1 **TITLE:**

2 Onion thrips *Thrips tabaci* [Thysanoptera: Thripidae] reduces yields and THC in indoor grown

3 cannabis

4 **IDENTIFICATION OF AUTHORS:**

5 Frédéric McCune[1], Chad Murphy[2], James Eaves[3], and Valérie Fournier[1]

[1] Centre de recherche et d'innovation sur les végétaux, Université Laval, Québec, QC, Canada G1V 0A6; corresponding author: frederic.mccune.1@ulaval.ca.
[2] GreenSeal Cannabis Co., Stratford, ON, Canada N4Z 1H3
[3] Département de management/ Department of Management, FSA ULaval – Faculté des sciences de l'administration, Université Laval, Québec, QC, Canada G1V 0A6

ABSTRACT

Cannabis (*Cannabis sativa* L. [Rosales: Cannabaceae]) is a newly legalized crop and requires deeper insights on its pest communities. In this preliminary study, we identified a thrips species affecting indoor grown cannabis in Canada and tested its impact on plant yield. We used three levels of initial infestation (zero, one, and five thrips) on individual plants grown in two growing 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 that fresh yields were higher for the plants that initially received zero thrips compared to those that initially received five thrips. Moreover, the biostimulant did not help reduce the impact of thrips. We highlight the importance for growers to carefully monitor thrips infestations in indoor grown cannabis. Finally, we emphasize the need for more research related to the impact of pests on cannabis yields and safe means of pest control for this strictly regulated crop.

RÉSUMÉ

Le cannabis (*Cannabis sativa* L. [Rosales: Cannabaceae]) est une culture nouvellement légalisée et qui requiert des connaissances approfondies sur ses ravageurs. Dans cette étude préliminaire, nous avons identifié une espèce de thrips affectant le cannabis cultivé à l'intérieur au Canada et testé son impact sur le rendement des plants. Nous avons testé trois niveaux initiaux d'infestation (zéro, un, et cinq thrips) sur des plants individuels cultivés dans deux terreaux : un substrat normal ou un substrat contenant le biostimulant *Bacillus pumilus*, Meyer and Gottheil [Bacillales: Bacillaceae]. Nous avons observé que le thrips de l'oignon, *Thrips tabaci* (Lindeman) [Thysanoptera: Thripidae] prolifère dans le cannabis cultivé à l'intérieur. Nos résultats montrent que le rendement des plants de cannabis est plus élevé pour les plants n'ayant pas reçu de thrips comparativement aux plants sur lesquels cinq thrips ont initialement été inoculés. De plus, le biostimulant n'a pas permis de réduire l'impact des thrips. Nous mettons en lumière l'importance pour les producteurs de cannabis cultivé à l'intérieur de faire un suivi rigoureux de leurs populations de thrips. Finalement, nous soulignons les besoins importants en recherches concernant les ravageurs du cannabis, leurs impacts et le développement de méthodes de lutte dans cette culture hautement règlementée.

INTRODUCTION

Cannabis (*Cannabis sativa* L. [Rosales: Cannabaceae]) was 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). This includes research related to the impact of pest species and the means of controlling them (Cranshaw et al., 2019). Under the Cannabis Regulations and the Pest Control Products Act, Health Canada only allows cannabis growers to use a limited number of pesticide products. Consequently, companies rely mostly on biological control, but these techniques are very costly, increase the risk of contaminating the final product with dead insect parts, and yield uneven results.

More than 300 arthropod species have been identified on hemp and cannabis (Cranshaw et al., 2019; McPartland, 1996a). On cannabis, the most predominant ones are sap-sucking arthropods such as aphids, whiteflies, leafhoppers, mealybugs and various mites (Lago & Stanford, 1989; McPartland, 1996a; Wilson et al., 2019). Recent reports of potential pests in cannabis include the marmorated stink bug (*Halyomorpha halys*) (Britt, Pagani, & Kuhar, 2019) and two aphid species (*Phorodon cannabis* and *Rhopalosiphum rufiabdominale*) (Lagos-Kutz, Potter, DiFonzo, Russell, & Hartman, 2018). Despite this, it is believed that very few insects can actually cause significant losses in commercial cannabis production (Dewey, 1913; McPartland, 1996a). In a recent survey, growers from California reported from zero to over 25% crop damage caused by arthropods (Wilson et al., 2019). Nonetheless, a large proportion of cannabis production occurs indoor or in greenhouses, which provide environments that are particularly favourable for pests. If fact, in Canada, Health Canada only started licensing outdoor area in October of 2019. 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.

Thrips have been shown to be a major pest for many crops, most notably in greenhouses (Stuart, Gao, & Lei, 2011) and can inflict both direct and indirect damage (Hao, Shipp, Wang, Papadopoulos, & Binns, 2002; Pereira et al., 2017). Damage resulting from sucking or ovipositing in the marketable plant parts, like fruits, correspond to direct damage (Shipp, Hao, Papadopoulos, & Binns, 1998), while damage caused on non-marketable plant parts, like leaves, are considered indirect damage (Diaz-Montano, Fuchs, Nault, Fail, & Shelton, 2011; German, Ullman, & Moyer, 1992). Thrips are found in indoor cannabis facilities (McPartland, 1996a) but 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) [Thysanoptera: Thripidae] have been frequently found on hemp in Colorado and can cause important foliage damage 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, 1996a).

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 (de-Bashan, Hernandez, Bashan, & Maier, 2010;

79 Gutiérrez-Mañero et al., 2001; Probanza, Lucas, Acero, & Gutierrez Mañero, 1996) and antifungal
80 (Pérez-García, Romero, & de Vicente, 2011) properties. Furthermore, *B. pumilu*s successfully
81 suppressed larvae of *Scirpophaga incertulas* and *Bruchus dentipes* in laboratory conditions
82 (Rishad, Rebello, Shabanamol, & Jisha, 2017; Tozlu, Dadasoglu, Kotan, & Tozlu, 2011). These
83 results are likely explained by its high production of chitinase, an enzyme that can degrade the
84 chitin containing cell walls of insects and thus induce death (Rishad et al., 2017). Chitinase has
85 shown insecticidal properties against weevils (Laribi-Habchi, 2014) and aphids (Kim & Je, 2010).
86 Growing mediums enhanced with entomopathogenic bacteria represents a promising avenue
87 toward pest control and reduced use of pesticides. When added to a growing medium, *B. pumilus*
88 reduces the infestation level of fungus gnats (Diptera) in greenhouses, but shows inconclusive
89 results for the western flower thrips (Gravel & Naasz, 2019).

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

94 MATERIALS AND METHODS

95 Experiment

96 The experiment was conducted in the autumn of 2019 in the commercial cannabis production
97 facility of GreenSeal Cannabis Company located in Stratford, Ontario, Canada. We used 60 clones
98 (approximately two weeks old) of cannabis (*C. sativa* var. Green Crack) to test the impact of three
99 initial levels of thrips infestation (zero, one or five thrips) and two growing substrates (normal or
100 biostimulant). Ten cannabis clones, acting as ten replicas, were randomly assigned to each

101 combination of infestation level and growing substrate. All clones were planted in seven inches
102 square pots (4L) using one of the two types of substrate. The first ("normal substrate") was a
103 fibrous, peat-moss substrate with perlite (Pro-Mix HP Mycorrhizae, Premier Tech). The second
104 ("biostimulant substrate") was the normal substrate with the addition of the biostimulant *Bacillus*
105 *pumilus* (strain GHA180) (Pro-Mix HP Biostimulant + Mycorrhizae, Premier Tech). Plants were
106 propagated in a quarantine room and we visually inspected them for predatory mite or thrips. As
107 an additional precaution, we carefully used a spray-bottle and cloth to wipe each individual leaf to
108 ensure no arthropods were on them.

109 As thrips can reproduce asexually (Morison, 1957; Stuart et al., 2011) inoculating a single
110 immature thrips can lead to an significant population overtime. It is thus not possible to control for
111 their final infestation levels. Even though we do not think that controlling the initial infestation
112 levels will result in consistent levels of infestation at the end of the experiment, we consider that it
113 provides a valuable insight about the impact of a pest (Torres-Vila, Rodríguez-Molina, & Lacasa-
114 Plasencia, 2003). In this way, we inoculated the plants with zero, one and five thrips to represent
115 respectively control, low and high levels of infestation (Hao et al., 2002). Thrips used for the
116 experiment were collected directly from the production area of the facility with entomological
117 mouth aspirators. We targeted what we believed to be late instar larvae. Thrips were carefully
118 inoculated on each plant with fine brushes. All plants, including the controls with no inoculated
119 thrips, were then covered with Nitex (150 µm mesh) bags that were supported by stainless-steel
120 frames and tightly secured around the pots by elastics bands. The fine meshes of the Nitex bags
121 help prevent thrips from escaping and non-experimental pests or predatory mites from entering.
122 Nonetheless, since this experiment was conducted in a production facility, rather than inside a
123 university lab (It is still very difficult to receive a cannabis research license in Canada.), we did

124 expect some cross contamination and thus recorded leaf damage for all plants. Two drippers were
125 threaded under the elastic bands for irrigation purpose. We considered monitoring the thrips levels
126 over the course of the experiment but decided the risk of allow arthropodes to enter or escape was
127 too high.

128 The plants were grown in the facility's quarantine room, where all plants were placed on two-
129 levels shelves. Five plants of each treatment were placed on each level of the shelves following a
130 completely randomized design, so that 30 plants were located on the top level and 30 plants on the
131 bottom level. Plants were placed in two rows of 15 plants on each level. Shelves were equipped
132 with broad-spectrum LED lighting (Voltserver High-Intensity Lighting Platform). Light intensity
133 was gradually increased each week from 25% intensity (average of around 500 PPFD) to a
134 maximum of 50% intensity (average of around 1,080 PPFD). Plants were then kept under
135 commercial cultivation conditions (day/night temperature of 25°C/21°C +/- 2, 12 hours of daylight,
136 50% +/- 5 RH, and CO2 at ambient levels). Using a short vegetative period combined with these
137 environmental conditions are typical for growers who follow a "sea of green" growing strategy, as
138 GreenSeal Cannabis does. The exception is CO2 concentration levels, which were maintained at
139 ambient levels for the experiment. Plants were watered by drip irrigation about every two days
140 with approximately 1 L of water/plant.

141 All 60 plants were grown in the cages under aforementioned conditions for eight weeks. At the
142 end of the eight-week period, the plants had reached the end of the flower stage. The fresh
143 inflorescences were then harvested following normal commercial methods and weighed for each
144 plant (Pennsylvania 7600 Series Bench Scale 4536 g x 0.5 g). In order to measure total THC levels,
145 we took flower samples from three plants from each treatment with zero or five thrips. The samples

146 for each treatment were then blended together and analyzed using HPLC analysis conducted by
147 A&L Canada Laboratories Inc. located in London, Ontario. We therefore obtained a single THC
148 measure for each control and high infestation treatment.

149 Multiple rows of plants in the two production rooms of the cannabis facility were checked to
150 collect and identify all thrips species occurring in the facility. All collected specimens were
151 observed under a stereomicroscope and appeared to be similar. Multiple thrips specimens at both
152 adult and larval stages were collected and sent to the expert insects taxonomist from the Laboratoire
153 d'expertise et de diagnostic en phytoprotection (LEDP) of the Ministère de l'Agriculture, des
154 Pêcheries et de l'Alimentation du Québec for identification (Palmer, Mound, Du Heaume, & Betts,
155 1989), who has stored the vouchers. No other pests than thrips were observed in the facility.

156 **Statistical analyses**

157 Fresh yield data were analyzed with R (R Core Team, 2019). We used generalized least squares
158 fitted linear models and linear mixed-effects models (package "nlme" (Pinheiro, Bates, DebRoy,
159 Sarkar, & R Core Team, 2019)). Fresh yield was used as the response variable, while both the
160 number of thrips initially inoculated and the substrate type were explanatory variables. All models
161 included all interactions between our explanatory variables. We first computed generalized least
162 squares fitted linear models and then compared these to linear mixed-effects models in which the
163 shelves' level (upper or lower) was treated as a random effect. This allows us to take into account
164 a potential difference in temperature or growing conditions between levels. We thereafter
165 compared both types of models based on their Akaike information criterion (AIC). The more
166 complex linear mixed-effects models including the shelves' level had a higher AIC than the more
167 simple models, indicating less accuracy of the model. We therefore only present results from the
168 generalized least squares fitted linear models in the result section. We used an ANOVA to test the

169 effect of both the number of inoculated thrips and the type of substrate on the fresh yield. We
170 changed the reference level and fitted models once more to evaluate the effect of the multiple levels
171 of our explanatory variables that were identified as significant in the ANOVA.

172 **RESULTS**

173 All thrips specimens collected in the GreenSeal Cannabis Company's facility were identified as
174 being onion thrips, *Thrips tabaci* (Lindeman). The final fresh yield of our individual plants varied
175 between 36.81 g and 195.84 g. Three plants died at the start of the experiment from transplant
176 shock. We did not have replacement plants. They were respectively under treatments zero thrips-
177 biostimulant, one thrips-normal, and five thrips-normal. Five others grew far more slowly than the
178 average plant, which may indicate they were somehow stunted. Those were respectively under
179 treatments zero thrips-biostimulant, zero thrips-normal (two plants), one thrips-biostimulant, and
180 one thrips-normal. The plants that died or had a reduced growth were thus represented in almost
181 all treatments, but slightly more in controls and low infestation treatments. Nonetheless, a boxplot
182 revealed that only three of these observations were actual outliers. We removed the dead plants
183 from our dataset and performed all analyses with and without the stunted plants. We obtained very
184 similar results both times and thus decided to include the stunted plants in all analyses. We observed
185 thrips on most plants at the end of the experiment, even on many control plants. However, we are
186 not very concerned about this contamination since the relative amount of damage is representative
187 of our desired levels of infestation. For example, control plants had very little damage compared
188 to what was observed on the treatment plants. For the plants inoculated with five thrips, the total
189 THC level was of 17.6% for the normal substrate and 17.77% for the biostimulant substrate. For
190 the zero thrips treatment, the total THC level was 19.34% for the normal substrate and 19.62% for
191 the biostimulant substrate. We limited our THC measurements to those four samples and thus

192 cannot provide statistics here. Those THC levels nevertheless indicate a possible reduction in THC
193 when plants are under high infestation of thrips.

194 The number of thrips had a significant effect on the final yield of the cannabis plants (ANOVA
195 $F(2, 51) = 7.1062$, $P = 0{,}0019$; Fig. 1). Specifically, the yields were lower for plants that were
196 initially inoculated with five thrips compare to the plants that had no thrips ($t(51) = -2.569502$,
197 $P = 0.0132$). Average yield was 30.68% higher for plants that were not inoculated with thrips
198 (130.15 g per plant compared to 90.22 g). This result highlights the relevance of keeping stunted
199 plants in the analysis. Indeed, as the prevalence of stunted plants was slightly higher in the control
200 and low infestation treatment, the effect of including these low yielding plants in the models was
201 to reduce the potential negative impact of thrips. As we found an opposite trend, it therefore
202 reinforces the hypothesis that thrips negatively impact yields. The effect of the substrate type was
203 not significant (ANOVA $F(1, 51 = 3.5769$, $P = 0.0643$; Fig. 2) while the interaction between the
204 infestation level and the substrate type (ANOVA $F(2, 51 = 0.0506$, $P = 0.9507$) had no effect on
205 the final yields.

206 **DISCUSSION**

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

214 2002). A multitude of factors such as the crop nutritional status, its growing condition and the
215 prevalence of pests and diseases influence yields in a crop and thus make describing yields as a
216 function of a precise pest infestation very difficult (Pereira et al., 2017). This is even more true for
217 pests inflicting indirect damage such as thrips (Hao et al., 2002; Pereira et al., 2017). However, as
218 growing conditions are highly controlled in indoor cannabis production, as they were standardized
219 in between our plants, as climatic variations are minimal, and as our plants were all clones equally
220 treated, we believe the differences observed in this study are most likely due to differences in
221 infestations rates.

222 The onion thrips have a very diversified range of hosts (Diaz-Montano et al., 2011; Nault, Kain,
223 & Wang, 2014; Stuart et al., 2011) and has long been recognized as a greenhouse pest (Morison,
224 1957). Considering it has been found on hemp (Cranshaw et al., 2019), it is not surprising that
225 indoor grown cannabis can be added to this long list of hosts. Nonetheless, this is a new piece of
226 valuable information for producers. In onions, it can notably impair bulb weight (Ghosheh & Al-
227 Shannag, 2000) and reduce yields, sometime by more than 50% (Diaz-Montano et al., 2011;
228 Fournier, Boivin, & Stewart, 1995), especially since as little as 10 thrips per plant is sufficient to
229 decrease yields by 7% in greenhouses (Kendall & Capinera, 1987). Onion thrips are particularly
230 known to feed on leaves, causing photosynthesis reduction, distorted plant parts, and reduced bulbs
231 size as well as transmitting viruses, such as the Iris yellow spot virus (family Bunyaviridae, genus
232 *Tospovirus*, IYSV) (Diaz-Montano et al., 2011; Gent et al., 2006; Wu et al., 2013). Damage
233 consisting of yellow dot-shaped scars were observed both on the leaves of our experimental plants
234 and on production plants under outbreak pressure (Fig. 3). These injuries can be considered indirect
235 damage and were almost certainly inflicted by the onion thrips. We believe our observations
236 correspond to the "serious foliage damage" reported by Cranshaw et al. (2019) on hemp. Even

237 though we did not investigate damage extent or photosynthetic rate in our cannabis plants, it can
238 be expected that our reduced yields originate from those indirect feeding damage. Similar injuries
239 and scars are known to reduce the photosynthetic ability of leaves in onions (Diaz-Montano et al.,
240 2011). Little information is available about the transmission of viruses to cannabis plants by thrips
241 but McPartland (1996b) mention that viruses can greatly reduce yields in cannabis and that the
242 onion thrips is one of the worst vector of viruses in this crop. Besides yield losses, we also highlight
243 potential decreases in product value through reduced levels of total THC from highly infested
244 plants.

245 The use of a growing medium with *B. pumilus* did not improved the plants' strength. It is
246 consistent with previous experiments with the same enhanced growing medium on the control of
247 the western flower thrips (*F. occidentalis*) (Gravel & Naasz, 2019). However, our results were
248 nearly significant, and we still consider that growing medium enhanced with microbial
249 biostimulants represents a promising avenue for integrated pest management in greenhouse or
250 indoor productions. Their use should be investigated more in indoor cannabis production and for
251 various pests.

252 In conclusion, we showed that the onion thrips is present in indoor grown cannabis in Canada
253 and that it represents an economic threat. We observed damage caused by thrips feeding on leaves
254 and experimentally found a link between infestation levels and final fresh yields, in addition to a
255 possible reduction of total THC levels under high infestation. This study was preliminary and
256 should motivate further experiments. As chemical means of control are very limited for indoor
257 grown cannabis, we recommend strict monitoring programs for indoor cannabis producers to avoid
258 economic losses. We show that thrips potentially represent a major threat to product quality and

yields. In this way, we suggest more research on cannabis pests to identify all pest species in various growing setting, including the range of damage they can cause and their economic thresholds. Only this can subsequently lead to the development of management programs and the development of safe and affordable control methods.

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365 **LEGENDS OF FIGURES**

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

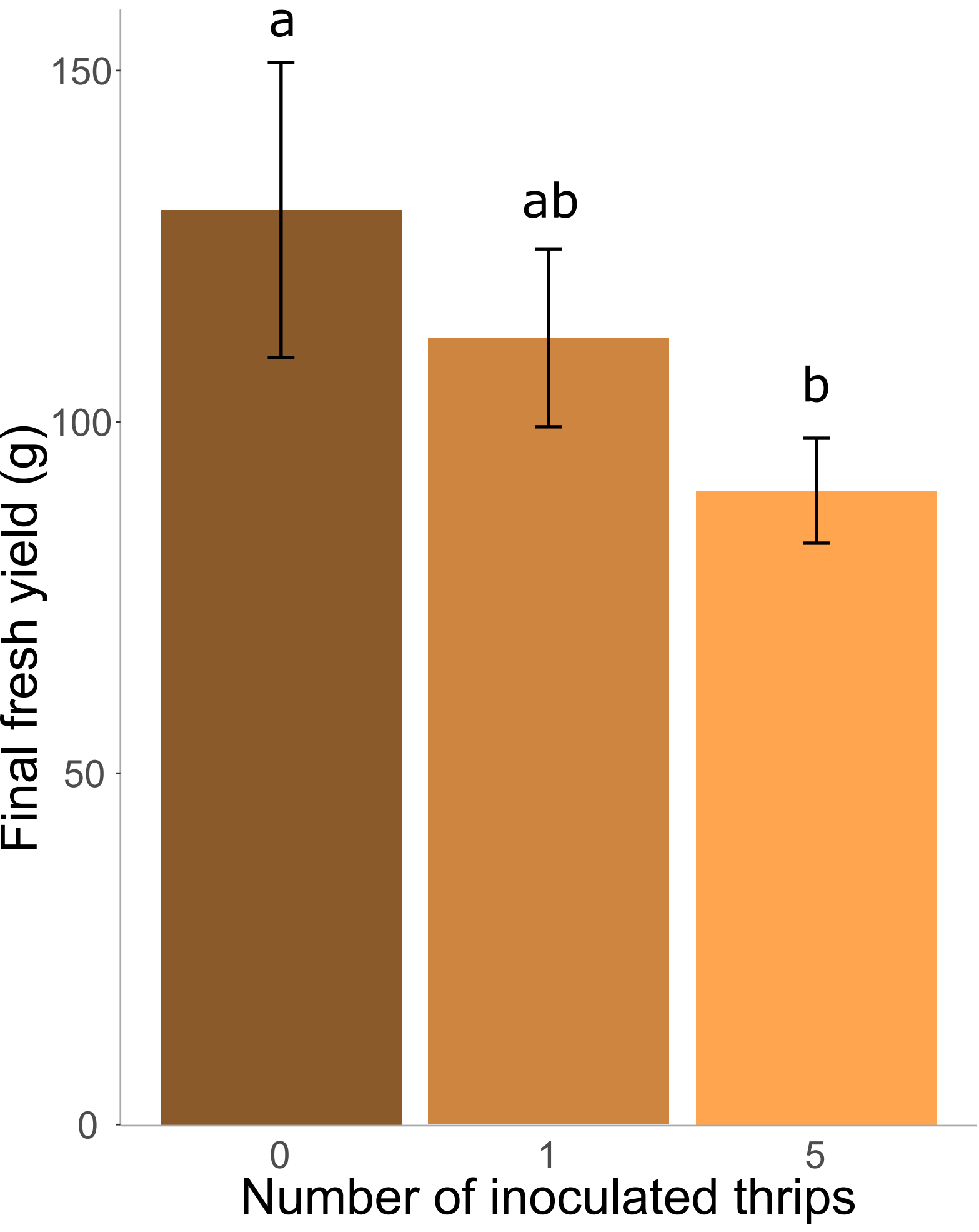
368 Fig. 2 Mean final fresh yields of the cannabis plants after eight weeks according to the
369 substrate type used (normal or with *Bacillus pumilus* biostimulant). Error bars represent 95%
370 confidence intervals.

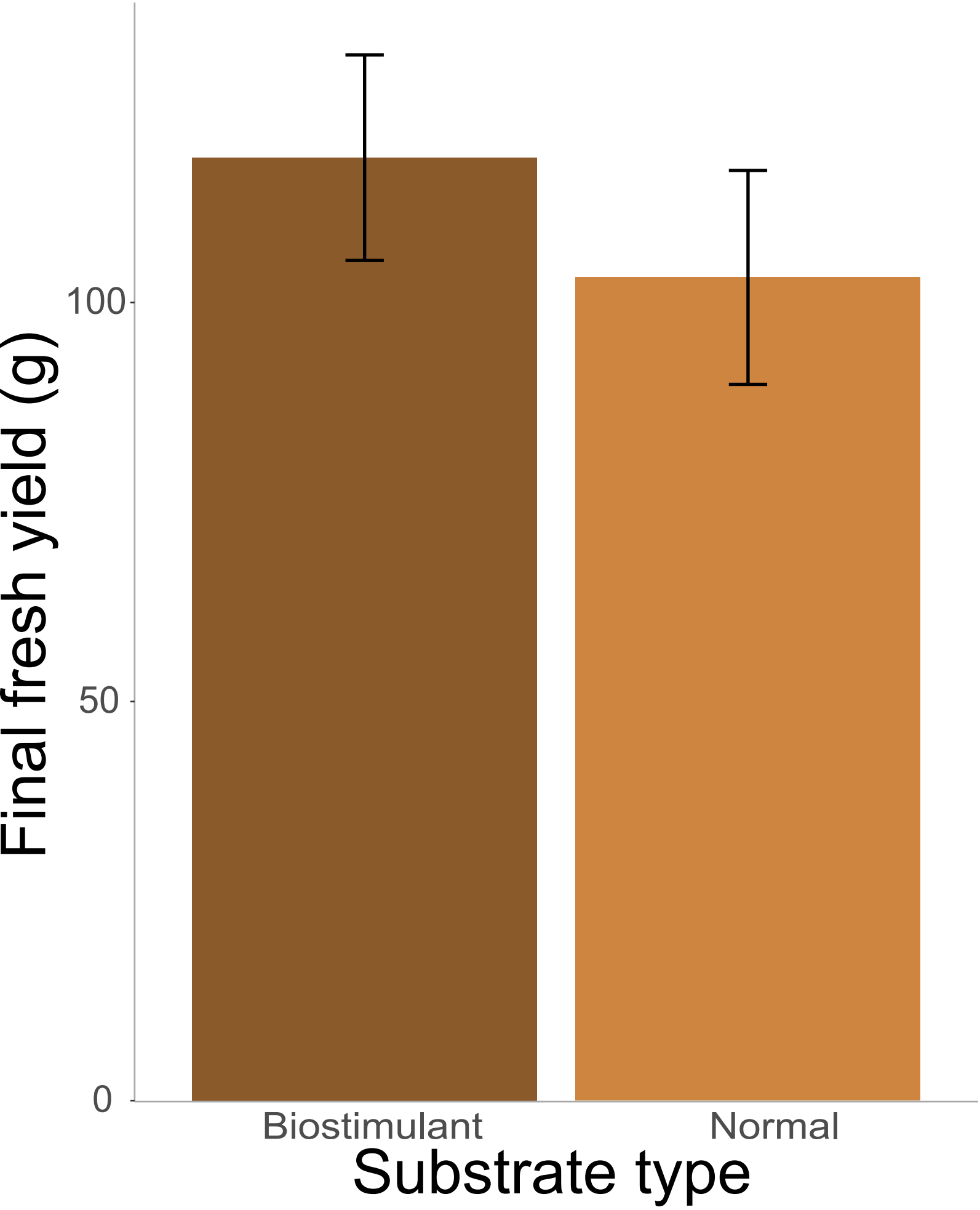
371 Fig. 3 Example of leaf damage observed on the experimental plants and on production
372 plants in the facility.

373

RUNNING TITLE:

Onion thrips in cannabis


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