Proceedings of the IX International Symposium on Biological Control of Weeds, pp. 297-299. V.C. Moran and J.H. Hoffmann (eds). 19-26 January 1996, Stellenbosch, South Africa. University of Cape Town.

Development of application techniques for biological weed control using rhizobacteria

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Abstract. Inundative biological control of weeds with microbial agents offers an ecological alternative to chemical pest control. Historically, research has focused on foliar-applied pathogens for reducing weed populations. However, soil-borne microorganisms, such as rhizobacteria, have recently been evaluated for control of grassy weeds. Of crucial importance to the success of rhizobacteria for weed control is the development of effective delivery systems which can be readily integrated into existing farming practices. Therefore, research into development of potential application techniques for control of green foxtail using rhizobacteria was initiated. Three application methods were investigated: (i) spray application onto crop residues; (ii) encapsulation of bacteria into alginate granules; and (iii) seed treatment. Under conservation tillage, crop residues left in the field support populations of rhizobacteria. Enumeration studies revealed that these crop residues provide a food source for rhizobacteria and maintain stable populations of these organisms, with larger population numbers occurring at 5°C than at 15°C. This has implications for both fall and early-spring applications. Rhizobacteria encapsulated into sodium alginate beads provided a stable system for introduction of these microbes for weed control. Some rhizobacterial isolates were shown to be effective root colonizers and may therefore be introduced into the field as a treatment on crop seeds. Evaluation of these application techniques in weed-crop competition studies is necessary to determine whether they will be effective delivery systems for rhizobacteria, to control weeds.

Introduction

Although herbicides account for over half of all annual pesticide sales, weeds continue to be a major yield loss factor in many western farming systems (Swanton *et el.* 1993). Herbicide resistance coupled with growing public concern over the use of synthetic chemicals has prompted researchers to investigate biological weed control as an additional strategy to standard weed-management practices. Several rhizobacteria, associated with the roots and rhizosphere of weeds such as downy brome, *Bromus tectorum* L., leafy spurge, *Euphorbia esula* L., wild oats, *Avena fatua* L. and green foxtail, *Setaria viridis* (L.) Beauv., have caused significant deleterious effects to root growth and plant development (Boyetchko and Mortensen 1993; Kennedy *et al.* 1991; Souissi and Kremer 1994). Of crucial importance to the success of rhizobacteria for weed control is the development of effective delivery systems that can be integrated into existing farming practices.

The objective of the project was to evaluate three application systems (crop residue, encapsulation into alginate beads, and powder formulation) for biological weed control with soil microorganisms using a rhizobacterial isolate that causes significant reductions in root growth and root dry-weight of green foxtail.

Materials and methods

Crop residue
Rhizobacteria (spontaneous rifampicin-resistant mutant) were applied to air-dried spring wheat straw, according to methods outlined by Stroo *et al.* (1988), in glass storage dishes. The effects of two moisture regimes (1 and 2g H_2O/g dry straw) and two incubation temperatures (5°C and 15°C) on rhizobacterial survival were tested. Straw samples were taken 2, 4, 7, 14, 21, 28 and 35 days after inoculation, shaken in sterile distilled water and the liquid serially diluted to obtain a measurement of the bacterial population, expressed as colony-forming units (cfu) per gram of dry straw.

Encapsulation into alginate beads

Rhizobacteria (spontaneous rifampicin-resistant mutant) were encapsulated into alginate beads, according to methods outlined by Bashan (1986). A bacterial suspension was mixed with 2% sodium alginate and added drop by drop into 0.1M $CaCl_2$ solution. Half the beads were air-dried overnight and stored at 5°C while the remainder was stored wet in sterile distilled water at 5°C. Rhizobacterial populations were enumerated 2, 4, 7, 14, 21, 28 and 35 days after bead formation. Beads were dissolved in phosphate buffer and serially diluted to obtain the population level of rhizobacteria in each alginate bead, expressed as cfu per bead.

Powder formulation

A powder formulation was prepared, similar to that described by Caesar and Burr (1991). Approximately 5 ml of methylcellulose (1%, w/v) was added to a rhizobacterial culture (spontaneous rifampicin-resistant mutant), mixed, added to talc (1:1 ratio), spread on a flat surface to dry, ground with a mortar and pestle, and stored at 5°C. One gram samples were taken 2, 4, 7, 14, 21, 28 and 35 days after preparation, shaken in 10 ml sterile distilled water and serially diluted to determine rhizobacterial populations (cfu per gram dry powder).

Results and discussion

Crop residue

Bacteria survived on crop residue for up to 35 days in all treatments, without the addition of external nutrients or moisture (Fig. 1). Regardless of the initial amount of bacteria applied to the straw, the overall population trends remained the same and the carrying capacity of the straw was approximately log 10 cfu/g dry straw.

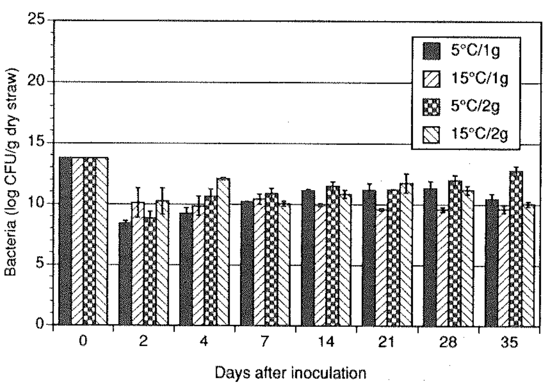

Fig. 1. Growth and survival of inhibitory bacteria (rifampicin-resistant) on wheat straw residue at 5°C and 15°C and two moisture regimes. Vertical bars indicate SE.

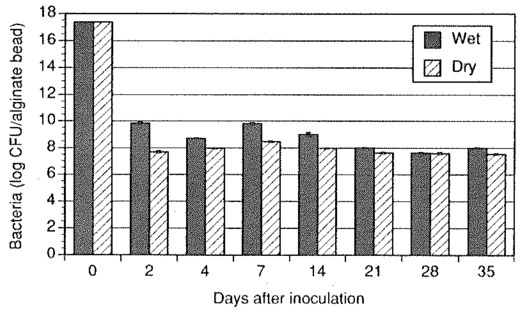

Fig. 2. Survival of bacterial populations (rifampicin-resistant) in sodium alginate beads (wet and dry beads) after storage at 5°C. Vertical bars indicate SE.

Encapsulation into alginate beads

Bacteria survived for up to 35 days when encapsulated in both wet and dry alginate beads (Fig. 2). Despite an initial decline, the populations stabilized around log 8 cfu/alginate bead. Wet beads supported a slightly higher population of bacteria than dry beads.

Powder formulation

Bacteria survived for up to 35 days in a powder formulation (Fig. 3). Maximum population decline occurred within the first four days and stabilized around log 5 cfu/g dry powder.

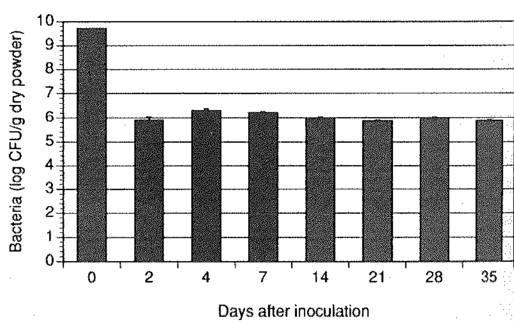

Fig. 3. Effect of powder formulation on survival of inhibitory bacteria (rifampicin-resistant) after storage at 5°C. Vertical bars indicate SE.

Conclusion

Rhizobacteria, with weed suppressive properties, have potential as biological weed control agents. One major challenge is to develop effective delivery systems for these soil microorganisms. The results from this study have demonstrated that rhizobacteria can survive on crop residues, alginate beads, and in powder formulation. These three carriers have potential to serve as application methods for delivery of rhizobacteria to the soil for green foxtail control.

Acknowledgements

We wish to thank the Natural Sciences and Engineering Research Council of Canada, Western Grains Research Foundation, and DowElanco Canada, Inc. for financial support of this project.

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