The effect of cut seed tubers and *Metarhizium brunneum* on wireworm damage in potato

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Objective

If whole or cut seed potatoes, with or without *M. brunneum*, to determine if the attract-and-kill strategy would protect an organic potato crop from wireworm damage without reducing yield.

Introduction

Wireworms are polyphagous soil-dwelling larvae of click beetles (Coleoptera: Elateridae) that can cause significant damage to agricultural crops including cereals, legumes and potato (Eckard, 2014). Their lifecycle varies according to species, geography, and weather; however, they can transition through as many as 13 larval instars over 2-5 years terminating as short-lived adults (Kabaluk, 2014). Eggs are generally laid singly, and are widely scattered, however, wireworm populations are often confined to certain areas of the field (Reddy, 2014). These adult click beetles typically emerge from March to June, to feed and reproduce (Kabaluk, 2014). Thirty-nine species are known to attack potatoes, the most common wireworm pest species, however, include *Agriotes, Conodenis, Ctenicera, Liminius, and Melanotus* (Brandl, 2017,Wraight *et al*, 2009). Species of *Agriotes* are abundant and considered a serious pest indigenous to Europe, and accidentally introduced to North America (Kabaluk, 2014). Potato tubers are vulnerable to cosmetic damage from wireworms (Wraight *et al*, 2009). Tubers with as little as two holes can be deemed unmarketable for table grade potatoes (Wraight *et al*, 2009). The most crop damage occurs during the larval stage of the click beetle, due to feeding damage to the sprouts emerging from the seed tuber, affecting stem growth and new tuber production, especially when considering the length of time the larvae inhabit the soil (Wraight *et al*, 2009). Average yield loss of potatoes can range from 5-25% in the US and the UK, even when wireworm specific insecticides are applied (Eckard, 2014). This is concerning when taking into account that wireworm damage assessed to be greater than 10-15% is often deemed to be uneconomical to sort and therefore entirely rejected (Ansari, 2008).

Potato damage due to wireworm pests is increasing. Wraight *et al*, attribute this to increasing demand for unblemished tubers, and an increase in the planting of potatoes in areas that were previously planted with perennial grasses (2009). In addition, many organochlorine and organophosphate soil insecticides that target wireworms with reliable efficacy have been withdrawn due to concerns about human health and adverse effects on the environment (Eckard, 2014). This has led to pesticides targeting wireworms being widely unavailable in Canada, due to their low market potential, environmental concerns, and high-risk assessments (Kabaluk and Ericsson, 2014). Preventative control of wireworms is possible using integrated pest managements strategies including adjusted crop rotations, repeated tillage, or the use of chemical pesticides (Eckard, 2014). The success of these strategies, however, varies greatly (Eckard, 2014).

The exploration of new reduced risk insecticides, such as naturally occurring entomopathogenic fungi (EPF) are considered as promising biocontrol agents in augmentative biocontrol strategies (Eckard, 2014). This includes microbial insecticides for wireworm management, such as *Metarhizium* (Wraight *et al*, 2009). Wireworms are natural hosts of EPF's (Eckard, 2014). It has been suggested that wireworm problems in agriculture are due to a deficiency of *M. brunneum* in the soil (Wraight *et al*, 2009).

Metarhizium fungi occurs naturally in the soil, and has already been tested with varying success (Eckard, 2014). It has been found to contribute to the natural control of a wide range of insects (Eckard, 2014). Based on the findings of Bischoff *et al*, (2009) the former type species *Metarhizium anisopilae* (Metschinikoff) Sorokin which is widely known and used as a biocontrol agent (BCA) has been divided into four subspecies, these include *Metarhizium pingshaense, Metarhizium anisopliae, Metarhizium robertsii, and Metarhizium brunneum* (Eckard, 2014). The *M. brunneum* isolate in particular, has been found to infect wireworms under field conditions in Canada (Kabaluk and Ericsson, 2014). An isolate of *M.brunneum,* LRC112, obtained from an infected wireworm cadaver near Agassiz, B.C. has been mass-produced and used in field and laboratory trials (Kabaluk, 2014). Some strains of *Metarhizium spp.* are already in commercial use, such as BIPESO 5, a BCA against black vine weevils (Eckard, 2014). Fungi can infect the host without being ingested (Reddy, 2014). For infection to occur, however, the EPF needs to germinate and penetrate the cuticle wall of the host (Kabaluk and Ericsson, 2014).

Efficacy of BCAs is influenced by pest behavior and by environmental factors such as temperature, soil moisture, and nutrient availability (Kabaluk and Ericsson, 2014). Extreme high and low temperatures have been shown to reduce the success of *M. brunneum* and other EPF as a pest control product for wireworms (Kabaluk and Ericsson, 2014). The best results can be expected during periods when soil temperature is conductive to infection causing substantial mycosis, which can be seen at least 18^{O}C (Kabaluk, and Ericsson, 2014). For south coastal British Columbia, optimal infection temperature begins in June for deep seed crops such as potato (Kabaluk, and Ericsson, 2014). *Metarhizium* brunneum has been known to cause 90-100% mortality of *A.*

lineatus, three weeks after inoculation (Ansari, 2008). Although insects can detect and avoid *M. anisopliae,* attractive food sources may offset the repellent effect sufficiently. Carbon dioxide is known to attract many soil dwelling pests (Kabaluk and Ericsson, 2014, Vemmer *et al*, 2016). The mechanisms that enable this recognition are still not well understood (Kabaluk and Ericsson, 2014). Reduced feeding due to infection with EPF has been reported in several insects (Reddy, 2014).

Applications of an artificial carbon dioxide source was found to reduce wireworm tuber damage by 37-75%, thereby enhancing the efficacy of the application of *M.brunneum* by up to 35% through an attract-and-kill method (Brandl, 2017). An attract-and-kill method using wheat as a carbon dioxide source was also found to increase the efficacy of chemical pesticides against wireworm in potatoes (Brandl, 2017). Seed potatoes also emit carbon dioxide in the soil, and cut seed potatoes have been found to produce significantly more carbon dioxide in the soil, compared to whole seed potatoes (Kabaluk, 2016).

Another aspect to consider is the lack of research in targeting adult click beetles, a strategy that could reduce mating, oviposition, and larvae in the soil (Kabaluk, 2014). No pesticides are currently registered for this purpose, and while many species-specific pheromones for click beetles have been identified, and are synthesized (Kabaluk, 2014). These synthetic pheromones are only being used, however, for monitoring to predict species composition, predicting larval populations, and mass trapping (Kabaluk, 2014).

In this study, the effects of whole or cut seed potatoes were tested, with and without the presence *M. brunneum,* to determine if the amount of carbon dioxide emitted from the seed potatoes can protect the crop from wireworm damage through the attract-and-kill method, without a significant reduction in yield.

Methods

This study was conducted at “The KPU Orchard,” in Richmond, B.C., Canada, in partnership with Kwantlen Polytechnic University, and Agriculture and Agri-Food Canada. Whole and cut seed tubers were tested to see if the cut seed tubers better protect the potato crop from wireworm damage.

Adult click beetles were monitored before the seed tubers are planted. This was accomplished with pitfall and funnel traps with a source of carbon dioxide and pheromones to attract the click beetles. The monitoring was used to determine which species of click beetles are present on the site, as well as abundance to estimate larval populations. Species present in this location were determined to be *Agriotes obscurus,* and *Agriotes lineatus* (Kabaluk, Hume and van Herk, 2018).

Seed tubers were in half, and left for several days to suberize, in order to reduce the likelihood of disease for the tubers once they are planted. Potatoes were planted on June 20, 2018. The field was tilled, and the furrows were made using a BCS before planting. Any sprouts were removed, immediately before planting, from both the cut and whole seed potatoes to ensure all potatoes were at the same stage of growth at the time of planting. There were four treatments total, they include: whole seed tubers with and without *Metarhizium brunneum* and cut seed tubers with and without *Metarhizium brunneum*. The *Metarhizium brunneum* was provided by Agriculture and Agri-Food Canada, it was mass produced with rice, dried, and ground into a powder.

The quantity of *M. brunneum* per potato used was determined using Todd Kabaluk's ideal concentration of 10^{14} spores per hectare. The *M. brunneum* obtained from Kabaluk had a viability of 92%, and 7.24% moisture content, and 1.96×10^9 spores per gram. 1.96×10^9 spores per gram multiplied by 0.92 viability equals 1.803×10^9 viable spores per gram. Grams per plot was calculated by dividing spores per hectare by viable spores per gram for a total of 25.0g per plot. This was divided by the number of potatoes per plot for a total of 1.19g of *M. brunneum* per potato.

The *M.* brunneum was pre-weighed into plastic portion cups, one cup per potato, and remained in a styrofoam cooler until used to protect it from the high environmental temperatures. It was directly applied in the soil before the seed tuber at planting. The *M. brunneum* was lightly covered with soil before the seed tuber was placed on top, half seed pieces were planted cut side down. The treatments were replicated six times, for a total of twenty-four plots. Each plot will be 4 m wide and 2.25 m long. Each seed tuber was planted 30 cm a part, 21 seed potatoes per plot total (Stewart, 2017). There was 1.25 m between each row, and 1 m between each plot. An additional 1 m potato buffer was planted surrounding the edges experiment to help ensure consistency. Drip irrigation was laid down in each row before hilling. The furrows were hilled using a BCS, immediately after planting, and again 42 days after planting. Plots were weeded as necessary throughout the duration of the trial. All tops were removed with a weed whacker 10 days before harvesting.

The potatoes were harvested using a tractor mounted potato harvester. The weight of each plot, number of potatoes, and number of wireworm feeding holes per potato was assessed for each plot.

Results

An analysis of variance (ANOVA) was calculated using R statistical software. Whole seed pieces were determined to produce a higher yield than half seed pieces (p=0.01). There was no significant influence detected of the biocontrol on yield (Figure 1). An interaction was observed between seed and biocontrol effects on wireworm damage (p=0.03) (Figure 2). *M. brunneum* application reduced the number of holes per tuber in potatoes grown from half seed pieces, but not in potatoes grown from whole seed pieces (Figure 2).

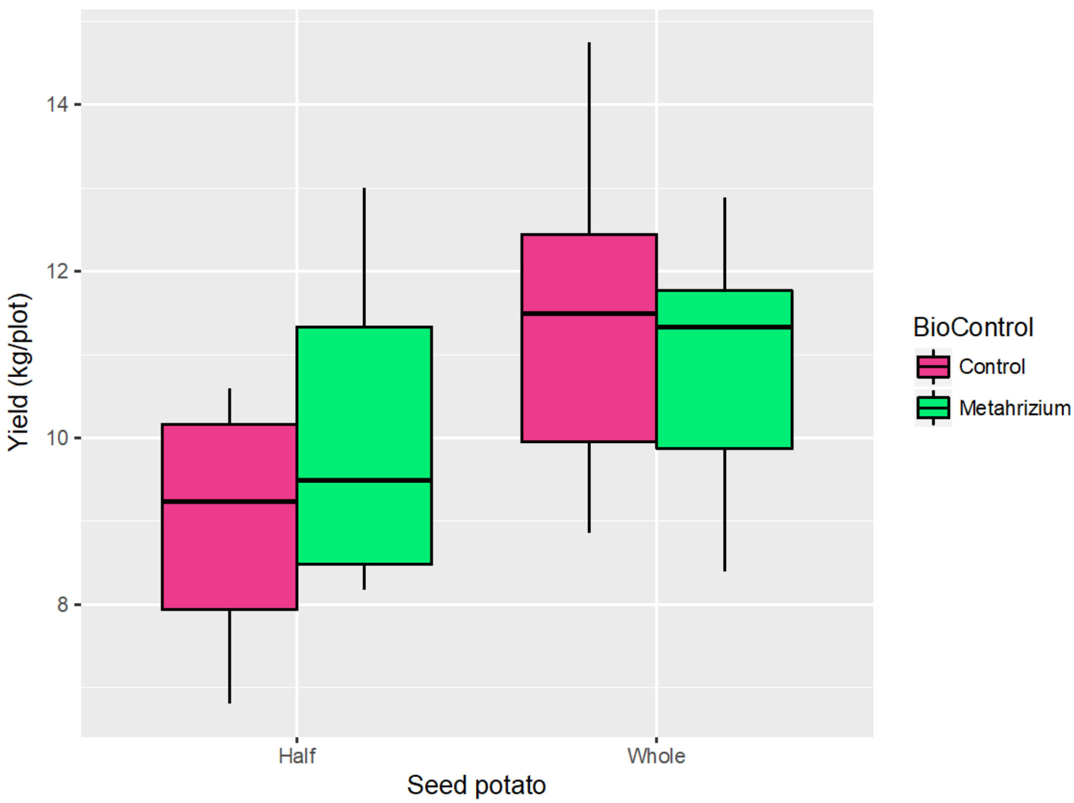

Figure 1: Yield of potatoes according to seed potato type and biocontrol.

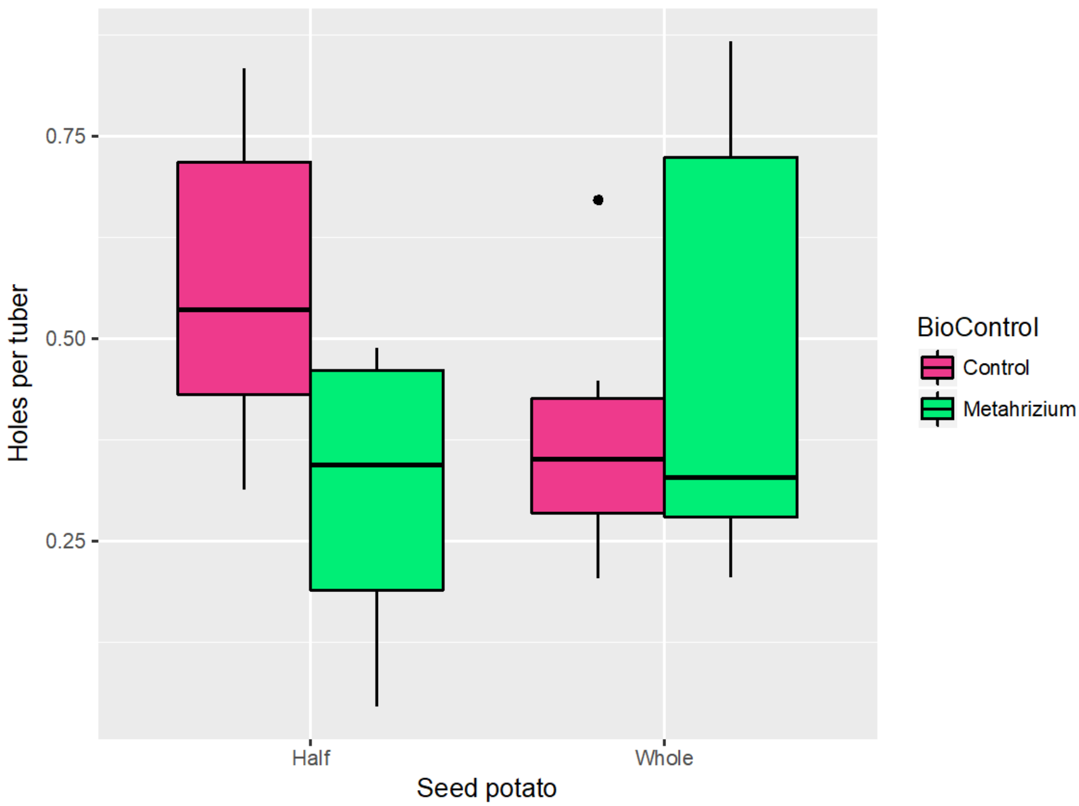

Figure 2: Wireworm damage according to seed potato type and biocontrol.

Conclusion:

Potatoes grown from whole seed pieces yielded more, and had no more damage than potatoes grown from half seed pieces with *M. brunneum*. It was determined that *M. brunneum* reduced wireworm damage to potatoes grown from half seed pieces. This observation is consistent with the hypothesis that cut seed pieces attract more wireworm larvae that can be killed by *M. brunneum*. This suggests that it is more beneficial to apply *M. brunneum* to the soil before planting potatoes using half seed pieces, than if you were to use whole seed pieces. Although it was determined that your crop would yield more with whole seed pieces, it is possible that it could be more economical to use half seed pieces with the application of *M. brunneum* as the cost of seed potatoes is high.

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