Onion thrips Thrips tabaci (Thysanoptera: Thripidae) reduces yields in indoor grown cannabis

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ABSTRACT

Cannabis (Cannabis sativa L. (Rosales: Cannabaceae)) is a newly legalized crop and requires deeper insights on its pest communities. Here we identified the thrips species affecting indoor grown cannabis in Canada and tested its impact on plant yield. We used three levels of initial infestation (0, 1, and 5 thrips) on individual plants grown in two mediums: normal substrate or substrate containing the biostimulant Bacillus pumilus, Meyer and Gottheil (Bacillales: Bacillaceae). We found that the onion thrips, Thrips tabaci (Lindeman) (Thysanoptera: Thripidae) is proliferating in indoor grown cannabis. Furthermore, our results showed fresh yields were higher for the plants that initially received zero thrips compared to

those who initially received five thrips. Moreover, the biostimulant had only a marginal effect on the reduction of the thrips' impacts. We highlight the importance for growers to carefully monitor thrips infestations in indoor grown cannabis. Finally, we emphasize the need for more research regarding the impact of pests on cannabis yields and safe means of pest control for this strictly regulated crop.

INTRODUCTION

Cannabis (Cannabis sativa L. (Rosales: Cannabaceae)) has been legalized for recreational purposes in October 2018 in Canada and is still under strict prohibition in most of the world. Thus, there is a severe lack of information regarding its growing practices (Eaves, Eaves, Morphy, & Murray, 2020; Wilson et al., 2019), including regarding the impact of pest species and the means of controlling them (Cranshaw et al., 2019). Under the Cannabis Act and the Cannabis Regulations, Health Canada only allows growers to use a limited number of pesticide products. Consequently, companies rely mostly on biological control, but these techniques are very costly and yield uneven results.

More than 300 pest species have been identified on hemp and marijuana (Cranshaw et al., 2019; McPartland, 1996). On marijuana, the most predominant ones are sap-sucking arthropods such as aphids, whiteflies, leafhoppers, mealybugs and various mites (Lago & Stanford, 1989; McPartland, 1996; Wilson et al., 2019). Recent reports of potential pests in cannabis include the marmorated stink bug (Halyomorpha halys) (Britt, Pagani, & Kuhar, 2019) and aphids (Lagos-Kutz, Potter, DiFonzo, Russell, & Hartman, 2018). Despite this, it is believed that very few insects can actually cause important losses in commercial cannabis production (Dewey, 1913; McPartland, 1996). In a recent survey, growers from California reported from zero to over 25% crop damage caused by arthropods (Wilson et al., 2019). Nonetheless, the vast majority of

cannabis production occurs indoors or in greenhouses, which provide environments that are particularly favourable for pests. Since most published studies have focused on outdoor production, our current estimates of pest-risk posed to cannabis producers may greatly underestimate the actual risk.

Though thrips have been shown to be a major pest for many crops, most notably in greenhouses (Stuart, Gao, & Lei, 2011) and are commonly found in indoor cannabis facilities, we are not aware of any studies investigating their impact on cannabis yields. Nevertheless, Cranshaw et al. (2019) reports that thrips are common pests in hemp farms. For instance, Onion thrips, Thrips tabaci (Lindeman) have been frequently found on hemp in Colorado and can cause important foliage damages on indoor grown plants (Cranshaw et al., 2019). Western flower thrips, Frankliniella occidentalis (Pergande), tobacco thrips, Frankliniella fusca (Hinds) and greenhouse thrips, Heliothrips haemorrhoidalis (Bouché) have also been found in hemp farms (Cranshaw et al., 2019; Lago & Stanford, 1989; McPartland, 1996).

Biostimulants are biological products that improve the productivity of plants. These products are often a mixture of compounds derived from various organisms, such as bacteria, fungi, algae, higher plants or animals, and frequently possess unexplained modes of action (Calvo, Nelson, & Kloepper, 2014; Conant, Walsh, Walsh, Bell, & Wallenstein, 2017; Yakhin, Lubyanov, Yakhin, & Brown, 2017). Specifically, the bacterium Bacillus pumilus, Meyer and Gottheil, (Bacillales: Bacillaceae) is known for its growth promoting properties (de-Bashan, Hernandez, Bashan, & Maier, 2010; Gutiérrez□Mañero et al., 2001; Probanza, Lucas, Acero, & Gutierrez Mañero, 1996). Furthermore, B. pumilus, which is already known for its antifungal properties (Pérez-García, Romero, & de Vicente, 2011), recently showed good potential for insect control. It notably showed suppression against larvae of Scirpophaga incertulas and

Bruchus dentipes in laboratory conditions (Rishad, Rebello, Shabanamol, & Jisha, 2017; Tozlu, Dadasoglu, Kotan, & Tozlu, 2011), results that highlighted its high production level of chitinase. This enzyme can degrade the chitin containing cell walls of insects and thus induce death (Rishad et al., 2017). Chitinase previously showed insecticidal properties against weevils (Laribi-Habchi, 2014) and aphids (Kim & Je, 2010). Growing medium enhanced with entomopathogenic bacteria represents a promising avenue toward pest control and reduced use of pesticides. When used as a growing medium, B. pumilus reduces the infestation level of fungus gnats (Dipetera) in greenhouses, but shows inconclusive results for the western flower thrips (Gravel & Naasz, 2019).

Hence, the objectives of this preliminary study were to identify the thrips species affecting indoor cannabis production in Canada, to determine the potential yield and quality losses associated with their infestations, and, finally, to evaluate the impact of adding the biostimulant B. pumilus to the growing substrate on the impacts of thrips.

MATERIALS AND METHODS

Experiment

The experiment was conducted in the autumn of 2019 in the commercial cannabis production facility of GreenSeal Cannabis Company located in Stratford, Ontario, Canada. We used 60 clones of cannabis (C. sativa var. Green Crack). All clones were planted in seven inches square pots (1.1 gallons) using two types of substrate. The first ("normal substrate") was a fibrous, peat-moss substrate with perlite (Pro-Mix HP Mycorrhizae, Premier Tech). The second ("biostimulant substrate") was the normal substrate with the addition of the biostimulant Bacillus pumilus (strain GHA180) (Pro-Mix HP Biostimulant + Mycorrhizae, Premier Tech). Plants were

checked and rinsed with water to remove any potential predatory mite or thrips. The plants were grown in the facility's quarantine room, where all plants were placed on shelves equipped with broad-spectrum LED lighting (Voltserver High-Intensity Lighting Platform). Light intensity was gradually increased each week from 25% intensity (average of around 500 PPFD) to a maximum of 50% intensity (average of around 1,080 PPFD). Two rows of 15 plants where placed on each of the two levels of the shelves. Plants were then kept under commercial cultivation conditions (day/night temperature of 25°C/21°C +/- 2, 12 hours of daylight, 50% +/- 5 RH, and CO2 at ambient levels). Plants were watered by drip irrigation approximately every two days with approximately 1 L of water/plant.

As thrips can reproduce asexually (Morison, 1957; Stuart et al., 2011), it is not possible to control for their final infestation levels. In this way, we inoculated the plants with zero, one and five thrips to represent respectively control, low and high realistic levels of infestation. Ten cannabis clones (approx. 2-w old) were randomly assigned to each combination of infestation level (zero, one or five thrips) and growing substrate (normal or biostimulant). Thrips used for the experiment were collected directly from the production area of the facility with entomological mouth aspirators. We targeted fourth and fifth instar larvae (L4-L5). Thrips were carefully inoculated on each plant with fine brushes. Plants were then covered with Nitex (150 µm mesh) bags that were supported by a stainless-steel frame and tightly hold around the pots by elastics bands. Two drippers were threaded under the elastic bands for irrigation purpose. Plants were grown in the bags under aforementioned conditions for eight weeks. At the end of the eight-week period, the bags were removed, and the fresh yield was weighed for each plant (Pennsylvania 7600 Series Bench Scale 4536 g x 0.5 g). In order to measure THC levels, we took flower samples from three plants from each treatment with zero or five thrips. Each treatment

sample was then blended together and analyzed using HPLC analysis conducted by A&L Canada Laboratories Inc in London, Ontario. Thrips specimens (adults and larvae) were collected and sent to the expert insects taxonomist from the Laboratoire d'expertise et de diagnostic en phytoprotection (LEDP) of the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec for identification (vouchers are stored therein).

Statistics

Fresh yield data were analysed with R (R Core Team, 2019). We used linear mixed-effects models (package “nlme” (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019)) and ANOVA to test the effect of both the number of inoculated thrips and the type of substrate on the fresh yield. The models included all interactions between our explanatory variables. To take into account a potential difference in temperature or growing conditions, the shelves’ level (upper or lower) was treated as a random effect.

RESULTS AND DISCUSSION

The thrips species present in GreenSeal Cannabis Company’s facility was identified as the onion thrips, Thrips tabaci (Lindeman). The final fresh yield of our individual plants varied between 36.81 and 195.84 g. Three plants died before the end of the experiment. Five others grew far more slowly than the average plant and we believe they were somehow stunted. Nonetheless, the stunted plants were still included in all analyses, but the dead plants were not. We observed thrips on most plants at the end of the experiment, even on many control plants. However, we observed damages and populations representative of our desired levels of infestation. For the plants inoculated with five thrips, the THC level was of 17.6% for the normal substrate and 17.77% for the biostimulant substrate. For the zero thrips treatment, the THC level was 19.34% for the normal substrate and 19.62% for the biostimulant substrate.

The number of thrips had a significant effect on the final yield of the cannabis plants (ANOVA $F(2, 50) = 7.1062$, $P = 0{,}0019$; Fig. 1). Specifically, the yields were lower for plants that were initially inoculated with five thrips compare to the plants that had no thrips ($t(50) = -2.569502$, $P = 0.0132$). Average yield was 30.68% higher for plants that were not inoculated with thrips (130.15 g per plant compared to 90.22 g). The effect of the soil type was marginally significant (ANOVA $F(1, 50 = 3.5769$, $P = 0.0644$; Fig. 2), while the interaction between the infestation level and the soil type (ANOVA $F(2, 50 = 0.0506$, $P = 0.9507$) had no effect on the final yields.

Fig. 1 Mean final fresh yields of the cannabis plants after eight weeks according to the number of inoculated onion thrips (Thrips tabaci). Error bars represent 95% confidence intervals.

Fig. 2 Mean final fresh yields of the cannabis plants after eight weeks according to the substrate type used (normal or with Bacillus pumilus biostimulant). Error bars represent 95% confidence intervals.

In this preliminary article, we report the first quantification of yield loss from damage caused by onion thrips for indoor grown cannabis. We estimated yield losses that are higher than those reported for outdoor cannabis growers in California in a survey on all pests (Wilson et al., 2019). Indoor growing environment are particularly favourable for thrips and, thus, likely increase the risk associated with thrips' outbreaks. A similar study using three initial infestation levels of thrips found that the western flower thrips (F. occidentalis (Pergande)) can significantly reduce yields for greenhouse grown cucumbers, as well as the plant's growth and photosynthesis rate (Hao, Shipp, Wang, Papadopoulos, & Binns, 2002).

For its part, the onion thrips have a diversified range of hosts, including onion, leek, broccoli, cabbage, tobacco, cotton, and ornamentals (Diaz-Montano, Fuchs, Nault, Fail, & Shelton, 2011; Nault, Kain, & Wang, 2014; Stuart et al., 2011) and has long been recognized as a greenhouse pest (Morison, 1957). In onions, it can notably reduce yields, sometime by more than 50% (Diaz-Montano et al., 2011; Fournier, Boivin, & Stewart, 1995), and as little as 10 thrips per plant is sufficient to decrease yields by 7% in greenhouses (Kendall & Capinera, 1987). A comparison of two thrips infestation levels on onions led to a reduction in bulb weight for the higher level (Ghosheh & Al-Shannag, 2000). Onion thrips are particularly known to feed on leaves, causing photosynthesis reduction, distorted plant parts, and reduced bulbs size as well as transmitting viruses such as the Iris yellow spot virus (family Bunyaviridae, genus Tospovirus, IYSV) (Diaz-Montano et al., 2011; Gent et al., 2006; Wu et al., 2013). Damages consisting of yellow dot-shaped scars were observed both on the leaves of our experimental plants and on production plants under outbreak pressure (Fig. 3). Besides yield losses, we also highlight potential decreases in product quality by reduced levels of THC in flowers from highly infested plants.

Fig. 3 Example of leaf damages observed on the experimental plants and on production plants in the facility.

The use of growing medium added with B. pumilus only marginally improved the plants strength, especially at higher levels of infestation. It is impossible with our design to determine if this observed effect is due to the growth enhancement or chitinase production properties of the bacterium. However, we consider that growing medium enhanced with microbial biostimulants represent a promising avenue that should be investigated more in indoor cannabis production.

In conclusion, we showed that the onion thrips is present in indoor grown cannabis in Canada and that it represents an economic threat. We observed damage caused by thrips feeding on leaves and experimentally found a strong link between infestation levels and final fresh yields, in addition to reduced THC levels under high infestation. This study was preliminary and will lead to further experiments. We recommend strict monitoring programs for indoor cannabis producers to avoid economic losses and more research in developing safe, more affordable, control methods.

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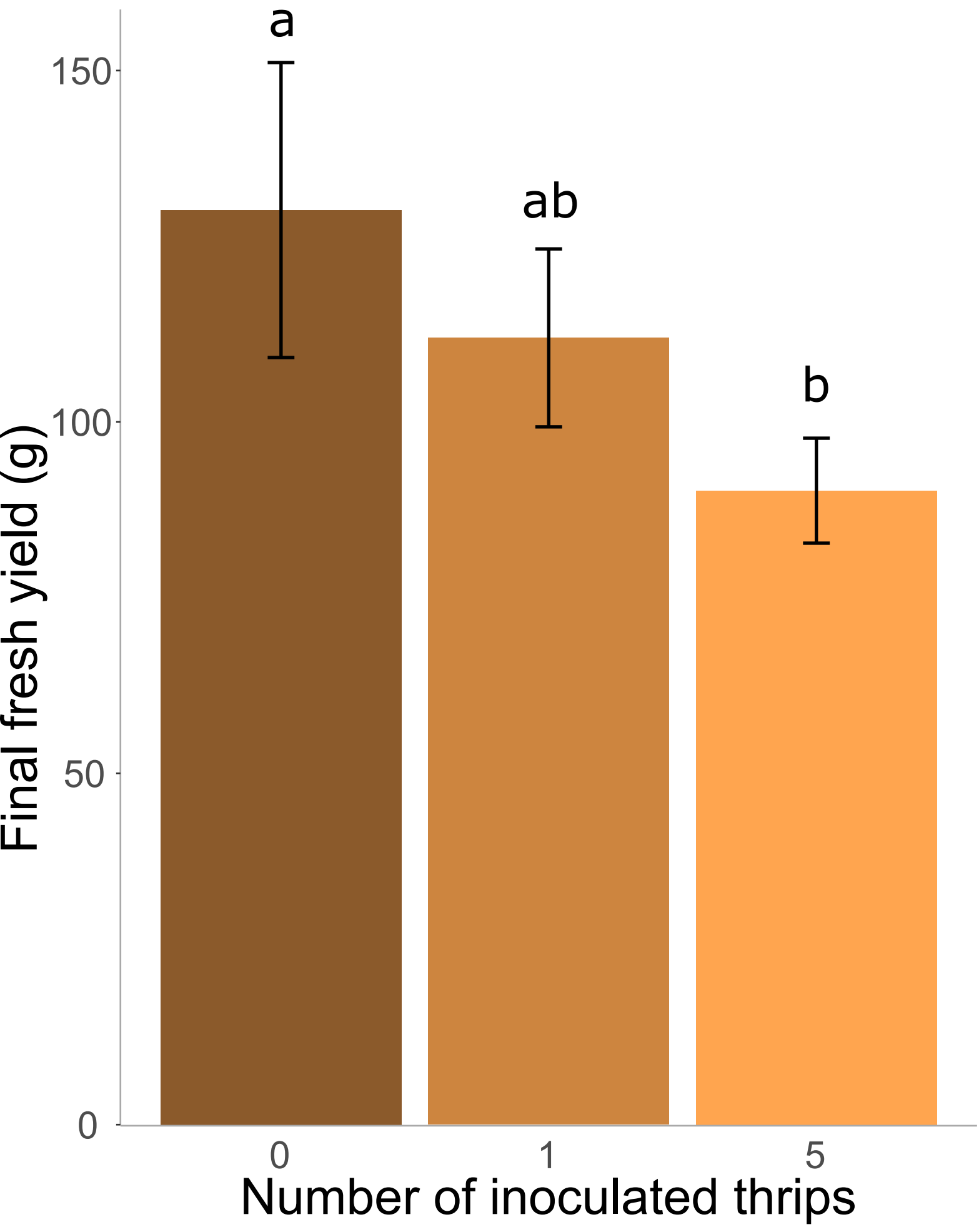
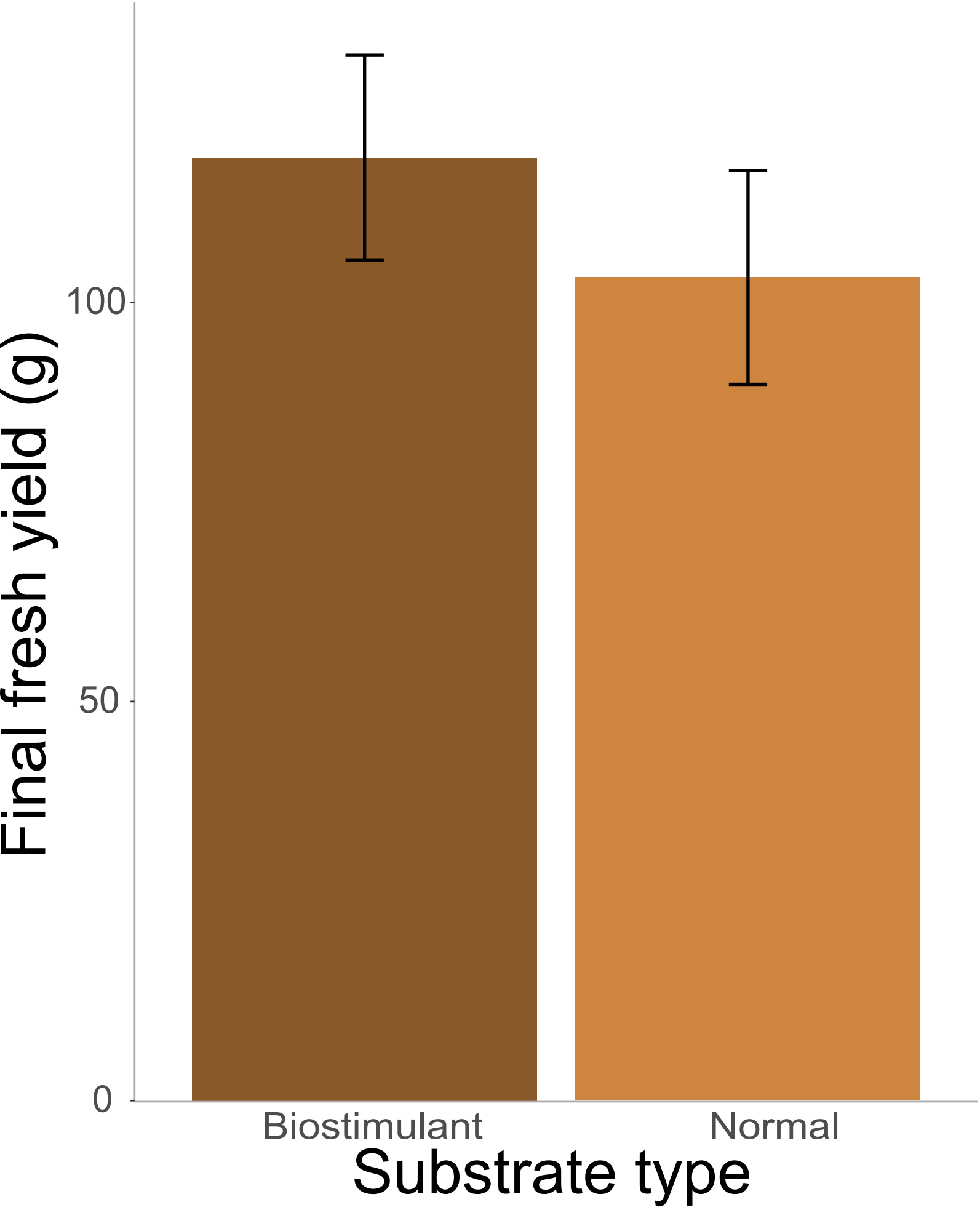

a
ab
b
150
100
50
0
Final fresh yield (g)
0
1
5
Number of inoculated thrips


Final fresh yield (g)
100
50
0
Biostimulant
Normal
Substrate type