Wireworm Control in Scallions: Attract-and-Kill Tactic Using *Metarhizium brunneum* Granules and Rolled Oats

Hazel Chan

Kwantlen Polytechnic University, Department of Sustainable Agriculture and Food Systems
December 11, 2018

Abstract

Wireworms damage has been an increasing issue over the years across many parts of the world. Options for wireworm control have been very few and limited to chemical insecticides. Many of those chemical controls are now deregistered from use due to their negative environmental impacts, and this has created a significant need for development of more sustainable alternatives. Research efforts on such alternatives have been growing but mainly targeted towards a narrow range of susceptible crops. This study aimed to expand the scope of the research by testing the effectiveness of an attract-and-kill tactic using *Metarhizium brunneum* LRC112 granules and rolled oats in reducing wireworm damage in scallions. Three band-applied treatments were tested: *Metarhizium brunneum* and rolled oats, rolled oats alone, and an untreated control. A significant treatment effect was detected on dry weight of scallions. Scallions in the *Metarhizium* treated plots tended to have fewer feeding holes, higher dry weights, and higher number of stems compared to the oats treated plots untreated control plots, suggesting that inter-row treatment application of *Metarhizium* and oats may help reduce wireworm damage in susceptible crops.

Keywords Wireworms · Elateridae · *Metarhizium brunneum* · Scallions · Attract-and-kill · Rolled oats · Carbon dioxide

1. Introduction

Wireworms are click beetle larvae belonging to the *Elateridae* family (Rogge et al., 2017) that reside in the soil and feed on plant roots or seeds upon hatching (Government of Alberta, 2014). Several pest species have greatly affected countries in the northern hemisphere (Rogge et al., 2017). Major pest species that have impacted Canada, Europe, and western Asia include *Agriotes obscures* and *Agriotes lineatus* (LaGasa et al., 2001). Wireworms are generalist feeders that attack a wide range of crops (Antwi et al., 2017), including potatoes, corn, lettuce, canola, sugar beets, and onions (Government of Alberta, 2014). Damage is often localized within a field and can result in plant stand losses up to 70% (Reddy et al., 2014). Typical signs of wireworm damage include hollowed out seedlings, shredded stems, wilting, discoloration, missing row sections (Vernon, 2010), and round feeding holes (University of Nebraska-Lincoln, 2018).

Management of wireworms has been challenging due to some of the characteristics of wireworms and the options available for control. Some problematic characteristics include the long life cycle and polyphagous, hardy nature of wireworms (Ericsson et al., 2007). Additionally, the ability of wireworms to respond quickly to abiotic changes to the soil environment has hindered the effectiveness of short-term controls that often target the upper part of the soil (Rogge et al., 2017). Available options for wireworm control have also historically been very few, and the most effective control of wireworms had been broad-spectrum insecticides, primarily organophosphates and carbamates (Reddy et al., 2014; Antwi et al., 2017; Furlan et al., 2010). However, these treatments are a concern due to their environmental impacts, and most have now been deregistered (Ericsson et al., 2007; Vernon et al., 2015). The current chemical controls such as pyrethroids, neonicotinoids and phenylpyrazoles differ greatly in their effects on wireworm populations and their persistence in the soil, some countries have not approved of their use in wireworm control (Brandl et al., 2016). Neonicotinoid seed treatments are currently the primary chemical controls for wireworms; however, their effectiveness is limited in that they are only capable of repelling the target wireworm population (Antwi et al., 2017).

The concern over the loss of effective wireworm controls and the negative environmental effects of the current options have raised awareness of the need for more environmentally sustainable and cost-effective alternatives (Furlan et al., 2010; Vernon et al., 2015), such as biopesticides (Antwi et al., 2017). *Metarhizium* is a fungal entomopathogen capable of infecting over 200 species of arthropods, whose biocontrol potential against insects has been explored since 1879 (Cornell University, n.d.). There has been growing research on the use of entomopathogens for controlling wireworms, including studies on the application of entomopathogens to spring wheat (Reddy et al., 2014), cover crop application of *Metarhizium brunneum* (Rogge et al., 2017), drench application of *Metarhizium ansiopliae* and spinosad (Ericsson et al., 2007), application of encapsulated baker's yeast and *M. brunneum* (Brandl et al., 2016), and an attract- and-kill strategy based on *M. brunneum* and pheromone granules (Kabaluk et al., 2015). Several studies make note of limitations to using *Metarhizium*, such as the possibility of temperature, soil moisture, and duration of exposure influencing the effectiveness of the fungus (Ericsson et al., 2007; Antwi et al., 2017). There is also room for more research on refinement of some of the treatments studied. Brandl et al. note that conidia suspensions of *Metarhizium* have limited control efficacy (2016). Rogge et al. note that drought stress may hinder viability and persistence of the conidia (2017).

As most of the studies (Antwi et al., 2017; Brandl et al., 2016; Ericsson et al., 2007; Morton & Pino, 2017; and Reddy et al., 2014) have focussed wireworm control research on potato and wheat crops, there is limited research available on wireworm control of other susceptible crops. Expanding the research to a greater diversity of crops may lead to a better understanding of the mechanism of wireworm migration, and the environmental effects that may affect migration, the extent of crop damage, and treatment efficacy. Based on the concept that wireworms are attracted to sources of carbon dioxide (Government of Alberta, 2014), such as decaying rolled oats, and based on lethality of *Metarhizium* to wireworms, placing rolled oats alongside *Metarhizium*-coated granules can serve as an attract-and-kill tactic to control wireworms (Kabaluk, 2018).

This study tested the central hypothesis that an attract-and-kill tactic using *Metarhizium brunneum* (strain LRC112) granules and rolled oats will reduce wireworm damage in scallions. The objectives of the study were to increase options for wireworm control that have lower environmental impacts, to promote research on organic methods of wireworm control over a wider range of susceptible crops, and to test the effectiveness of an attract-and-kill tactic using *Metarhizium* granules and rolled oats in reducing wireworm damage in scallions.

2. Materials and methods

2.1 Experimental Design

The experiment was conducted at the Kwantlen Polytechnic University Orchard, an 8-acre certified organic farm located along the South Arm of the Fraser River in Richmond, BC. The experiment took place from May to July over a 35 m x 60 cm study area in the form of a randomized complete block design with six replicates and three treatments, for a total of 18 experimental plots. The three treatments used were *Metarhizium* granules and rolled oats, rolled oats alone, and an untreated control. Allocation of treatments were randomized using the Agricolae package in the R statistical software.

Each experimental plot was 100 cm long x 60 cm wide, measured using a reel measuring tape and marked using surveyor flags on May 31st. Each plot contained 3 rows of scallions. Rows were spaced 20 cm apart, and each contained 10 scallions planted 7 cm apart. Each plot received 4 inter-row band applications of assigned treatment. Plots were separated by a 1 m buffer zone.

2.2 Treatment preparation and application

Metarhizium brunneum strain LRC112 was sourced from wireworm cadaver near Agassiz, BC, and mass produced by Agriculture and Agri-Food Canada. Treatments were measured in the laboratory using the ScoutPro Balance on May 30th. 1.11 g portions of *M. brunneum* strain LRC112 granules were weighed into 24 solar cups for a total of 26.6 g. 10.0 g portions of No name® rolled oats were weighted into 48 solar cups for a total of 48.0g.

Treatments were band applied on May 31st. A 1 m long landscape rake was used to create four 1.5" deep trenches, spaced 20 cm apart, at each treatment plot. The respective treatments were added into the trenches: 1.1 g of *M. brunneum* (LRC112) and 10.0 g of rolled oats were added to each trench at the *Metarhizium and* oats treatment plots, 1.1 g of *M. brunneum* (LRC112) was added to each trench at the *Metarhizium* treatment plots, and no treatment was added to the untreated control plots. After all the treatments were applied, the rake was used to refill the trenches with soil.

2.3 Scallions

On April 30th, Pacific-22 scallion seeds were seeded 1" deep into the soil into two 128-cell transplant trays at 4-5 scallion seeds per cell for a total of 236 cells. The seeded trays were watered and placed under grow lights. Scallion transplants were exposed to 12 hours of light each day for 3 weeks. On May 23rd, transplants were placed in the cold frame and hardened off. Compost tilled into the study area on May 30th. On June 14th, scallion transplants were planted at each experimental plot. Plants received water from a drip-irrigation system running along the length of the plots. Plant growth was monitored and plots were manually weeded each week.

2.4. Data collection

2.4.1 Wireworm sampling

Two soil core samples were taken at the 2 middle treatment bands at each plot and placed in plastic bags labelled with the treatment and replicate. The soil was spread over a flat surface and large soil masses were broken down by hand and examined for wireworms. The number of dead and live wireworms were counted for each sample, placed in solar cups labelled with the treatment and replicate, and placed in the freezer.

2.4.2 Scallion health and damage assessment

On July 25-26, the number of scallion stems were counted for each plant, and the length of the scallion stems for each plant were measured from the soil level to tip of the stem to the nearest 0.5 cm. The number and length of stems for any missing scallion plants were recorded as 0 stems and 0 cm. On July 30th, scallions were hand harvested from all plots and both dead and live wireworms that were present around the plant were collected. The scallions were placed in clear plastic bags labelled with the replicate and treatment. The wireworms collected were placed into solar cups labelled with the replicate and treatment. On July 31st, the number of feeding holes along the base of the each scallion plant were counted. Scallion fresh weights were measured using the ScoutPro balance on July 31st. Scallions were oven dried at

55°C for 24 hours on August 1st. Dry weights of the oven-dried scallions were measured with the ScoutPro balance on August 2nd.

2.5 Statistical Analysis

Data were analyzed with the Analysis of Variance (ANOVA) test (α = 0.05) and Tukey's Honestly Significant Differences (Tukey HSD) Test (α = 0.05), using the R statistical software.

3. Results

3.1 Wireworm sampling

Treatment	Dead wireworms	Live wireworms
Metarhizium+ Rolled Oats	1	1
Rolled Oats	1	3
Untreated Control	1	0

Table 1. Total dead and live wireworm counts for soil core samples of the *Metarhizium* and rolled oats, rolled oats, and untreated control plots.

3.2 Scallion health and damage assessment

Treatment	Dead wireworms	Live wireworms
Metarhizium+ Rolled Oats	1	5
Rolled Oats	0	3
Untreated Control	0	2

Table 2. Total dead and live wireworm counts at harvest for the *Metarhizium* and rolled oats, rolled oats, and untreated control plots.

	***Metarhizium* and rolled oats**	**Rolled oats**	**Untreated control**
Replicate 1	8	16	12
Replicate 2	7	5	7
Replicate 3	4	3	15
Replicate 4	3	2	9
Replicate 5	4	7	7
Replicate 6	3	2	1
Total	29	35	51

Table 3. Number of missing scallion plants for the *Metarhizium* and rolled oats, rolled oats, and untreated control plots.

3.3 Statistical Analysis

ANOVA and Tukey's HSD test of scallion dry weight indicate that treatment had a significant effect on dry weight of scallions. Scallion dry weight was significantly higher in the *Metarhizium* treated plots than in the control plots (p=0.011) (Fig. 1). The effect of treatment approached statistical significance for the number of holes per scallion stem (p=0.0604) (Fig. 4), and for the dry weight per scallion stem (p=0.068) (Fig. 3): Scallions in the *Metarhizium* treated plots tended to have a higher number of stems (Fig. 2), higher dry weight (Fig. 3), and fewer feeding holes (Fig. 4) than scallions in the untreated control or oats treated plots, although these effects were marginally significant (p<0.1). Analysis of Variance of scallion dry weights and dry weight per scallion stem generated p-values of 0.0011 and 0.0033 for replicate.

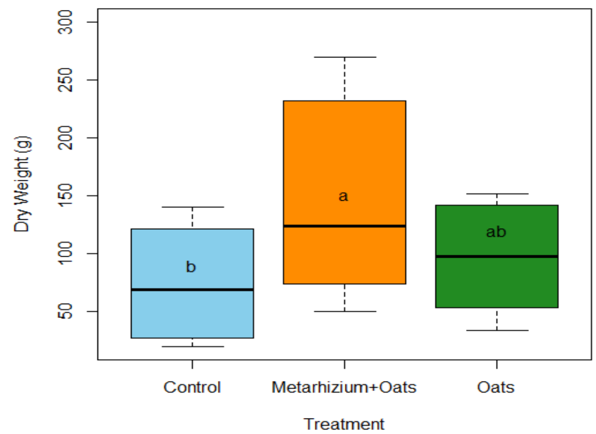

Fig 1. Dry weight of scallions harvested from untreated plots (Control), and plots treated with *Metarhizium* and rolled oats (Metarhizium+Oats), or rolled oats alone (Oats). Treatments labeled with the same letter do not differ significantly (Tukey's HSD test, α = 0.05).

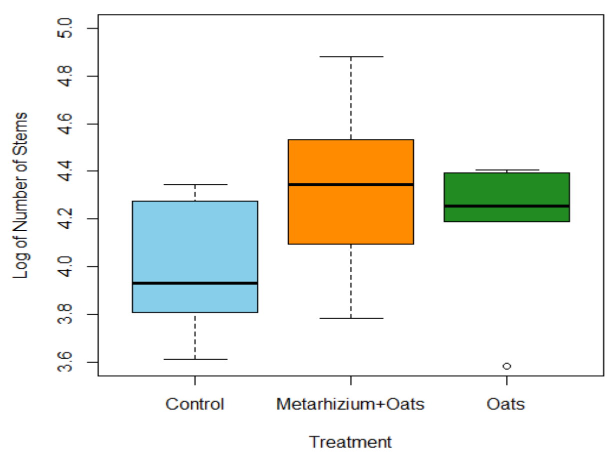

Fig 2. Number of stems for scallions harvested from from untreated plots (Control), and plots treated with Metarhizium and rolled oats (Metarhizium+Oats), or rolled oats alone (Oats). Data were log-transformed for normalization. No significant difference was found between treatments.

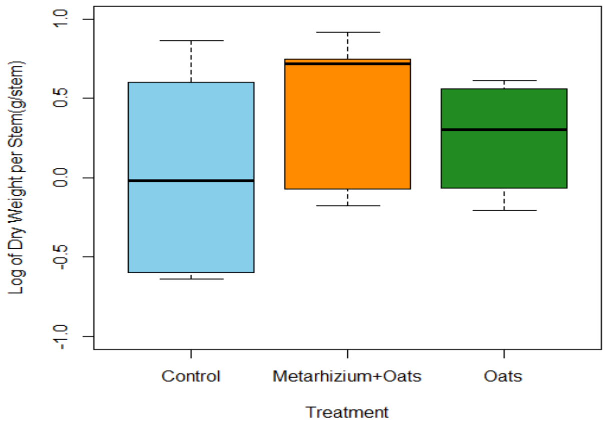

Fig 3. Dry weights of scallions per stem of scallions harvested from untreated plots (Control), and plots treated with Metarhizium and rolled oats (Metarhizium+Oats), or rolled oats alone (Oats). Data were log-transformed for normalization. No significant difference was found between treatments.

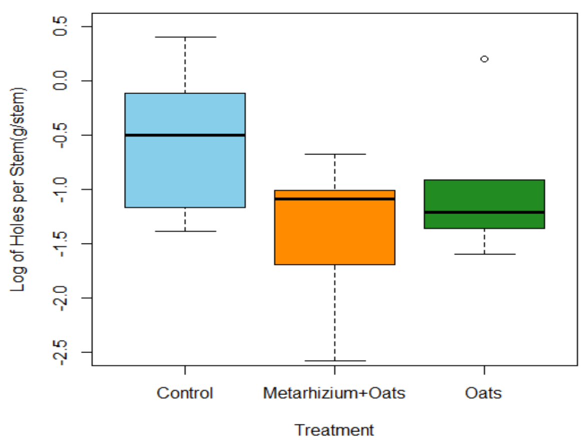

Fig 4. Number of feeding holes per scallion stem for scallions harvested from untreated plots (Control), and plots treated with *Metarhizium* and rolled oats (Metarhizium+Oats), or rolled oats alone (Oats). Data were log-transformed for normalization. No significant difference was found between treatments

Discussion

The untreated control plots had the highest number of missing scallions compared to the oats treated plots and *Metarhizium* treated plots (Table 3). This would lower the values for dry weight, feeding hole counts, and stem counts for the untreated control plots compared to the *Metarhizium* treated plots. Consequently, the significant difference in the dry weights between the *Metarhizium* treated plots and untreated control plots (Fig. 1) may have resulted from missing scallion plants rather than from decreased wireworm damage to the plants.

The lack of scallion growth in some of the plots may be indicative of a varietal effect, as scallions of a different variety planted at an adjacent field were nearly all significantly larger in size and experienced faster rate of growth. Additionally, the loss of opportunity to uptake nutrients during the 3 week time gap when compost was spread to the soil and when the scallions were transplanted into the soil may have contributed to the slower and weaker growth in the scallion plants observed. This combined with wireworms present throughout the soil (Table 1 and Table 2) may significantly weaken the scallion plants and increase susceptibility to damage and disease. Most plots had scallions that had dried out stems and browned tips, which may also be a response to heat damage from the sun. The scallions in the nine experimental plots towards the north end of the study were also severely infected with downy mildew.

The overall higher mean number of stems, dry weight, dry weight per stem and feeding holes per stem in the *Metarhizium* treated plots compared to the oats treated plots suggest that the scallions exposed to the *Metarhizium* treatment had healthier growth and a higher yield, but also greater amount of feeding holes at the base of the plant. Weather variances may have also affected the amount of feeding damage. Warm, dry soil conditions particularly near during the heat wave near the end of the study, may have caused wireworms to migrate deeper into the soil (Queen's Printer For Ontario, 2016). At times of cooler temperatures and drier conditions, there may have been more restricted movement of wireworms towards the soil surface (Campbell, 1937; Milosavljevic et al., 2016; Antwi et al., 2017).

In two of the *Metarhizium* treated plots, the scallions were visibly much taller and the plots had much fewer missing scallion plants compared to the other experimental plots. Having a higher amount of scallion plants would also generate more CO_2 at the plot and potentially attract more wireworms towards the plot. Overall, the *Metarhizium* treated plots had a higher number of wireworms compared to the oats treated plots or untreated control (Table 2 and Table 3). Additionally, only one of the wireworms collected was infected by *Metarhizium* and was not found until harvest at the *Metarhizium* treated plot (Table 2). It was also found alongside 3 live wireworms It is possible that the wireworm species composition in the study area was not as susceptible to *Metarhizium brunneum LRC112* compared to other strains of *Metarhizium*, as fungal pathogenicity may differ for different host species, or a longer time may be needed for the fungal infection to kill the host (Reddy et al., 2014).

Live wireworms were also found in the oats treated plot and untreated control plot at harvest, both of which were adjacent to the *Metarhizium* treated plot (Table 2). CO_2 released from the oats in the *Metarhizium* treated plots along with the CO_2 released from the oats in the adjacent oat treated plots may have increased the migration of wireworms both between the plots and from the guard rows towards the treatment plots, resulting in a higher number of live wireworms and lower infection efficacy. A similar migration effect was also noted in an attract-and-kill study by Brandl et al. (2016). The same study found that the use of *Metarhizium* alone reduced potato tuber damage more significantly compared to an attract-and-kill co-application of *Metarhizium* and bakers yeast and co-application did not increase infection (Brandl et al., 2016), which may suggest that *Metarhizium* treatment plots treated with *Metarhizium* alone rather than a co-application of *Metarhizium* and oats, would help result in a greater reduction in wireworm damage. Yet, there are limitations to the use of *Metarhizium* for wireworm control, as Ericsson et al., 2007 found that applying *Metarhizium* alone was insufficient for effective control at higher levels of wireworm damage. The effects of the treatments on post-experiment wireworm larvae populations remain to be explored (Reddy et al., 2014).

Future studies may include post-experiment wireworm sampling or explore the extent of damage to subsequent crops planted in the area. Future studies may also further explore the effect of timing and method of planting the host crop, and timing of treatment application on treatment efficacy.

Since the p-values for replicate was smaller than 0.05 for scallion dry weights ($p=0.0011$) and dry weight per scallion stem ($p=0.0033$), there is strong evidence to conclude that replicate has a significant effect on dry weight of scallion plants and dry weight per scallion stem, and that blocking was justified for the experiment.

Conclusion

The numerical trends in the overall data also suggest that '*Metarhizium*+oats' may have had some effect in reducing wireworm damage on scallions, and may have a role in wireworm control for a wider range of susceptible crops. There is room for more research on experimenting different ways and different crops for which *Metarhizium* can be used in wireworm control.

Acknowledgements Mike Bomford provided valuable support, guidance and assistance throughout the study. Todd Kabaluk provided valuable support, guidance, and the needed *Metarhizium* and experiment supplies. Torin Boyle and James Reinert assisted with seeding, spreading compost, and setting up irrigation. Lindsay Dodds procured the *Metarhizium* and experiment supplies.

References

Antwi, F. B., G. Shrestha, G. V. Reddy, and S. T. Jaronski. 2017. Entomopathogens in conjunction with imidacloprid could be used to manage wireworms (Coleoptera: Elateridae) on spring wheat. Canadian Entomologist 150: [doi: 10.4039/tce.2017.58].

Brandl M. A., M. Schumann, M. Przyklenk, A. Patel, and S. Vidal. 2016. Wireworm damage reduction in potatoes with an attract-and-kill strategy using Metarhizium brunneum. Journal of Pest Science 90: [doi: 10.1007/s10340-016-0824-x].

Campbell, R.E. 1937. Temperature and moisture preferences of wireworms. Ecology 18: 479–489.

Cornell University. n.d. *Metarhizium.* College of Agriculture and Life Sciences. https://biocontrol.entomology.cornell.edu/pathogens/Metarhizium.php

Ericsson, J. D., J. T. Kabaluk, M. S. Goettel, and J. H. Myers. 2007. Spinosad interacts synergistically with the insect pathogen Metarhizium anisopliae against the exotic wireworms Agriotes lineatus and Agriotes obscurus (Coleoptera: Elateridae). Journal of Economic Entomology 100:31-38.

Furlan, L., C. Bonetto, A. Finotto, L. Lazzeri, L. Malaguti, G. Patalano, and W. Parker. 2010. The efficacy of biofumigant meals and plants to control wireworm populations. Industrial Crops & Products 31: 245-254.

Government of Alberta. 2014. Wireworm. Alberta Ag-Info Centre. https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex14740

Kabaluk, J. T., J. P. Lafontaine, and J. H. Borden. 2015. An attract and kill tactic for click beetles based on Metarhizium brunneum and a new formulation of sex pheromone. Journal of Pest Science 88:707-716.

Kabaluk, T. 2018. Biological Control of Wireworms. PowerPoint.

LaGasa, E., Vernon, B., Wraspir, J., Hertzog, P., and Kamping, H. 2001. 2000 Western Washington Exotic Wireworm Survey, a Preliminary Detection and Delimiting Survey for Agriotes obscures and A. lineatus (Coleoptera: Elateridae). Washington State Department of Agriculture. https://agr.wa.gov/PlantsInsects/insectpests/Exotics/Surveys/exotic_wireworm2000.pdf

Milosavljevic, I., Esser, A.D., and D.W. Crowder. 2016. Seasonal population dynamics of wireworms in wheat crops in the Pacific Northwestern United States. Journal of Pest Science 90: 77–86.

Morton, A., and F. Garcia-del-Pino. 2017. Laboratory and field evaluation of entomopathogenic nematodes for control of Agriotes obscurus (L.) (Coleoptera: Elateridae). Journal of Applied Entomology 141: [doi: 10.1111/jen.12343].

Queen's Printer For Ontario. 2016. Managing Wireworms in Vegetable Crops. Ministry of Agriculture, Food and Rural Affairs. http://www.omafra.gov.on.ca/english/crops/facts/00-047.htm

Reddy, G. V., K. Tangtrakulwanich, S. Wu, J. H. Miller, V. L. Ophus, J. Prewett, and S. T. Jaronski. 2014. Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. Journal of Invertebrate Pathology 120:43-49.

Rogge, S. A., J. Mayerhofer, J. Enkerli, S. Bacher, and G. Grabenweger. 2017. Preventive application of an entomopathogenic fungus in cover crops for wireworm control. Biocontrol 62:613-623.

Vernon, B. 2010. Wireworm Field Guide- A guide to the identification and control of wireworms. Syngenta Crop Protection Canada, Inc. http://www.syngentacropprotection.com/assets/assetlibrary/wireworm_booklet_2010_web.pdf

Vernon, R. S., W. G. van Herk, M. Clodius, and J. Tolman. 2015. Companion planting attract-and-kill method for wireworm management in potatoes. Journal of Pest Science 89:375-389.