disease severity was assessed every two weeks, following Aquino et al. (2008b), and these results were used to calculate the AUDPC. The plots contained five-5 m rows spaced 0.9 m apart. The experiment was arranged in a randomized block design with three replicates. The disease severity was estimated for 50 plants from each of the two central rows of each plot.

2.4. Integrated management of Ramularia leaf spot with cotton genotypes, a chemical fungicide and Trichoderma asperellum

Based on the results of the experiments described above, the genotypes BRS 293 and BRS 372 were cultivated in Sapezal, Mato Grosso State, in the 2012–2013 crop season. The treatments were either the fungicide Tetraconazole (Emerald^R) at the rate of 0.5 L ha^{-1} or the biocontrol agent *T. asperellum* (Quality WG – Laboratório Farroupilha) at the rate of 0.1 kg ha^{-1}, and treated plots were compared with untreated plots. The plants were sprayed with the chemical or biofungicide 25, 40, 55, 70, 90, 105 and 120 DAE. The same sprayer describes before was used to spray the products. The disease severity was assessed 40, 55, 70, 90, 105, 120 and 135 DAE. To evaluate disease severity and calculate the AUDPC, the identical methodology was used as described previously. The plots and the experimental design were also identical to those described above.

2.5. Statistical analyses

The design of each experiment was a randomized block, which was repeated three times. Data for disease severity were examined using analysis of variance (ANOVA), and treatment means were compared with Tukey's tests ($P<0.05$).

3. Results

3.1. Assessment of cotton genotypes for Ramularia leaf spot management

Interaction was detected between cotton genotypes and *Ramularia* isolates ($P < 0.05$). The genotypes LD CV 03, BRS 336, IAC 08-2031, IPR Jataí and IMA CD 03–1661 were the most susceptible to both isolates, with AUDPC values between 385.9 and 541.8 when inoculated with isolate IMA 244 and between 257.7 and 508.2 for isolate IMA 237 (Table 1). The genotypes IMA CD 05-8276 and CNPA GO 2007-419 had higher resistance to *R. areola* strains IMA 237 and IMA 244 (Table 1) than that of the other genotypes. The AUDPC values for IMA CD 05-8276 were 5.2 and 11.5 when inoculated with strains IMA 237 and IMA 244, respectively. For the genotype CNPA GO 2007-419, the AUDPC values were 13.2 and 17.8, respectively, when inoculated with these two *Ramularia* strains.

3.2. Integrated management of Ramularia leaf spot with cotton genotypes and a chemical fungicide

In the assay performed in São Desidério, the genotype CNPA GO 2008-1265, untreated with the fungicide, had the lowest severity rating when compared with that of the other genotypes (Table 2).