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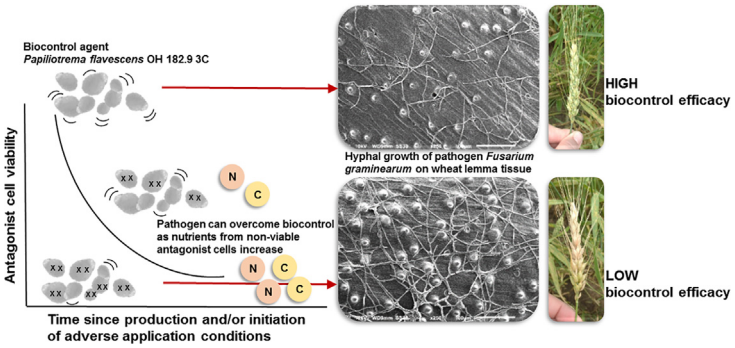
Nonviable biomass of biocontrol agent *Papiliotrema flavescens* OH 182.9 3C enhances growth of *Fusarium graminearum* and counteracts viable biomass reduction of Fusarium head blight

David A. Schisler, Miho Yoshioka, Martha M. Vaughan, Christopher A. Dunlap, Alejandro P. Rooney*

National Center for Agricultural Utilization Research (NCAUR), United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Peoria, IL 61604, United States

GRAPHICAL ABSTRACT



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ABSTRACT

Microbially-based plant disease control products have achieved commercial market success, but the efficacy of biocontrol products is sometimes deemed inconsistent. Declines in product viability before or after application results in a mixture of non-viable and viable antagonist cells being present on infection courts. We hypothesized that these non-viable cells could provide a nutritive benefit to plant pathogen propagules and thereby reduce the efficacy and consistency of performance of a biocontrol product. We tested this hypothesis on the pathogen *Fusarium graminearum*, an important causal agent of Fusarium head blight (FHB) of wheat, and a yeast antagonist *Papiliotrema flavescens* (formerly *Cryptococcus flavescens*) strain OH 182.9 3C. When *F. graminearum* was grown in a liquid basal salts medium without carbon or nitrogen but supplemented with increasing levels of non-viable cells of strain OH 182.9 3C, the amount of pathogen biomass produced increased ($P \leq 0.05$) with each sequentially higher level of non-viable cell amendment tested. Total conidial germination of *F. graminearum* on membranes was initially greatest in the control and least when conidia were combined with live cells of antagonist OH 182.9 3C. Though the presence of dead cells initially resulted in reduced conidial germination, the treatment increased bipolar germination of conidia after 6 h compared to the control (89% and 45%, respectively) ($P \leq 0.05$, FPLSD). Treatment with a combination of dead and live cells supported more bipolar germination than live cells alone (51% and 29%, respectively). After 12 h, dead cells had a similar stimulating effect on the branching of germ tubes and negated the reduction in branching seen when treating with live cells alone. Scanning electron microscopy indicated that the presence of non-viable cells of *P. flavescens* OH 182.9 3C

* Corresponding author at: 1815 N. University Street, NCAUR, USDA-ARS, Peoria, IL, United States.
E-mail address: Alejandro.Rooney@ars.usda.gov (A.P. Rooney).

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modified the ectotrophic growth of *F. graminearum* on wheat lemma tissues. In greenhouse experiments, live cells of *P. flavescens* tended to reduce FHB incidence, severity and the deoxynivalenol content of spring wheat grain compared to the control but treatment with live cells amended with dead cells increased all of these disease parameters compared to the live cells only treatment ($P \leq 0.05$, FPLSD). Results indicate that non-viable antagonist cells present in a biocontrol cell treatment can reduce disease control efficacy or even cause the biocontrol treatment to support a net adverse effect on plant health.

1. Introduction

The use of naturally occurring microorganisms to reduce disease on agricultural crops continues to gain acceptance (Jensen et al., 2016; Leggett et al., 2011; O'Brien, 2017; Pieterse et al., 2014). Increasing grower use of biocontrol products as alternatives or supplements to traditional pesticides is based, in part, on product performance, the ability to use such products after minimum preharvest intervals for chemical pesticides and the possibility of reducing residues in the agricultural commodities produced. However, wholesale adoption of biocontrol solutions to plant diseases is hampered by concerns regarding variability in the performance of biological control agents (Cunniffe and Gilligan, 2011; Mari et al., 2014). Variable or reduced performance of biocontrol products in field applications has been attributed to inadequate inoculant establishment or activity on host tissues that differ in metabolic state, chemistry, microclimate, microbial communities and nutrient availability compared to greenhouse grown plants (Guetsky et al., 2001; Marín et al., 2017).

However, narratives accounting for variable biocontrol product performance have largely failed to address the deleterious impact dead biomass of biocontrol agents could have on plant disease control. A mixture of non-viable and viable biomass is delivered to infection courts when a biocontrol product has lost significant viability since production or when a portion of the viable cells die from stresses encountered during product handling and application to plant surfaces. In the case of applying biocontrol cells to wheat heads, live cells are immediately subjected to rapid drying, ultraviolet light, and microbial competition which can significantly increase the levels of moribund biomass on wheat head tissues soon after the application. While examples of nutrients exuded from plants or that are experimentally applied reducing biocontrol efficacy are reported (Bautista-Rosales et al., 2013; Di Francesco et al., 2017; Janisiewicz and Korsten, 2002), studies of the impact of nutrients from moribund cells of a biocontrol agent on efficacy are lacking.

A variety of yeast antagonists have been used to reduce disease on field grown crops (Schisler et al., 2011). The dosages applied in field, greenhouse and post-harvest applications of yeasts for biocontrol of plant disease commonly range from 1×10^7 to 1×10^9 CFU/mL (Utkhede and Mathur, 2006; Zeng et al., 2015). In our laboratory, we have studied the yeast *Papiliotrema flavescens* (NRRL 50378, formerly *Cryptococcus flavescens*) strain OH 182.9 3C as a biocontrol agent against *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch), a causal agent of Fusarium head blight (FHB) of wheat. The progenitor strain of *Papiliotrema flavescens* OH182.9 3C was originally isolated from the anthers of flowering wheat heads (Khan et al., 2001). It can reduce FHB and the mycotoxin deoxynivalenol (DON) in greenhouse and field trials when applied at doses ranging from 5×10^7 to 5×10^8 CFU/mL (Khan et al., 2004; Schisler et al., 2014, 2015) but significant reductions are not always obtained with these doses. Viable counts of strain OH 182.9 can drop up to 1 log unit after inoculation of wheat heads and anthers in field studies but tend to recover after a few days (Schisler et al., 2014) and persist at reduced levels through the growing season (Rong et al., 2017).

We hypothesize that non-viable antagonist cells, when in sufficient quantity within a formulation or that result when viable cells die from stresses encountered during handling and after application, can provide a nutritive benefit to plant pathogen propagules and therefore reduce the efficacy of a biocontrol product. Our objectives for this study were to (1) quantify the growth of *Fusarium graminearum* in, and the composition of, a basal salts liquid medium containing dead cells of OH 182.9 3C as the sole source of nitrogen and carbon, (2) evaluate the influence of dead cells alone or in combination with live cells of biocontrol agent OH 182.9 3C on *F. graminearum* conidial germination and subsequent hyphal growth, and (3) determine the influence of these same treatments on the development of FHB disease on wheat in greenhouse bioassays.

2. Materials and methods

2.1. Biomass production by F. graminearum in liquid basal medium amended with non-viable cells of Papiliotrema flavescens

A minimal base medium lacking in carbohydrate and nitrogen was prepared that contained, per L, 4.0 g KH_2PO_4, 0.6 g $MgSO_4$, 0.8 g $CaCl_2$, 0.1 g $FeSO_4$, 0.028 g $ZnSO_4$, 0.031 g $MnSO_4$, 0.073 g $CoCl_2$, 0.5 mg each of vitamins thiamine, riboflavin, calcium pantothenate, niacin, pyridoxamine, and thioctic acid and 0.05 mg each of vitamins folic acid, biotin, and B_{12}. Four media types were then prepared from the base medium and contained 3×10^8, 6×10^8, 9×10^8 non-viable cells/mL or no non-viable cells of *P. flavescens*. Cells of *P. flavescens* were obtained from concentrated cell pastes that had lost viability after storage for more than 3 years at −20 °C. These cell pastes of *P. flavescens* were produced as described previously (Rong et al., 2017). Briefly, cells were cultured in a B Braun D-100 fermentor charged with 80 L of SDCL medium (Slininger et al., 2010). Biomass was harvested and then concentrated using a continuous flow tubular bowl centrifuge. The biomass was resuspended in sterile weak PO_4 buffer (pH 7.2, 0.004% wt/vol KH_2PO_4 buffer with 0.019% wt/vol $MgCl_2$) to approximately 2×10^9 CFU/mL, and confirmed similar in count using a hemacytometer, prior to freezing at −20 °C (Schisler et al., 2002). For the present test, all media and treatment ingredients were autoclaved, except vitamins, which were filter sterilized. Media were transferred aseptically at 50 mL per 500 mL baffled Erylenmeyer flask equipped with a metal closure. There were four replicates per treatment. A nutrient analysis of unamended and amended at 9×10^8 non-viable cells/mL media used to assay for growth of *F. graminearum* was conducted by the Cornell Nutrient Analysis Laboratory (Ithaca, New York, USA).

Conidia of *F. graminearum* Z-3639 for inoculating media were produced on clarified V-8 juice agar (CV8 agar) at 24 °C with 12 h/day fluorescent light for 8 days (Schisler et al., 2002). Five days after plates were initially inoculated, all fungal growth on each plate was rubbed multi-directionally using a sterile disposable loop to promote subsequent conidial formation. Colonized plates were flooded with weak PO_4 buffer to obtain conidial suspensions of the pathogen (Schisler et al., 2002) and used to inoculate treatment flasks to a concentration of 500 conidia/mL. Flasks were incubated at 250 rpm, 2.5 cm orbit and 25 °C for 6 days. Once daily, each flask was hand-shaken to release any mycelia growth that had built up on the inside wall of the flask.

At harvest, each flask of colonized broth was individually processed by pouring the content of a flask through a Cole Palmer (Vernon Hills, IL, USA) standard test sieve (No. 325, 45 μm) to collect mycelial biomass. Two liters of deionized water then was poured over the mycelial biomass collected in order to remove any adhering dead cells of *P. flavescens*. Fungal biomass was collected into a 1-L glass beaker by