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Evaluation of field application of fungi-inoculated bioplastic granules for reducing herbicide carry over risk

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A B S T R A C T

Previous studies have shown that starch-based bioplastic granules can serve as an effective formulation for delivering biocontrol and bioremediation fungi. This field study evaluated the feasibility of this approach for reducing injury potential and/or carry over risk caused by the in season application of the herbicide Bicyclopyrone (BIR) on two sensitive crop species.

Planting soybean in field plots simultaneously sprayed with BIR at 50, 100 and 200 g ha^{-1} resulted in an average plant injury of 60, 80 and 93%, respectively. Plant injury decreased when the two sensitive crops were planted at increasing time interval from BIR treatment. Application of bioplastic granules inoculated with *Beauveria bassiana*, *Rhizopus oryzae* or *Trichoderma harzianum* led to a significant reduction of carry over risks. In 2012, at 7 days after treatment, plots amended with inoculated granules showed a reduction of plant injury higher than 50%. Reduced plant injury was recorded in 2013. However the effect of inoculated granules was consistent with that observed in the previous year.

Findings from this field study showed that this technology is promising in reducing herbicide carry over risk. This novel triketone herbicide BIR was used here as a model for evaluating this approach, thus not excluding the possibility to be extended to other classes of residual herbicides.

1. Introduction

From the beginning of their use in the late 1940s, synthetic herbicides have played a crucial role in the control of weeds in cultivated crops (Shaner, 2014). In recent years, increasing environmental concerns and wide acceptance of soil conservation practices, selection of weed populations resistant to herbicides, and many other factors, have renewed the demand for novel and more effective herbicide active ingredients (Duke, 2012).

Bicyclopyrone (BIR) is a new selective herbicide which acts by inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an essential enzyme for carotenoid biosynthesis (Baalouch et al., 2013; Lins et al., 2014). Similar to other HPPD-inhibiting triketone herbicides, BIR causes bleaching symptoms and then necrosis of meristematic tissues on the leaves of susceptible plants (Riddle et al., 2013a). This residual pre- and post-emergence herbicide is effective against a wide range of broadleaf weeds in corn and is expected to be commercialized for the 2015 growing season. BIR is also intended to be applied as a herbicide premix to provide complete pre- and post-emergence control of grass and broadleaf weeds and to reduce herbicide selection pressure (Lins et al., 2014).

Among the different techniques and strategies for mitigating the evolution of herbicide resistance, crop rotation remains a valuable and additional option (Cardina et al., 2002; Monjardino et al., 2003). However, beside economic and other technical considerations, remaining residues in soil of herbicides applied in the previous years and the subsequent potential occurrence of

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injures on sensitive crops in the rotation has to be seriously considered in planning crop rotation schemes (Greenland, 2013; Felix et al., 2007). This aspect is well discussed in the technical and scientific literature and attempts to predict the risk of crop injuries caused by carry over of biologically active herbicide residues have also been focused on triketone herbicides, including Mesotrione and Sulcotrione (Riddle et al., 2013a, 2013b; Maeghe et al., 2004). Since persistence of herbicides in soil depends on many uncontrolled factors (e.g., soil type, soil temperature and water regimes, diversity and dynamic of the soil microbial population, etc.), under real agricultural conditions, the risk of crop injuries caused by herbicide carry over is not easily predictable. As such, the current study used in-season applications of BIR to maximize crop injury potential on the susceptible crops tested rather than a true carry over scenario which would have several months between application and planting with a much reduced risk of injury.

Recent advancements in applied biology and microbiology may offer novel and alternative solutions for reducing the risk of herbicide carry over and crop injury. Starting in late 2006, a project focusing on the use of starch-based bioplastic as a formulation for delivering biocontrol microorganisms was initiated at the USDA-ARS in Stoneville, Mississippi, in collaboration with the University of Bologna (Accinelli and Abbas, 2011). Laboratory and field scale experiments demonstrated that the concept of using starch-bioplastic granules inoculated with specific fungal strains is effective and reliable in the biocontrol of different pathogens and pests (Accinelli et al., 2009, 2012, 2014; Portilla et al., 2014). More recently, this approach was also considered as an option for reducing the risk of herbicide carry over. A series of laboratory and greenhouse studies were then conducted with selected herbicides, including BIR, to develop the technology and to select more promising fungal strains. Greenhouse studies have shown that soil application of inoculate granules was able to significantly reduce injuries caused by BIR to up 80% on soybean (Accinelli C, unpublished). In the research summarized here, bioplastic granules inoculated with different fungal strains, including *Beauveria bassiana*, *Rhizopus oryzae* and *Trichoderma harzianum*, were evaluated under field conditions. Effects of granule application timing, BIR concentration and time interval from herbicide application and planting on crop injury were included in the study.

2. Materials and methods

2.1. Fungal isolates and inoculation of bioplastic granules

The study was carried out using the following fungal isolates: *B. bassiana* DSM 1344, *R. oryzae* DSM 905, *T. harzianum* ATCC 20847. Fungi were grown on potato dextrose agar (PDA) at 25 °C for 7–10 days, then ten 5-mm agar plugs were transferred to autoclavable plastic bags containing 1 kg of pre-wetted and sterilized granules (diameter 3 mm) made of a starch-based bioplastic type PE01S. The bags were capped with cotton plugs, fixed to steel rings and incubated in vertical position for 10 days at 25 °C. Inoculated granules were then dried at 60 °C in a ventilated oven for two days. Potency of the four different formulations were then evaluated following the procedure described in Accinelli et al. (2009). Briefly, triplicate granules were transferred into centrifuge tubes containing 10 mL of phosphate buffer saline (PBS) and 5 g of glass beads and shaken for 1 h at 200 rpm. Suspensions were serially diluted in PBS and plated onto acidified PDA. After incubation at 25 °C for 7–10 days, colonies were counted. Potency was then adjusted to the final value of 10^7 cfu g^{-1} by spraying the granules with spore suspensions. Granules were then redried as described above and stored at room temperature for ≤ one week before use.

2.2. Experimental scheme and treatments

The experiment was conducted during two consecutive years (2012–2013) in a 2-ha field located at the experimental station of the University of Bologna (Cadriano, Italy). Properties of the soil are reported elsewhere (Accinelli et al., 2009). A complete randomized block design with 3 blocks was used in each year. Granules were uniformly spread on the ground surface at 0 or 14 days before treatment (DBT) at the dosage of 30 kg ha^{-1}. BIR (20% a.i. SL; Syngenta Crop Protection AG, Basel, Switzerland) was applied at the beginning of June using a sprayer mounted on a small-size tractor operating a 300 L ha^{-1}. BIR doses were the following: 50, 100 and 200 g a.i. ha^{-1}. Untreated seeds of soybean (cv Nikko; Asgrow Vegetable Seed, Lodi, Italy) and field pea (cv Pepone; SIS S.p.A., Bologna, Italy) were planted at 0, 7, 14, 28 and 56 days after herbicide treatment (DAT). Plots consisted of 2 m row long and 2 m apart with 6 rows for each plant species. Plots

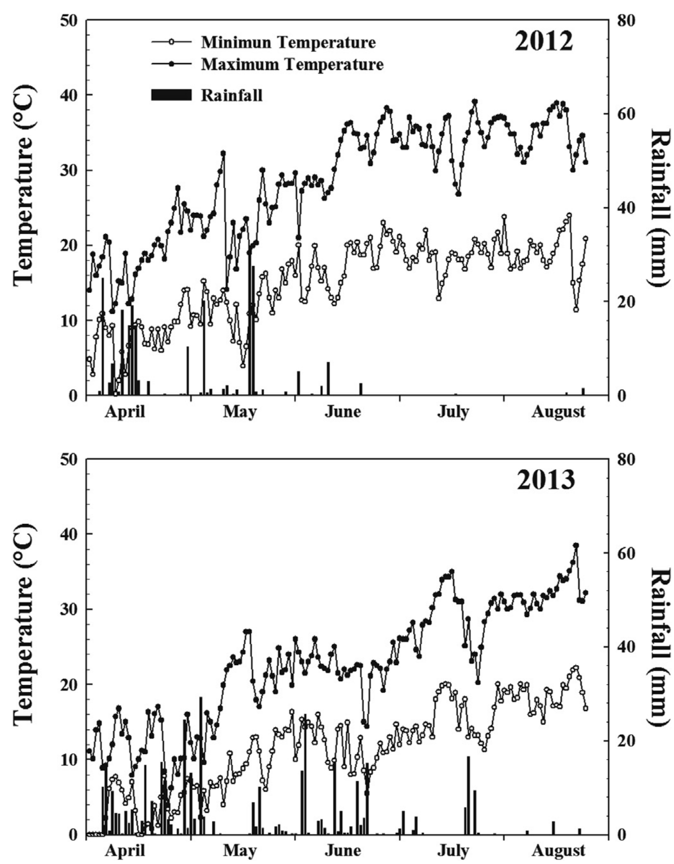

Fig. 1. Meteorological data recorded at the experimental site during the 2012 and 2013 crop season. Maximum daily temperature is shown with closed circles and minimum daily temperature is shown with open circles; rainfall is shown as solid bars.