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Wang L, Pokharel SS, Chen F. 2019. Arbuscular mycorrhizal fungi alter the food utilization, growth, development and reproduction of armyworm (*Mythimna separata*) fed on *Bacillus thuringiensis* maize. PeerJ 7:e7679 <https://doi.org/10.7717/peerj.7679>

# Arbuscular mycorrhizal fungi (AMF) alter the food utilization, growth & development and reproduction of armyworm, *Mythimna separata* fed on *Bt* maize

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## Abstract

**Background:** The cultivation of *Bt* maize (maize genetically modified with *Bacillus thuringiensis*) continues to expand globally. Arbuscular mycorrhizal fungi (AMF), a kind of beneficially microbial community, closely related to soil fertility and plant nutrition, may influence the resistance risk of *Bt* crops against target lepidopteran pests.

**Methods:** In this study, transgenic *Bt* maize (Line IE09S034 with *Cry1Ie* vs. its parental line of non-*Bt* maize cv. Xianyu335) was inoculated with a species of AMF, *Glomus caledonium* (GC) and its outcomes on the food utilization, reproduction and development of armyworm, *Mythimna separata* were conducted in a potted experiment from 2017 to 2018.

**Results:** AMF inoculation showed favorable influence on the GC colonization of both modified and non-modified maize, and marked increase in the grain weight per plant and 1000-grain weight of modified and non-modified maize. Meanwhile, the cultivation of *Bt* maize didn't significantly affected the AMF colonization. The feeding of *M. separata* with *Bt* maize resulted in a notable decrease in the parameters RCR, RGR, AD, ECI and ECD as compared with those observed in larvae fed with non-*Bt* maize in 2017 and 2018 regardless of GC inoculation. Furthermore, remarkable shortening of the adult longevity, remarkable prolongation of larval life span and remarkable decrease in the rate of pupation, weight of pupa, rate of eclosion and fecundity of *M. separata* was observed in *Bt* treatment regardless of GC inoculation during the two year experiment. Also, when *M. separata* was given *Bt*, a significant prolongation of larval life and shortening of the adult longevity, and a significant decrease of the pupal weight and fecundity of *M. separata*, was observed when inoculated with GC. However, it was just the opposite for larvae fed with non-*Bt* maize and inoculated with GC. The increased percentage of larval life-span, and the decrease percentages of the food utilization and the other indexes of reproduction, growth, and development of *M. separata* fed on *Bt* maize relative to non-*Bt* maize were all obviously lower under GC inoculation in contrast to the CK.

**Discussion:** It is presumed that *Bt* maize has marked adverse impact on the *M. separata* development, reproduction and feeding, especially in combination with the GC inoculation. And the GC inoculation favors the resistance efficiency of *Bt* maize against *M. separata* larvae through reducing their food utilization ability, and then negatively affecting the development and reproduction of the armyworm. So *Bt* maize inoculated with AMF (here, GC) can lessen the severe threats arising from armyworms, and hence the AMF inoculation may play important ecological functions in the field of *Bt* maize ecosystem with potentially high control efficiency for the target lepidopteran pests.

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24 significantly affected the AMF colonization. The feeding of *M. separata* with *Bt* maize resulted  
25 in a notable decrease in the parameters RCR, RGR, AD, ECI and ECD as compared with those  
26 observed in larvae fed with non-*Bt* maize in 2017 and 2018 regardless of GC inoculation.  
27 Furthermore, remarkable shortening of the adult longevity, remarkable prolongation of larval life  
28 span and remarkable decrease in the rate of pupation, weight of pupa, rate of eclosion and  
29 fecundity of *M. separata* was observed in *Bt* treatment regardless of GC inoculation during the  
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42 severe threats arising from armyworms, and hence the AMF inoculation may play important  
43 ecological functions in the field of *Bt* maize ecosystem with potentially high control efficiency  
44 for the target lepidopteran pests.

45 **Keywords:** Arbuscular mycorrhizal fungi; Transgenic *Bt* maize; *Mythimna separata*; Food  
46 utilization; Growth & development and reproduction

47

## 48 Introduction

49 The cultivation of *Bt* maize (maize genetically modified with *Bacillus thuringiensis*) continues to  
50 expand globally (James, 2012). The transgenic *Bt* maize is one of the most produced genetically  
51 modified (GM) crops which has been genetically engineered to express one or more  
52 insecticidal toxins derived from the soil bacterium *B. thuringiensis* (Alyokhin, 2011; Huang et  
53 al., 2014). These toxins are Cry (crystalline proteins) from *B. thuringiensis* that affect a number  
54 of insect groups. The number of Cry proteins identified is at least 60 (Icoz & Stotzky, 2008;  
55 Sanchis, 2011). These insecticidal toxins (i.e., *Bt* toxins) thus mitigate the damage by some target  
56 lepidopteran insects (Walters et al., 2010; Pardo-López et al., 2013; Huang et al., 2014), such  
57 as *Ostrinia nubilalis*, *Ostrinia furnacalis* and *Mythimna separata* (Sanahuja et al., 2011; Guo et  
58 al., 2016; Jia et al., 2016).

59 Symbiotic relationships with plant roots are formed by arbuscular mycorrhizal fungi (AMF)  
60 for the purpose of improving the uptake of water and nutrients, accelerate plant growth, and help  
61 to build soil structure and function (Smith & Read, 2008). On the other hand, AMFs require an  
62 adequate plant host. The fungi get carbon from plants, and in return, fungi give nitrogen,  
63 phosphorus and other nutrients. Also, fungi can improve soil stability and resistance to disease  
64 (Singh et al., 2012; Steinkellner et al., 2012). Plants associated with AMF may have their  
65 interactions with insects, pollinators or herbivores altered, and this will affect the plant health  
66 (Vannette & Rasmann, 2012; Koricheva et al., 2009; Wolfe et al., 2005). AMF colonization often  
67 affects insect herbivores (Koricheva et al., 2009). AMF influence on defense chemicals, nutrient  
68 contents, or plant biomass. (Bennett & Bowers, 2009). For instance, the interaction with  
69 mycorrhizal fungi may give the plant resources against herbivores, although it may instead made  
70 the plant a better food source (Vannette & Hunter, 2011).

71 Also, a number of researches have showed that nitrogen was the most active nutrient element  
72 in the crop growth process and was the main constituent of *Bt* protein. Plant nitrogen uptake and  
73 nitrogen metabolism levels can change the carbon-nitrogen ratio in plant tissues and also affect  
74 the production of *Bt* toxins (Jiang *et al.*, 2013; Gao *et al.*, 2009). The AMF can enhance plant  
75 absorption and utilization of soil nutrients (mainly N and P). Thus, the effects of AMF on *Bt*  
76 maize and target lepidopteran pest naturally become an interesting and significant research  
77 priority. There are some studies that focused on the influence of *Bt* on AMF colonization, the  
78 AMF community diversity, and soil ecology (Zeng *et al.*, 2014; Cheeke *et al.*, 2013). But, the  
79 effects of AMF on the resistance of *Bt* crops against target lepidopteran pests, have rarely been  
80 reported. In this work, we study the indirect effects of AMF on food utilization, growth, and  
81 development of armyworm *M. separata* feeding on transgenic *Bt* maize; and the direct influence  
82 of AMF on the yields of *Bt* and non-*Bt* maize. We expect that that this work will help reduce the  
83 risk of *Bt* crops resistance and ultimately serve the sustainable and ecological usage of *Bt* crops.

84

## 85 **Materials & Methods**

### 86 **Plant materials and arbuscular mycorrhizal fungi (AMF) inoculation**

87 A two-year study (2017–2018) was conducted in Ningjin County, Shandong Province of China  
88 (37°38'30.7"N, 116°51'11.0"E). The Institute of Crop Sciences, Chinese Academy of  
89 Agricultural Science provided us the transgenic *Bt* maize cultivar (Line IE09S034 with *CryIIe*,  
90 *Bt*) and its non-*Bt* parental line (cv. Xianyu 335, Xy). *Glomus caledonium* (strain number 90036,  
91 referred to as GC) was provided by the State Key Laboratory of Soil & Sustainable Agriculture,  
92 Institute of Soil Science, Chinese Academy of Sciences. The inoculum consisted of spores,  
93 mycelium, maize root fragments, and soil. Both genetically modified and non-modified maize  
94 were put in plastic buckets (45 cm height, 30 cm diameter) with 20 kg of soil sterilized in an  
95 autoclave and 300g GC inoculum, and 300g sterilized strains were added to each plastic bucket  
96 in the non-GC inoculation treatment (control group, ab. CK) on June 10 in each sampling year.  
97 The whole experiment involved four treatments: two maize cultivars (*Bt* and Xy), and two AMF  
98 inoculations (GC and CK), each bucket served as one replication and replicated fifteen times for  
99 each treatment. Therefore, there were 15 buckets for each maize cultivar × AMF inoculation  
100 treatment, and total 60 buckets in this study. In each bucket, three maize seeds were put at a  
101 depth of 2 cm. During the whole experimental period, special emphasis was given for the cut off  
102 of pesticides application and the manual weeding was preferred to keep the maize buckets free  
103 from incidence of weeds.

104

### 105 **Insect rearing**

106 The 3rd instar *M. separata* larvae were individually fed on excised leaves of the sampled maize  
107 plants until pupation. The experimental maize leaves were randomly chosen from 10 buckets of

108 each maize cultivar×AMF inoculation treatment during the heading stage for the feeding trials  
109 conducted in plastic dish (6 cm in diameter and 1.6 cm in height) until pupation. Each maize  
110 cultivar×AMF inoculation treatment consisted of five replicates (30 larvae one replicates).

111

### 112 **Food utilization of *M. separata* larvae**

113 The initial weights of the tested 3rd instar larvae of *M. separata* were individually determined  
114 with an electronic balance (AL104; METTLER-TOLEDO, Greifensee). The weights of the total  
115 feces from the 3rd instar until pupation (sixth instar), pupal weight, and the residual leaves were  
116 also carefully measured. Several food utilization indices of *M. separata* larvae fed on the excised  
117 leaves of *Bt* and non-*Bt* maize inoculated with AMF, *G. Caledonium* and without *G.*  
118 *Caledonium*, were determined. The indices included RCR (relative consumption rate), RGR  
119 (relative growth rate), AD (approximate digestibility), ECD (efficiency of conversion of digested  
120 food) and ECI (efficiency of conversion of ingested food) (*Li et al., 2018*). The indices  
121 calculations were done with formulas adapted from *Chen et al. (2005)*.

### 122 **Growth & development and reproduction of *M. separata***

123 Larval growth and development were evaluated from the 3rd instar to pupation by observing  
124 each petri dish every 8 hrs and recording the timing of larval ecdysis, pupation, and emergence  
125 of *M. separata* moths fed on the excised leaves of *Bt* and non-*Bt* maize inoculated with *G.*  
126 *Caledonium* and without *G. Caledonium*. After the eclosion, pairing of novel moths by  
127 maintaining the female: male ratio of 1: 1 in a metal screen cage and fed with a 10% honey  
128 cotton ball, covered with cotton net yarn and butter paper for oviposition which were replaced  
129 every day. Recording of survivorship and oviposition was continued on a daily basis until death.

130

### 131 **Yield of *Bt* and non-*Bt* maize**

132 On September 25, 2017 and 2018, eight maize plants were randomly taken from 5 pots of each  
133 maize cultivar×AMF inoculation treatment at the harvest stage to measure the grain weight per  
134 plant (g) and 1000-grain weight (g) with an electronic balance (AL104; METTLER-TOLEDO,  
135 Greifensee), in order to ascertain the effects of AMF inoculation on the yield of *Bt* and non-*Bt*  
136 maize inoculated with and without GC.

137

### 138 **Data analysis**

139 All experimental data were analysed with the software IBM-SPSSv.20.0 (IBM, Armonk, NY).  
140 Three-way repeated-measures ANOVA was used to study the impacts of treatment (*Bt* maize vs.  
141 non-*Bt* maize), AMF inoculation (GC vs. CK), sampling years (2017 vs. 2018), and their bi- and  
142 tri- interaction on the AMF colonization. Moreover, three-way ANOVA was used to analyze the

143 effects of treatment (*Bt* maize vs. non-*Bt* maize), AMF inoculation (GC vs. CK), sampling years  
144 (2017 vs. 2018), and their bi- and tri-interactions on the measured indices of growth,  
145 development, reproduction and food utilization of *M. separata*, and the yield of *Bt* and non-*Bt*  
146 maize inoculated with and without GC in 2017 and 2018. Finally, the means were separated by  
147 using the Turkey test to examine significant difference between/among treatments at  $P<0.05$ .

148

## 149 **Results**

### 150 **AMF colonization of *Bt* and non-*Bt* maize inoculated with and without *G. Caledonium***

151 Three-way repeated-measures ANOVAs indicated that GC inoculation ( $F=6673.63$ ,  $P<0.001$ )  
152 and sampling year ( $F=70.10$ ,  $P<0.001$ ) both significantly affected the GC colonization, and there  
153 were significant interactions between GC inoculation and sampling year, and between transgenic  
154 treatment and sampling year ( $F\geq 7.80$ ,  $P\leq 0.013$ ; Table 1). Compared with the non-GC  
155 inoculation, the GC inoculation significantly enhanced the AMF colonization of *Bt* and non-*Bt*  
156 maize in 2017 and 2018 respectively, with significant increases for the *Bt* maize during the  
157 seedling (2017: +653.9%; 2018: +284.1%), heading (2017: +589.6%; 2018: +491.0%) and  
158 harvest (2017: +457.6%; 2018: +409.6%) stages in 2017 and 2018, and for the non-*Bt* maize  
159 during the seedling (2017: +613.6%; 2018: +432.2%), heading (2017: +472.8%; 2018: +425.7%)  
160 and harvest (2017: +437.1%; 2018: +448.7%) stages in 2017 and 2018 ( $P<0.05$ ; Fig.1).

161

### 162 **Food utilization of *M. Separata* larvae fed on *Bt* and non-*Bt* maize inoculated with and 163 without *G. Caledonium***

164 Transgenic treatment significantly affected all the measured indexes of feeding of *M. separata*  
165 larvae ( $F\geq 418.12$ ,  $P<0.001$ ), AMF inoculation ( $F\geq 6.60$ ,  $P<0.05$ ) and the interactions between  
166 transgenic treatment and AMF inoculation ( $F\geq 91.07$ ,  $P<0.001$ ) had important effects on the  
167 RGR, RCR, ECI and AD of *M. separata* larvae, and there were significant differences in the  
168 RGR, ECD, ECI and AD of *M. separata* larvae between two sampling years ( $F\geq 9.49$ ,  $P<0.01$ ;  
169 Table 2). Moreover, there were significant interactions between transgenic treatment and  
170 sampling year on the RGR, RCR and AD of *M. separata* larvae ( $F\geq 7.83$ ,  $P<0.05$ ; Table 2).  
171 Furthermore, there were significant interactions between AMF inoculation and sampling year,  
172 and among transgenic treatment, AMF inoculation and sampling year on the RGR of *M. separata*  
173 larvae fed on the detached leaves of *Bt* maize and its parental line of non-*Bt* maize during the  
174 heading stage in 2017 and 2018.

175 Considering the case of *M. separata* larvae fed on *Bt* maize and non-*Bt* maize inoculated with  
176 GC in comparison with the CK in both sampling years, differing trends in the food utilization  
177 indexes were observed (Fig.2). In relation to the CK (i.e., non-GC inoculation), GC inoculation  
178 significantly reduced the RGR (-24.2% and -23.3%), RCR (-10.5% and -6.1%), ECI (-15.3% and

179 -18.2%) and AD (-9.0% and -16.4%) of *M. separata* larvae fed on the detached leaves of *Bt*  
180 maize during the heading stage in 2017 and 2018 ( $P<0.05$ ; Fig.2). However, GC inoculation  
181 significantly enhanced the RGR (+36.9% and +56.7%), RCR (+10.8% and +15.0%), ECI  
182 (+19.9% and +26.4%) and AD (+17.2% and +19.3%) of *M. separata* larvae fed on the detached  
183 leaves of non-*Bt* maize during the heading stage in 2017 and 2018 ( $P<0.05$ ; Fig.2).

184 Moreover, significant decreases in the RGR (2017: -64.2% and -35.4%; 2018: -68.6% and -  
185 35.9%), RCR (2017: -25.7% and -8.1%; 2018: -30.6% and -15.1%), ECD (2017: -32.2% and -  
186 25.4%; 2018: -22.1% and -15.5%), ECI (2017: -51.9% and -31.8%; 2018: -54.7% and -30.0%),  
187 and AD (2017: -29.2% and -8.7%; 2018: -41.8% and -17.1%) were found when *M. separata*  
188 larvae fed on the detached leaves of *Bt* maize in contrast to non-*Bt* maize as inoculated with and  
189 without GC in 2017 and 2018 ( $P<0.05$ ; Fig.2). And the decreased percentages in the RGR, RCR,  
190 ECD, ECI and AD of *M. separata* larvae fed on the detached leaves of *Bt* maize compared with  
191 non-*Bt* maize were all obviously lower under GC inoculation in contrast to CK.

192

### 193 **Reproduction, growth and development of *M. Separata* fed on *Bt* or non-*Bt* maize** 194 **inoculated with and without *G. Caledonium***

195 Transgenic treatment ( $F\geq 5.42$ ,  $P<0.05$ ) and AMF inoculation ( $F\geq 5.30$ ,  $P<0.05$ ) significantly  
196 affected all the calculated indexes of *M. separata* in two sampling years, and there were  
197 significant difference in pupal weight ( $F=19.11$ ,  $P<0.001$ ) of *M. separata* between two sampling  
198 years (Table 3). Moreover, there were significant interactions between transgenic treatment and  
199 sampling year on larval life-span ( $F=8.06$ ,  $P=0.008<0.01$ ), between AMF inoculation and  
200 sampling year on larval life-span ( $F=7.44$ ,  $P=0.011<0.05$ ), adult longevity ( $F=4.39$ ,  
201  $P=0.044<0.05$ ) and fecundity ( $F=9.82$ ,  $P=0.004<0.01$ ), between transgenic treatment and AMF  
202 inoculation on all the measured indexes except larval life-span ( $F\geq 7.87$ ,  $P<0.01$ ), and among  
203 transgenic treatment, AMF inoculation and sampling year on larval life-span ( $F=6.91$ ,  
204  $P=0.013<0.05$ ) and fecundity ( $F=7.63$ ,  $P=0.009<0.01$ ) for *M. separata* fed on the detached  
205 leaves of *Bt* maize and non-*Bt* maize inoculated with and without GC during the heading stage in  
206 2017 and 2018 (Table 3).

207 Opposite trends were also seen in the calculated indexes reproduction, growth, and  
208 development of larvae fed on the detached leaves of *Bt* and non-*Bt* maize inoculated with GC in  
209 contrast to the CK in both sampling years (Fig.3). In comparison with the CK, GC inoculation  
210 significantly extended the larval life cycle (+7.6% and +10.4%) and shortened the adult  
211 longevity (-14.7% and -15.2%), and decreased the pupal weight (-9.1% and -14.1%) and  
212 fecundity (-19.2% and -19.9%) of larvae fed on the detached leaves of *Bt* maize in 2017 and  
213 2018 ( $P<0.05$ ; Fig.3). At the same time, GC inoculation significantly shortened the larval life-  
214 span (-12.3% and -10.3%) and prolonged the adult longevity (+24.6% and +17.1%), and  
215 significantly increased the pupation rate (+8.4% and +11.9%), pupal weight (+10.5% and  
216 +11.9%) and fecundity (+35.7% and +14.1%) of larvae fed on the detached leaves of non-*Bt*



217 maize in 2017 and 2018, and significantly increased the eclosion rate (+16.3%) of larvae fed on  
218 the detached leaves of non-*Bt* maize in 2018 ( $P<0.05$ ; Fig.3).

219 In comparison with the non-*Bt* maize, *Bt* maize significantly prolonged the larval life cycle  
220 (2017: +31.6% and +7.3%; 2018: +31.1% and +6.6%) and shortened the adult longevity (2017: -  
221 44.2% and -17.7%; 2018: -32.6% and -13.4%), and significantly decreased the pupation rate  
222 (2017: -32.6% and -27.7%; 2018: -31.1% and -22.0%), pupal weight (2017: -26.9% and -11.2%;  
223 2018: -28.5% and -6.9%), eclosion rate (2017: -33.3% and -28.2%; 2018: -36.0% and -24.6%)  
224 and fecundity (2017: -48.1% and -12.8%; 2018: -43.0% and -18.7%) of larvae fed on the  
225 detached maize leaves inoculated with and without GC in 2017 and 2018 ( $P<0.05$ ; Fig.3). And  
226 the percentage increased in the larval life-span, and percentages decrease in the pupation rate,  
227 pupal weight, eclosion rate, adult longevity and fecundity of larvae fed on the detached leaves of  
228 *Bt* maize in comparison with to non-*Bt* maize were all obviously higher under the GC inoculation  
229 in contrast to CK.

230

### 231 **Yields of *Bt* and non-*Bt* maize inoculated with and without *G. Caledonium***

232 AMF inoculation and sampling year significantly affected the grain weight per plant (AMF  
233 inoculation:  $F=144.17$ ,  $P<0.001$ ; Sampling year:  $F=90.57$ ,  $P<0.001$ ), and 1000-grain weight  
234 were significantly affected by AMF inoculation and transgenic treatment (AMF inoculation:  
235  $F=92.54$ ,  $P<0.001$ ; Transgenic treatment:  $F=5.87$ ,  $P=0.028<0.05$ ), and there were significant  
236 interactions between sampling year and transgenic treatment on the grain weight per plant  
237 ( $F=4.96$ ,  $P=0.041<0.05$ ) and 1000-grain weight ( $F=17.96$ ,  $P=0.001<0.01$ ), and between  
238 sampling year and AMF inoculation on the grain weight per plant ( $F=8.11$ ,  $P=0.012<0.05$ ) of *Bt*  
239 and non-*Bt* maize inoculated with and without GC inoculation in 2017 and 2018 (Table 1).

240 Compared with the CK, GC inoculation significantly increased the grain weight per plant (*Bt*  
241 maize: +39.6% and +24.1%; non-*Bt* maize: +33.1% and +30.6%) and 1000-grain weight (*Bt*  
242 maize: +8.7% and +7.4%; non-*Bt* maize: +8.5% and +8.5%) of *Bt* and non-*Bt* maize in 2017 and  
243 2018 ( $P<0.05$ ; Fig.4).

244

## 245 **Discussion**

246 AMF are a group of fungi belonging to phylum Glomeromycota that penetrates the cortex of the  
247 roots of vascular plants (Parniske, 2008; Smith & Read, 2008). AMF promote the exchange of  
248 phosphorus, carbon, water, and other nutrients with the host plants (Hodge, 2010). AMF can  
249 induce changes in plant morphology, physiology, biochemistry, and even gene expression, which  
250 in turn may change the food quality of herbivorous insects, thus affecting their feeding tendency,  
251 growth, reproduction and harmfulness (Jung et al., 2012). Some researchers reached to the  
252 conclusion that cultivation of *Bt* crops had no significant impact on AMF colonization of roots

253 between *Bt* maize (MEB307) expressing Cry1Ab protein and the near isogenic non-*Bt* variety  
254 (Monumental) (Vaufleury et al., 2007; Knox et al., 2008). It was also found that the  
255 arrangements of AMF in roots in non-*Bt* were almost identical to those in *Bt* cultivars of cotton  
256 (Cry1Ac and Cry2Ab). In this study, the GC inoculation significantly enhanced the AMF  
257 colonization of *Bt* and non-*Bt* maize in two sampling years, with significant increases for the *Bt*  
258 maize ( $\geq +284.1\%$  and  $\leq +653.9\%$ ) and non-*Bt* maize ( $\geq +425.7\%$  and  $\leq +613.6\%$ ) from the  
259 seedling stage to the harvest stage. No significant difference was found in 2017 or 2018 for the  
260 AMF colonization of both types of maize (*Bt* or non-*Bt*). So, it is presumed that cultivation of *Bt*  
261 maize has no significant impact on the GC colonization between *Bt* maize (Line IE09S034)  
262 expressing Cry1Ie protein and the near isogenic non-*Bt* variety (cv. Xianyu335). An important  
263 negative effect of the *Bt* on the AMF community was reported (Castaldini et al., 2005; Turrini et  
264 al., 2005). However, the GC inoculation had positive effects on the AMF colonization regardless  
265 of *Bt* maize or non-*Bt* maize. Hodge (2010) reported that the AMF promoted the absorption and  
266 utilization of soil nutrients by maize plant, thus improving the nutrient levels of nitrogen,  
267 phosphorus and potassium of plant leaves. In this study, the GC inoculation significantly  
268 increased the grain weight per plant and 1000-grain weight regardless of the *Bt* maize or non-*Bt*  
269 maize. While no notable differences were found in the grain weight per plant of *Bt* maize  
270 compared with that of non-*Bt* maize, regardless of GC inoculation or non-GC inoculation.

271 In the world, transgenic *Bt* maize, an anti-insect crop, has been rapidly commercialized to  
272 control lepidopteran insects (for example: *Ostrinia nubilalis*, *Mythimna separata* and *Ostrinia*  
273 *nubilalis*) (James, 2012; ISAAA, 2017), but it's rarely reported that the defense responses of *M.*  
274 *separata* to transgenic *Bt* maize infected by AMF. Most studies have shown that Cry proteins  
275 have adverse effects on the life-table parameters of different herbivores (Lawo et al., 2010; Smith  
276 & Fischer, 1983). Li (2018) reported that *Bt* maize significantly affected the food utilization,  
277 reproduction, growth & development of the armyworm, *M. separata*. Several parameters were  
278 markedly decreased. The research of Prutz & Dettner (2005) showed that *Bt* maize decreased the  
279 rate of growth and increased the mortality of *Chilo partellus*. Here, important reductions in the  
280 RCR, RGR, AD and ECI happened when the larvae were fed on *Bt* maize relative to non-*Bt*  
281 maize inoculated with and without GC in 2017 and 2018. And the decreased percentages in the  
282 indexes of food utilization of the larvae fed on *Bt* maize relative to non-*Bt* maize were all  
283 obviously lower under GC inoculation in contrast to CK. Moreover, *Bt* maize also markedly  
284 extended the larval life-span and shortened the adult longevity, and significantly decreased the  
285 pupation rate, pupal weight, eclosion rate and fecundity of larvae regardless of *Bt* or non-*Bt*  
286 maize inoculated with or without GC in 2017 and 2018. And increased percentage of the larval  
287 life-span, and decreased percentages of the pupation rate, pupal weight, eclosion rate, adult  
288 longevity and fecundity of larvae fed on *Bt* maize compared with non-*Bt* maize were all  
289 obviously higher under the GC inoculation in contrast to CK. So *Bt* maize has marked negative  
290 effects on the food utilization, reproduction, growth, and development of *M. separata* especially  
291 in combination with the GC inoculation.

292 On the other hand, opposite trends were found in the food utilization, reproduction, growth,  
293 and development of *M. separata* fed on *Bt* maize and non-*Bt* maize inoculated with and without  
294 GC. For the measured indexes of food utilization, the GC inoculation significantly reduced the  
295 RGR, RCR, AD and ECI of larvae fed on *Bt* maize, while it was just the opposite for those of  
296 larvae fed on non-*Bt* maize in 2017 and 2018. For the measured indexes of growth, development  
297 and reproduction, there were also opposite trends for larvae fed on *Bt* and non-*Bt* maize  
298 inoculated with and without GC. The GC inoculation markedly extended the larval life-span and  
299 shortened the adult longevity, and significantly decreased the pupal weight and fecundity of fed  
300 on *Bt* maize in 2017 and 2018; while significantly shortened the larval life-span and prolonged  
301 the adult longevity, and significantly increased the pupation rate, pupal weight and fecundity of  
302 *M. separata* fed on non-*Bt* maize in 2017 and 2018, and significantly increased the eclosion rate  
303 of *M. separata* fed on non-*Bt* maize in 2018. This phenomenon can be explained that AMF  
304 inoculation promoted the absorption and utilization of soil nutrients by maize plant, thus  
305 improving the nutrient level (e.g., nitrogen, phosphorus and potassium) of plant leaves (Hodge,  
306 2010; Rodriguez & Sanders, 2015). For non-*Bt* maize, the leaf food source with high nutrition  
307 means the intake and utilization of high nutrient elements, which naturally had a more positive  
308 and beneficial effect on *M. separata*. For *Bt* maize, an increase in nutrient levels may also mean  
309 an increase in toxin protein expression, higher toxins were bound to make more damage to the  
310 *M. separata* which could account for the inverse trends on RGR, RCR, AD, and ECI fed on *Bt*  
311 maize and non-*Bt* maize inoculated with GC.

312

## 313 Conclusion

314 This research indicated that the inoculation of arbuscular mycorrhizal fungi (AMF), *G.*  
315 *caledonium* (GC) had positive effects on the AMF colonization of *Bt* maize or non-*Bt* maize,  
316 which was, in turn, resulting in higher yields of *Bt* maize relative to non-*Bt* maize regardless of  
317 GC inoculation or the CK (i.e., non-GC inoculation), and the cultivation of *Bt* maize didn't  
318 significantly affected the GC colonization. Moreover, *Bt* maize had marked adverse effects on  
319 the food utilization, the reproduction, growth, and development of *M. separata* especially in  
320 combination with the GC inoculation. Furthermore, the GC inoculation was viable for *Bt* maize  
321 against larvae due to its ability to reduce their food utilization ability, and then negatively  
322 affecting the reproduction, growth, and development of *M. separata*. Simultaneously, the GC  
323 inoculation had adverse effects on the production of non-*Bt* maize due to the high potential risk  
324 of population occurrence through enhancing their food utilization ability, and positively affecting  
325 the reproduction, growth, and development of *M. separata*. The results indicated that the AMF  
326 inoculation of GC was conducive to improve the performance of *Bt* maize for *M. separata*  
327 control, and it was also a very friendly and effective way for increasing the yield and reducing  
328 fertilizer use of crop plants, therefore, we considered that AMF will play important ecological  
329 functions in the future *Bt* maize ecosystem.

330

331 **Acknowledgements**

332 Many thanks to Prof. Dr Wenwei Xu, Texas A&M University, Agricultural Research and  
333 Extension Center, for his help in the design and treatment setup of this experiment, and revising  
334 the manuscript.

335

336 **References**

- 337 **Alyokhin A, 2011.** Scant evidence supports EPA's pyramided *Bt* corn refuge size of 5%. *Nat.*  
338 *Biotechnol* **29(7)**: 577–578.
- 339 **Bennett AE, Bowers JDBD. 2009.** Arbuscular mycorrhizal fungal species suppress inducible  
340 plant responses and alter defensive strategies following herbivory. *Oecologia* **160(4)**:771-779  
341 DOI 10.1007/s00442-009-1338-5.
- 342 **Castaldini M, Turrini A, Sbrana C, et al. 2005.** Impact of *Bt* corn on rhizospheric and soil  
343 eubacterial communities and on beneficial mycorrhizal symbiosis in experimental  
344 microcosms. *Applied and Environmental Microbiology* **71(11)**: 6719-6729 DOI  
345 10.1128/AEM.71.11.6719-6729.2005.
- 346 **Cheeke TE, Cruzan MB, Rosenstiel TN. 2013.** Field evaluation of arbuscular mycorrhizal  
347 fungal colonization in bacillus thuringiensis toxin-expressing (bt) and non-bt maize. *Applied*  
348 *& Environmental Microbiology* **79(13)**: 4078-4086 DOI 10.1128/AEM.00702-13.
- 349 **Chen FJ, Wu G, Ge F, Parajulee MN, Shrestha RB. 2005.** Effects of elevated CO<sub>2</sub> and  
350 transgenic *Bt* cotton on plant chemistry, performance, and feeding of an insect herbivore, the  
351 cotton bollworm. *Entomologia Experimentalis et Applicata* **115**: 341-350 DOI  
352 10.1111/j.1570-7458.2005.00258.x.
- 353 **Gao HJ, Xiao NW, Li JS, Chen FJ, Zhai BP. 2009.** Effects of double atmospheric CO<sub>2</sub>  
354 concentration on nitrogen metabolism of transgenic *Bt* cotton under different nitrogen  
355 fertilization levels. *Chinese Journal of Ecology* **28(11)**: 2213-2219 DOI 10.13292/j .1000-  
356 4890.2009.0378.
- 357 **Guo JF, He KL, Hellmich RL, Bai SX, Zhang TT, Liu YJ, Ahmed T, Wang ZY. 2016.** Field  
358 trials to evaluate the effects of transgenic *cryIIe* maize on the community characteristics of  
359 arthropod natural enemies. *Scientific reports* **6(12)**: 22102 DOI 10.1038/srep22102.
- 360 **Hodge A. 2010.** Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology* **3**:267-273  
361 DOI 10.1016/j.funeco.2010.02.002.
- 362 **Huang FN, Qureshi JA, Meagher JRL, Reisig DD, Head GP, Andow DA, Ni XZ, Kerns D,**  
363 **Buntin GD, Niu Y, Yang F, Dangal V. 2014.** *CryIF* resistance in fall armyworm  
364 *Spodoptera frugiperda*: single gene versus pyramided *Bt* maize. *Plos One* **9(11)**: e112958  
365 DOI 10.1371/journal.pone.0112958.
- 366 **Icoz I, Stotzky G. 2008.** Fate and effects of insect-resistant *Bt* crops in soil ecosystems. *Soil*  
367 *Biology & Biochemistry* **40(3)**:559 –586 DOI 10.1016/j.soilbio.2007.11.002

- 368 **ISAAA, 2017.** Global status of commercialized biotech/GM Crops in 2017: biotech crop  
369 adoption surges as economic benefits accumulate in 22 years. ISAAA Brief No. 53. ISAAA:  
370 Ithaca, NY.
- 371 **James C.** 2012. Global status of commercialized biotech/GM crops: 2011. ISAAA brief no. 43.  
372 International Service for the Acquisition of Agri-Biotech Applications.  
373 <http://www.isaaa.org/resources/publications/briefs/43/default.asp>. Accessed 15 November  
374 2012.
- 375 **Jiang Y, Huang S, Cai M, Li C, Kong X, Zhang F, Mohamed I, Cao C.** 2013. Yield changes  
376 of Bt-MH63 with Cry1C\* or cry2A\* genes compared with MH63 (*Oryza sativa*) under  
377 different nitrogen levels. *Field Crops Research* **151**: 101-106 DOI 10.1016/j.fcr.2013.06.017.
- 378 **Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ.** 2012. Mycorrhiza-induced  
379 resistance and priming of plant defenses. *Journal of Chemistry Ecology* **38(6)**: 651-664 DOI  
380 10.1007/s10886-012-0134-6.
- 381 **Koricheva J, Jones GT.** 2009. Effects of mycorrhizal fungi on insect herbivores: a  
382 metaanalysis. *Ecology* **90(8)**: 2088–2097 DOI 10.1890/08-1555.1.
- 383 **Knox OGG, Nehl DB, Mor T, et al.** 2008. Genetically modified cotton has no effect on  
384 arbuscular mycorrhizal colonisation of roots. *Field Crops Research* **109(1-3)**: 57-60 DOI  
385 10.1016/j.fcr.2008.06.005
- 386 **Li Z, Parajulee MN, Chen FJ.** 2018. Influence of elevated CO<sub>2</sub> on development and food  
387 utilization of armyworm *Mythimna separata* fed on transgenic Bt maize infected by nitrogen-  
388 fixing bacteria. *PeerJ* **6(3)**: e5138 DOI 10.7717/peerj.5138.
- 389 **Lawo NC, Wäckers FL, Romeis J.** 2010. Characterizing indirect prey-quality mediated effects  
390 of a Bt crop on predatory larvae of the green lacewing, *Chrysoperla carnea*. *Journal of Insect*  
391 *Physiology* **56(11)**: 1702-1710 DOI 10.1016/j.jinsphys.2010.06.012.
- 392 **Lu YH, Wu KM, Jiang YY, Guo YY, Desneux N.** 2012. Widespread adoption of Bt cotton and  
393 insecticide decrease promotes biocontrol services. *Nature* **487(487)**: 362-365 DOI  
394 10.1038/nature11153.
- 395 **Pardo-López L, Soberón M, Bravo A,** 2013. *Bacillus thuringiensis* insecticidal three-domain  
396 Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEMS*  
397 *Microbiol. Rev* **37(1)**: 3–22.
- 398 **Parniske M.** 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature*  
399 *Reviews Microbiology* **6(10)**763-775 DOI 10.1038/nrmicro1987.
- 400 **Prutz G, Dettner K.** 2005. Effects of transgenic *Bacillus thuringiensis*-maize on larval food  
401 consumption, utilization and growth in the grass-moth species *Chilo partellus* under  
402 laboratory conditions (Lepidoptera: Crambidae). *Entomologia Generalis* **28(3)**: 161-172 DOI  
403 10.1127/entom.gen/28/2005/161.
- 404 **Reynolds SE, Nottingham SF, Stephens AE.** 1985. Food and water economy and its relation to  
405 growth in fifth instar larvae of tobacco hornworm, *Manduca sexta*. *Journal of Insect*  
406 *Physiology* **31(2)**: 119-127 DOI 10.1016/0022-1910(85)90016-2.
- 407 **Rodriguez A, Sanders IR.** 2015. The role of community and population ecology in applying

- 408 mycorrhizal fungi for improved food security. *ISME Journal* **9**: 1053–1061 DOI  
409 10.1038/ismej.2014.207.
- 410 **Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P, 2011.** *Bacillus thuringiensis*  
411 : a century of research, development and commercial applications. *Plant Biotechnol. J* **9(3)**: 283–  
412 300.
- 413 **Sanchis V. 2011.** From microbial sprays to insect-resistant transgenic plants: history of the  
414 biospesticide *Bacillus thuringiensis*. *Agronomy for Sustainable Development* **31**: 217–231  
415 DOI 10.1051/agro/2010027.
- 416 **Singh PK, Singh M, Tripathi BN. 2012.** Glomalin: an arbuscular mycorrhizal fungal soil  
417 protein. *Protoplasma* **250(3)**: 663–669 DOI 10.1007/s00709-012-0453-z.
- 418 **Smith SE, Read DJ. 2008.** Mycorrhizal symbiosis. *Quarterly Review of Biology* **3(3)**: 273–281  
419 DOI 10.1097/00010694-198403000-00011.
- 420 **Smith CM, Fischer NH. 1983.** Chemical factors of an insect resistant soybean genotype  
421 affecting growth and survival of the soybean looper. *Entomologia Experimentalis Et*  
422 *Applicata* **33(3)**:343–345 DOI 10.1111/j.15 70-7458.1 983.tb03 278.x.
- 423 **Steinkellner S, Hage-Ahmed K, García-Garrido JM, Illana A, Ocampo JA, Vierheilig H.**  
424 **2012.** A comparison of wild-type, old and modern tomato cultivars in the interaction with the  
425 arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium*  
426 *oxysporum* f. sp. *lycopersici*. *Mycorrhiza* **22(3)**: 189–194 DOI 10.1007/s00572-011-0393-z.
- 427 **Stitt M, Krapp A. 1999.** The interactions between elevated carbon dioxide and nitrogen  
428 nutrition: the physiological and molecular background. *Plant Cell & Environment* **22(6)**:  
429 583–621 DOI 10.1046/j.1365-3040.1999.00386.x.
- 430 **Turrini A, Sbrana C, Nuti MP, Pietrangeli BM, Giovannetti M. 2005.** Development of a  
431 model system to assess the impact of genetically modified corn and aubergine plants on  
432 arbuscular mycorrhizal fungi. *Plant and Soil* **26 (6)** : 69-75 DOI 10.1007/s11104-005-  
433 4892-6.
- 434 **Vannette RL, Hunter MD. 2011.** Plant defence theory reexamined: nonlinear expectations  
435 based on the costs and benefits of resource mutualisms. *J. Ecol.* **99**:66–76 DOI  
436 10.1111/j.1365-2745.2010.01755.x.
- 437 **Vannette RL, Rasmann S. 2012.** Arbuscular mycorrhizal fungi mediate below-ground plant-  
438 herbivore interactions: a phylogenetic study. *Funct. Ecol* **5**: 1033–1042 DOI: 10.1111/j.1365-  
439 2435.2012. 02046.x.
- 440 **Vaufleury AD, Kramarz PE, Binet P, et al. 2007.** Exposure and effects assessments of Bt-  
441 maize on non-target organisms (gastropods, microarthropods, mycorrhizal fungi) in  
442 microcosms. *Pedobiologia* **51**:185-194 DOI 10.1016/j.pedobi.2007.04.005.
- 443 **Walters FS, Fontes CM, Hart H, Warren GW, Chen JS. 2010.** Lepidopteran-active variable-  
444 region sequence imparts Coleopteran activity in *eCry3.1Ab*, an engineered *Bacillus*  
445 *thuringiensis* hybrid insecticidal protein. *Applied & Environmental Microbiology* **76(10)**:  
446 3082-3088 DOI 10.1128/AEM.00155-10.
- 447 **Wolfe BE, Husband BC, and Klironomos JN. 2005.** Effects of a belowground mutualism on

448 an aboveground mutualism. *Ecol. Lett* **8**: 218–223 DOI 10.1111/j.1461-0248.2004.00716.x.  
449 **Zeng H, Tan F, Zhang Y, Feng Y, Shu Y, Wang J. 2014.** Effects of cultivation and return of  
450 bacillus thuringiensis (bt) maize on the diversity of the arbuscular mycorrhizal community in  
451 soils and roots of subsequently cultivated conventional maize. *Soil Biology and Biochemistry*  
452 **75**: 254-263 DOI 10.1016/j.soilbio.2014.04.024.

**Table 1** (on next page)

Three-way repeated-measures ANOVAs on the AMF colonization and three-way ANOVAs on the yields of *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)



**Table 1** Three-way repeated-measures ANOVAs on the AMF colonization and three-way ANOVAs on the yields of *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)

Factors	Colonization (%)	Grain weight per plant (dry; g)	1000-grain weight (dry; g)
Y <sup>a</sup>	70.10/<0.001***	90.57/<0.001***	0.05/0.83
Cv. <sup>b</sup>	0.49/0.49	0.23/0.64	5.87/0.028*
AMF <sup>c</sup>	6673.63/<0.001***	144.17/<0.001***	92.54/<0.001***
Y×Cv.	7.80/0.013*	4.96/0.041*	17.96/0.001**
Y×AMF	48.56/<0.001***	8.11/0.012*	0.12/0.73
Cv.×AMF	0.04/0.85	0.01/0.92	0.14/0.71
Y×Cv.×AMF	0.02/0.91	2.10/0.17	0.30/0.59

**Note:** \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; <sup>a</sup>: Year (2017 vs. 2018); <sup>b</sup>: Transgenic treatment (*Bt* maize vs. non-*Bt* maize); <sup>c</sup>: AMF inoculation (GC vs. CK).

**Table 2** (on next page)

Three-way ANOVAs on the food utilization of *M. separata* from the 3rd to the 6th instar larvae fed on *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)

**Table 2** Three-way ANOVAs on the food utilization of *M. separata* from the 3rd to the 6th instar larvae fed on *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)

Factors	RGR (mg g <sup>-1</sup> d <sup>-1</sup> )	RCR (mg g <sup>-1</sup> d <sup>-1</sup> )	ECD (%)	ECI (%)	AD (%)
Y <sup>a</sup>	9.49/0.007**	2.44/0.14	128.14/<0.001***	18.04/0.002**	222.90/<0.001***
Cv. <sup>b</sup>	1176.29/<0.001***	418.12/<0.001***	990.94/<0.001***	804.52/<0.001***	485.47/<0.001***
AMF <sup>c</sup>	65.72/<0.001***	6.60/0.021*	0.06/0.82	11.67/0.004**	9.20/0.008**
Y×Cv.	7.83/0.013*	10.06/0.006**	1.20/0.29	2.61/0.13	11.01/0.004**
Y×AMF	7.19/0.016*	4.16/0.058	0.61/0.45	0.51/0.49	0.80/0.39
Cv.×AMF	303.71/<0.001***	91.07/<0.001***	3.13/0.096	104.96/<0.001***	138.78/<0.001***
Y×Cv.×AMF	2.58/0.021*	0.01/0.94	0.05/0.83	2.54/0.13	0.44/0.52

**Note:** \**P*<0.05, \*\* *P*<0.01, \*\*\* *P*<0.001; <sup>a</sup>: Year (2017 vs. 2018); <sup>b</sup>: Transgenic treatment (*Bt* maize vs. non-*Bt* maize); <sup>c</sup>: AMF inoculation (GC vs. CK).

**Table 3** (on next page)

Three-way ANOVAs on the growth, development and reproduction of *M. separata* fed on *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)

**Table 3** Three-way ANOVAs on the growth, development and reproduction of *M. separata* fed on *Bt* and non-*Bt* maize inoculated with and without *G. caledonium* in 2017 and 2018 (*F/P* values)

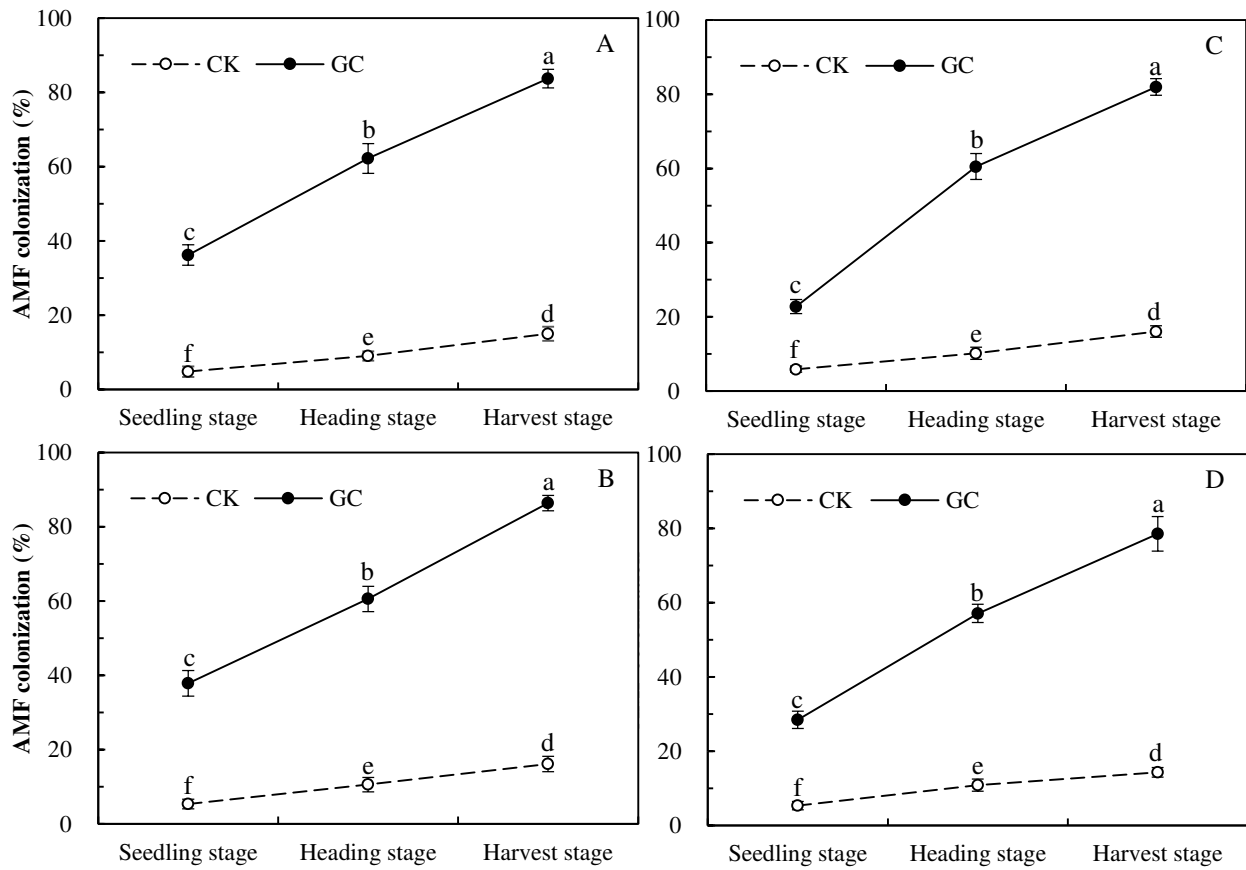
Factors	Larval life-span (day)	Pupation rate (%)	Pupal weight (g)	Eclosion rate (%)	Adult longevity (day)	Fecundity (eggs per female)
Y <sup>a</sup>	3.34/0.077	1.97/0.17	19.11/<0.001***	2.58/0.12	0.40/0.53	1.54/0.22
Cv. <sup>b</sup>	6.64/0.015*	275.83/0.008**	5.42/0.026*	320.94/<0.001***	934.67/<0.001***	516.08/<0.001***
AMF <sup>c</sup>	6.15/0.019*	7.87/0.008**	5.30/0.028*	6.73/0.014*	16.68/<0.001***	6.01/<0.020*
Y×Cv.	8.06/0.008**	0.87/0.36	1.52/0.23	0.14/0.71	1.80/0.19	0.14/0.71
Y×AMF	7.44/0.011*	0.06/0.82	0.51/0.48	2.58/0.12	4.39/0.044*	9.82/0.004**
Cv.×AMF	0.10/0.75	7.87/0.008**	27.38/<0.001***	11.13/<0.002**	341.13/<0.001***	162.70/<0.001***
Y×Cv.×AMF	6.91/0.013*	0.49/0.49	0.64/0.43	1.85/0.18	2.82/0.10	7.63/0.009**

**Note:** \* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; <sup>a</sup>: Year (2017 vs. 2018); <sup>b</sup>: Transgenic treatment (*Bt* maize vs. non-*Bt* maize); <sup>c</sup>: AMF inoculation (GC vs. CK).

**Figure 1**(on next page)

AMF colonization dynamics of transgenic *Bt* maize (A and C) and non-*Bt* maize (B and D) inoculated with and without *G. caledonium* in 2017 (A and B) and 2018 (C and D).

Different lowercase letters indicate significant differences between treatments of GC inoculation and the control group with maize growth stages as repeated measures in same sampling year by the Turkey test at  $P < 0.05$ .

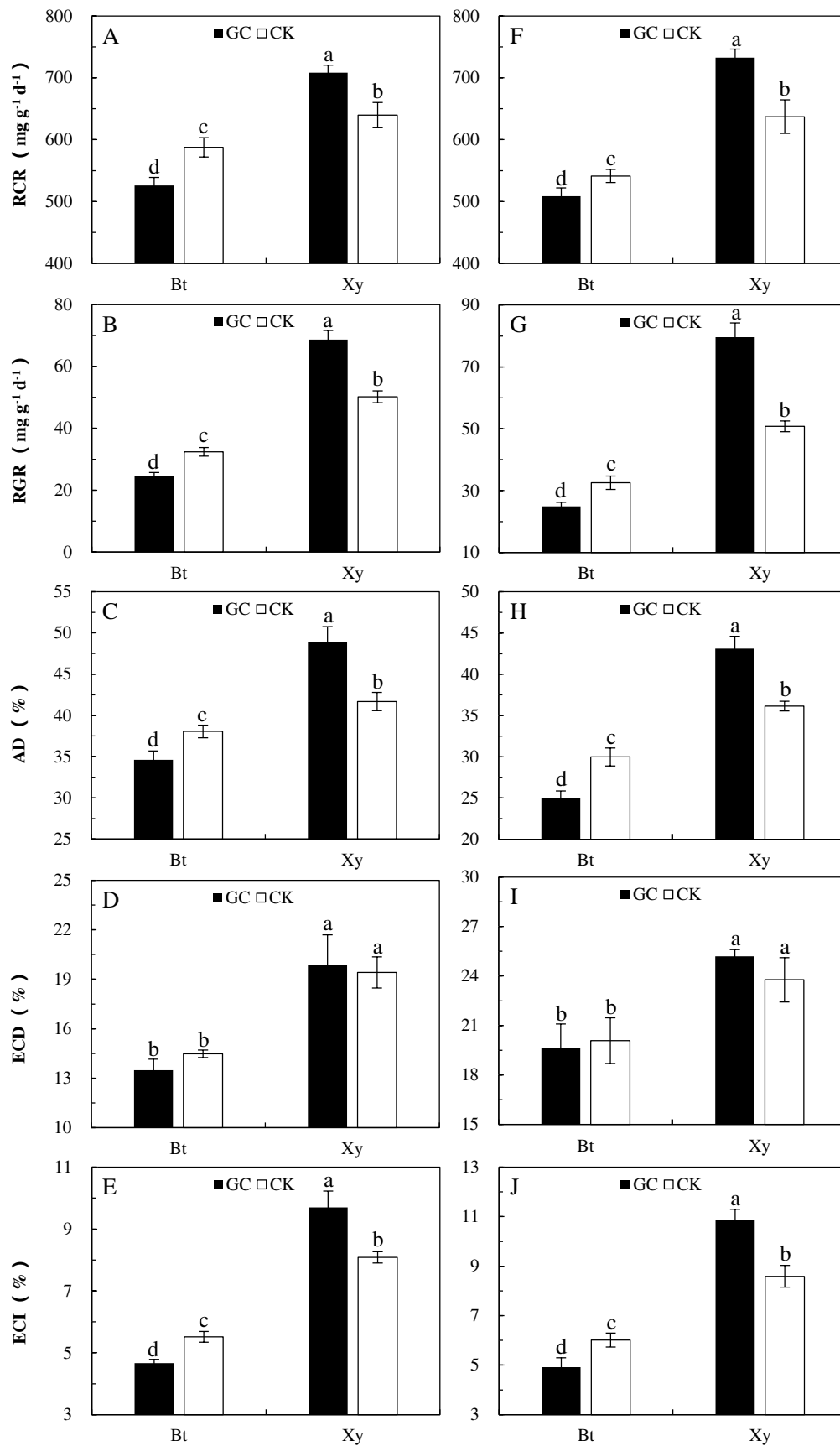


**Figure 2** (on next page)

Food utilization of *M. separata* from the 3rd to the 6th instar larvae fed on the *Bt* maize (Bt) and non-*Bt* maize (Xy) inoculated with and without *G. caledonium* in 2017 (A-E) and 2018 (F-J).

Different lowercase letters indicated significant differences between treatments in same sampling year by the Turkey test at  $P < 0.05$ .

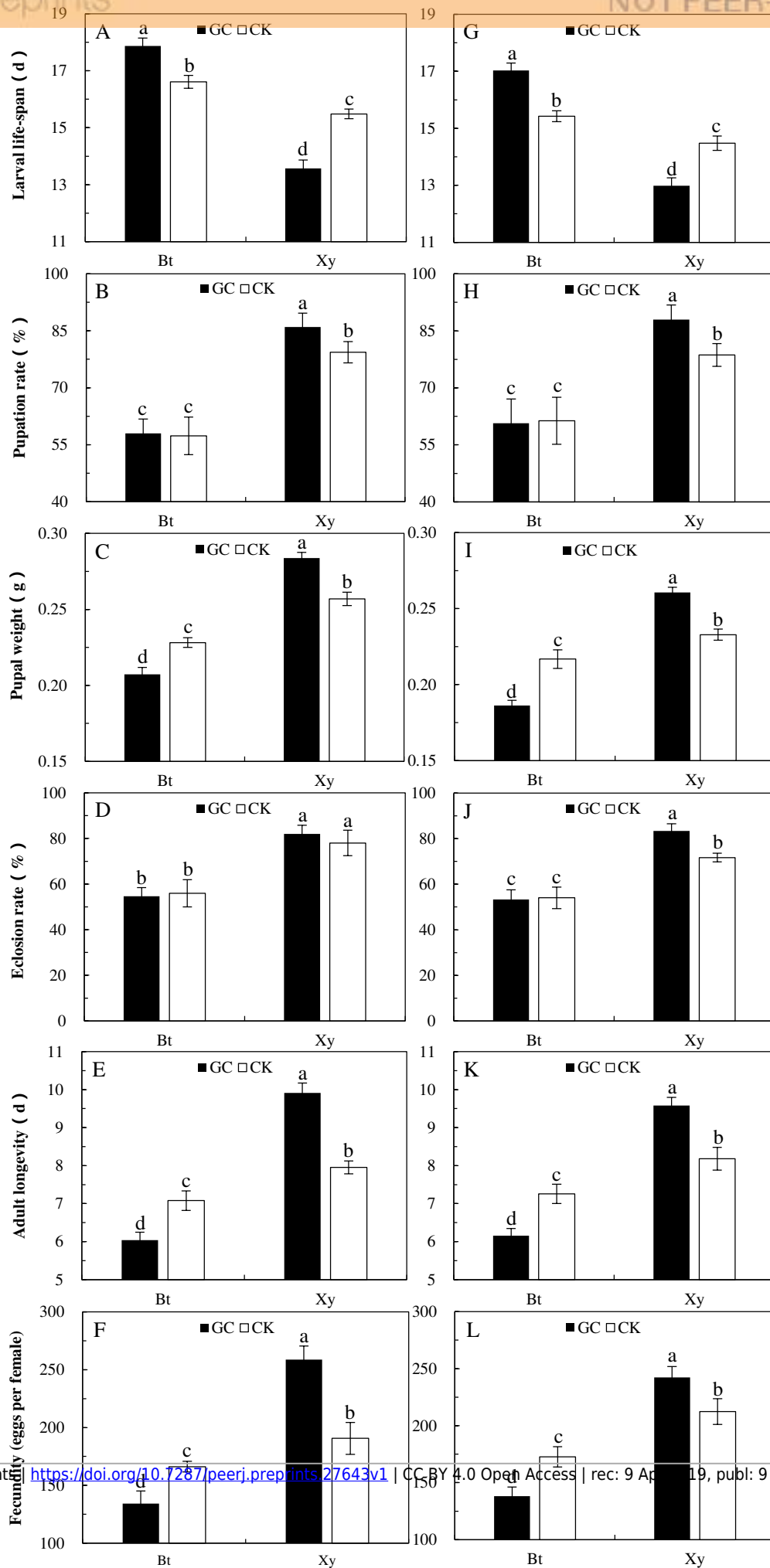




**Figure 3**(on next page)

Growth, development and reproduction of *M. separata* fed on *Bt* maize (Bt) and non-*Bt* maize (Xy) inoculated with and without *G. caledonium* in 2017 (A-F) and 2018 (G-L).

Different lowercase letters indicated significant differences between treatments in same sampling year by the Tukey test at  $P < 0.05$ .



**Figure 4**(on next page)

Grain weight per plant (A and C) and 1000-grain weight (B and D) of *Bt* maize (Bt) and non-*Bt* maize (Xy) inoculated with *G. caledonium* in 2017 (A and B) and 2018 (C and D).

Different lowercase letters indicate significant differences between treatments in same sampling year by the Tukey test at  $P < 0.05$ .

