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## Impact of control strategies on Thrips tabaci and its predator Aeolothrips intermedius on onion crops

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| 1        | Impact of control strategies on Thrips tabaci and its predator Aeolothrips intermedius on onion |
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### 15 Abstract

16 Thrips tabaci (Thysanoptera: Thripidae) is a major pest of onion worldwide. In 2011, research was 17 conducted in a commercial onion field in northwestern Italy to: (i) assess the presence of 18 autochthonous onion thrips predators on the crop; and (ii) evaluate the impact of the commonly 19 used insecticides and alternative pest management strategies on onion thrips and its autochthonous 20 predators. Toxicity of the active ingredients on local populations of onion thrips and its predatory 21 thrips was also evaluated in laboratory bioassays. During field surveys, the highest and lowest 22 thrips infestations were observed in plots treated with lambda-cyhalothrin and spinosad, 23 respectively. The effectiveness of spinosad on T. tabaci was also confirmed in laboratory bioassays. 24 The dominant zoophagous species Aeolothrips intermedius (Thysanoptera: Aeolothripidae) was 25 more adversely affected by treatment with lambda-cyhalothrin, confirmed by a decrease in 26 predator/prey ratios. The use of spinosad and acibenzolar-S-methyl is suggested as an alternative to 27 conventional insecticides for the preservation of A. intermedius, which proved to be a potential 28 biological control agent of T. tabaci.

29

30 Keywords Acibenzolar-S-methyl – Lambda-cyhalothrin – Onion thrips – Predator thrips –
 31 Spinosad – Thysanoptera –

32

#### 34 Introduction

35 Thrips tabaci Lindeman (Thysanoptera: Thripidae) is one of the most serious pests of onion (Allium cepa L.) and other Allium spp. in many parts of the world (MacIntyre Allen et al. 2005; Martin et 36 37 al. 2003; Nault & Shelton 2010). Moreover, T. tabaci is the vector of Iris yellow spot virus (IYSV), 38 genus Tospovirus (Bunyaviridae), a severe and widespread disease infecting onion, leek, iris and 39 wild Allium species (Gent et al. 2006; Nagata et al. 1999; Pappu et al. 2009). Chemical treatments 40 are the main method used by onion growers for thrips control. However, in Italy there is a restricted 41 range of authorized products, predominantly pyrethroids (e.g. lambda-cyhalothrin), to which onion 42 thrips can develop high levels of resistance (Martin et al. 2003; Shelton et al. 2006). Additionally, 43 some of these insecticides have side effects, affecting biological control agents either directly (*i.e.*, 44 physiological or behavioral effects) or indirectly (e.g. habitat destruction, oviposition, resting, 45 mating sites) (Desneux et al. 2007). Therefore, it is important to develop new reduced-risk 46 insecticides to use in Integrated Pest Management (IPM) programs with minimal impact on 47 beneficial arthropods (Jones et al. 2005). In this regard, spinosad is known to be effective against T. 48 tabaci (Gent et al. 2006; Shelton et al. 2006; Yarahmadi et al. 2009), with a low to moderate 49 toxicity to thrips' common natural enemies (Jones et al. 2005; Ludwig & Oetting 2001; Workman 50 & Martin 2002).

51 Pesticide impact on human and environmental health begs for alternative pest management 52 approaches, such as the use of straw mulch (Gent et al. 2006; Larentzaki et al. 2008; Momol et al. 53 2004), of intercrops (Trdan et al. 2006), and of thrips-resistant onion cultivars (Diaz-Montano et al. 2010), or the evaluation of new bio-insecticides (Patil et al. 2009) and biologically active plant 54 55 volatiles (Koschier et al. 2002) against T. tabaci. Moreover, activators of natural systemic acquired 56 resistance (SAR), such as acibenzolar-S-methyl, appear promising for reducing the use of 57 conventional insecticides (Mautino et al. 2012). Acibenzolar-S-methyl was used as an effective 58 alternative to many bactericides and fungicides (Gent et al. 2006; Momol et al. 2004; Pappu et al.

2000), and for the control of tospoviruses and their thrips vectors (Gent *et al* 2006; Momol *et al*.
2004).

61 Furthermore, the conservation and augmentation of predators feeding on thrips are important 62 control strategies. Different generalist predators are known to be effective biocontrol agents, and they are now commercially available. These predators include anthocorids of the genus Orius 63 64 (Hemiptera: Anthocoridae) (Bosco et al. 2008; Funderburk et al. 2000; Riudavets 1995), mirids of 65 the genera Dicyphus and Macrolophus (Hemiptera: Miridae) (Gabarra et al. 1995; Riudavets et al. 1993), and mites of the genus Amblyseius (Acarina: Phytoseiidae) (Wimmer et al. 2008). There are 66 67 comparatively few data on the predatory efficiency of the autochthonous thrips species belonging to 68 the genera Aeolothrips, Haplothrips and Franklinothrips (Thysanoptera: Aeolothripidae), that are 69 potential biological control agents of T. tabaci (Cox et al. 2006; Fathi et al. 2008; Kakimoto et al. 70 2006; Trdan et al. 2005a).

71 Integration of chemical, biological, agronomic and physical control options is increasingly 72 necessary in order to maintain pest populations below economic damage thresholds (Cox et al. 73 2006), particularly upon consideration of the weak efficacy of pyrethroids (especially lambda-74 cyhalothrin) that are widely used on onion (Herron et al. 2008; MacIntyre Allen et al. 2005; 75 Mautino et al. 2012). Moreover, the possible impact on beneficial arthropods should be carefully 76 monitored to preserve the presence and abundance of the natural populations in the field. 77 Independent of the type of insecticide employed, chemical control still remains the primary 78 strategy for pest control on onion crops; therefore, supervised control based on action thresholds or 79 tolerance levels consistent with IPM programs is always recommended for growers (Nault & Shelton 2010). 80

81 Consequently, this study focused on: (i) assessing the entity of autochthonous predators of *T*. 82 *tabaci* on the onion crop; and (ii) evaluating the impact of the commonly used insecticides and 83 alternative pest management strategies on onion thrips and its autochthonous predators.

#### 85 Materials and methods

86 *Field experiments* Research was conducted in 2011, in a commercial onion field of approximately 8 ha located in Castellazzo Bormida (province of Alessandria, Piedmont, 44°50'45" 87 N, 8°34'41" E, 105 m a.s.l.). The experimental site was flanked on all sides by at least 2 m of 88 insecticide-free onions within the grower's field. Experimental plots (10.5  $m^2$  each) consisted of six 89 90 onion rows 7 m in length, with rows spaced every 0.2 m. Plots were separated within rows by 1.0 91 m and spaced every 1.5 m. Onions of the golden onion cultivar 'Derek' were seeded in April; 92 diseases and weeds were controlled using products recommended for onion production 93 (pendimethanil, ioxynil, metalaxyl-M + copper, dimethomorph + pyraclostrobin, cyprodinil + 94 fludioxonil).

95 The trial was arranged in a randomized complete block design with four replicates for each 96 of six treatments (24 plots). The treatments consisted of one untreated control (T1); one treatment 97 based on four repeated pyrethroid applications (T2); one treatment based on one pyrethroid 98 application at the action threshold (*i.e.*, when mean number of thrips sampled by plant beating 99 exceeded two thrips per plant) (T3); one treatment based on two spinosad applications (T4); one 100 treatment based on four applications of the plant activator acibenzolar-S-methyl (T5); one treatment 101 based on two acibenzolar-S-methyl applications, followed by two spinosad applications (T6) (Table 1). The active ingredients and formulations that we tested were: acibenzolar-S-methyl 500 g a.i.  $kg^{-1}$ 102 (Bion<sup>®</sup> 50 WG, Syngenta Crop Protection, Milano, Italy); lambda-cyhalothrin 15 g a.i. l<sup>-1</sup> (Karate<sup>®</sup> 103 Zeon 1.5, Syngenta Crop Protection); spinosad 120 g a.i.  $l^1$  (Success<sup>®</sup> SC, Dow AgroSciences, 104 105 Milano, Italy). Chemicals were applied at the manufacturer's recommended field rates with a precision shoulder sprayer, using 600 l of solution ha<sup>-1</sup> of onion crop and producing a fine mist to 106 107 ensure effective coverage. The delivery pressure at the nozzle was 300 kPa. Rate and timing of 108 applications are listed in Table 1.

109 Commencing in May, onion plots were surveyed weekly for the presence of *T. tabaci* and 110 their predators. At the first occurrence of onion thrips infestation on the crop (pre-sampling, *i.e.*, 111 pre-S, June 15), insecticide applications were sprayed, in relation to the treatment, on June 19, June 112 29, July 9 and July 19. Sampling was carried out 3 days after each cluster of sprays (i.e., S1, June 113 23; S2, July 4; S3, July 13; S4, July 22). Thrips tabaci, thrips predators including anthocorid, mirid 114 bugs (Hemiptera: Anthocoridae, Miridae), and predatory thrips (Thysanoptera: Aeolothripidae) 115 were detected during field surveys. Plant beating was chosen as the sampling method for its high 116 feasibility, and because a strong relationship between this method and visual inspection has 117 previously been observed, and also for the larval population, generally underestimated with the 118 beating sampling method (Mautino et al. 2012). Five plants were randomly selected at three points 119 in each plot (15 plants per plot), and these were beaten over a plastic tray ( $350 \times 250$  mm). Thrips 120 adults and larvae, and their predators, were counted, collected with a mouth aspirator and 121 transferred to the laboratory. Subsequently, adult onion thrips were observed under a 122 stereomicroscope at 160× magnification and identified to the species level according to Mound et al. (1976). For predatory thrips, 10% to 20% of total adults sampled on each sampling date, and in 123 124 each treatment, were mounted on microscope slides and identified under a compound microscope according to Schliephake & Klimt (1979). Thrips larvae were observed under a stereomicroscope at 125 126 160× magnification and attributed to the family Aeolothripidae or Thripidae, according to 127 Vierbergen et al. (2010).

128 Data on local weather conditions during field experiments were provided by Rete

129 Agrometeorologica, Regione Piemonte, Settore Fitosanitario. In particular, the following average

130 temperatures were recorded: T mean 18.7°C, T max 27.4°C, T min 10.8°C in May; T mean 20.8°C,

131 T max 27.3°C, T min 15.5°C in June; T mean 22.2°C, T max 29.1°C, T min 16.1°C in July.

132 Rainfall was 34.2, 102.6 and 33.8 mm in May, June and July, respectively.

133 Laboratory bioassays Adults of T. tabaci and predatory thrips collected in untreated plots on

134 July 13 and July 22 (S3 and S4) were tested in laboratory bioassays. Field-collected thrips were

temporarily transferred to 1 *l* gauze-covered glass jars (approximately 200 thrips per jar), with

136 corrugated cardboard on the bottom to provide pupation sites, and paper to avoid humidity. To

provide food sources and oviposition sites, jars were supplied with pollen and green bean pods [*Phaseolus vulgaris* L. (Fabaceae)] for *T. tabaci*, and with leek leaves [*Allium porrum* L. (Alliaceae)], previously infested by *T. tabaci* (providing live prey), for predatory thrips. Mass rearing was conducted in growth chambers at  $25 \pm 1^{\circ}$ C,  $65 \pm 5\%$  r.h. and a 16h:8h L:D cycle (Tedeschi *et al.* 2001).

142 The toxicity of tested products was evaluated on *T. tabaci* and predatory thrips using the vial 143 bioassay method described by Mautino et al. (2012), which is an adaptation of the thrips 144 insecticides bioassay system (TIBS) described by Rueda & Shelton (2003). Thrips were collected 145 from rearing jars and placed into a plastic microcentrifuge tube previously treated with the product 146 being tested; the tube cap contained a small well with 10% sugar-water solution. The solution was 147 sealed into the well with a small piece of stretched parafilm through which thrips could feed on the 148 sugar solution. The tube, but not the cap, was treated with the product (or water for the untreated 149 control), and after 4 h the chemical solution (or water) was poured out and the tube was allowed to 150 dry overnight. Specifically, ten T. tabaci females and five predatory thrips females were introduced 151 separately into each treated tube. Five replicates were used for each of the four treatments: untreated 152 control, lambda-cyhalothrin, spinosad and acibenzolar-S-methyl. The active ingredients were used 153 in the same formulations and doses as those adopted in the field experiment. Acibenzolar-S-methyl 154 is a plant defense activator and it is supposed to have no effect by contact or fumigation on insects; 155 nonetheless, it was tested in the vial bioassay to evaluate a potential side effect (e.g. presence of 156 adjuvant compounds) on thrips, even in the absence of the plant. Thrips survival was assessed after 24 h and 48 h with the use of a stereomicroscope: thrips which did not move after 2 min of 157 158 observation were considered dead. The vial bioassay was replicated three times for both onion 159 thrips and predatory thrips.

Statistical analyses For the field data, the mean numbers of total (adults plus larvae) *T. tabaci* and
 predatory thrips per plant were log-transformed to achieve homogeneity of variance (Levene) and

162 normality (Shapiro-Wilk), and analyzed by Univariate Analysis of Variance (ANOVA) for

163 randomized blocks (treatments and blocks were the factors).

164 To describe the effect of treatments on the relationship between phytophagous and predatory 165 thrips, the predator/prey ratios were calculated from the mean number of total thrips per plant for each sampling date. Ratio values were log-transformed to achieve homogeneity of variance 166 167 (Levene) and normality (Shapiro-Wilk), and analyzed by ANOVA and Tukey's post hoc test. 168 Percentage survival data obtained in the laboratory bioassays for predatory thrips and T. 169 tabaci were separately transformed to arcsine square-root values before analysis; the non-170 parametric Kruskal-Wallis was chosen since data were non-homogeneous, and means were 171 compared using the Mann-Whitney U test. For each treatment, differences between the survival 172 data of T. tabaci and predatory thrips were analyzed by ANOVA after tests of homogeneity of 173 variance (Levene) and normality (Shapiro-Wilk).

All statistical analyses were performed using SPSS statistical package (version 17.0; SPSS,
Chicago, IL, USA).

176

#### 177 **Results**

178 Field experiments

179 *Treatment efficacy against onion thrips Thrips tabaci* was the dominant phytophagous species 180 collected on onions, with populations composed of both adults and larval stages. Over 96% of the 181 total sampled adult thrips (n = 2,787) belonged to this species, while the remaining 23 and 79 adult 182 thrips belonged to Frankliniella intonsa Trybom and to Haplothrips spp., respectively. Overall, 183 larval stages were 13% of the total thrips sampled by plant beating (n = 3,208); among the 184 treatments, percentages of Thripidae larvae varied from 7.6% in T6 to 19.0% in T2. Moreover, the 185 average percentages of larvae varied throughout the growing season: 1.6%, 6.0%, 31.6%, 6.7%, and 186 20.8% of total specimens sampled on June 15, June 23, July 4, July 13 and July 22, respectively.

187 The mean numbers of total (adults plus larvae) onion thrips collected in the plots of each 188 treatment are reported in Table 2. On June 15 (Pre-S), before the first chemical application, mean 189 numbers per plant beating ranged between 1.0 and 1.5 thrips without any significant differences between the treatments (ANOVA: df = 5, 63; F = 1.182; P = 0.327; n = 12). On June 23 (S1), after 190 191 the first chemical application of lambda-cyhalothrin (T2) and acibenzolar-S-methyl (T5 and T6), 192 independently of the product, numbers of *T. tabaci* per plant in the sprayed plots (*i.e.*, T2, T5, T6) 193 were significantly lower than in the unsprayed plots (*i.e.*, T1, T3, T4) (ANOVA: df = 5, 63; F =194 20.438; P < 0.001; n = 12). On July 4 (S2), thrips populations naturally decreased in the untreated 195 control (T1) and in all of the unsprayed plots (T3, T4), whereas in the sprayed plots (T2, T5, T6) 196 populations increased despite the second chemical application of lambda-cyhalothrin (T2) and 197 acibenzolar-S-methyl (T5 and T6). Nevertheless, on this sampling date no significant differences 198 between the treatments were recorded (ANOVA: df = 5, 63; F = 1.347; P = 0.256; n = 12) (Table 199 2). On July 13 (S3) in treatments T3, T5 and T6, and on July 22 (S4) in the other treatments, thrips 200 populations started to increase and reached maximum infestation levels. On July 13 (S3), the 201 maximum numbers of T. tabaci per plant beating were recorded after the third application of 202 acibenzolar-S-methyl in treatment T5; mean numbers were significantly higher than in the untreated 203 control (T1), and in the treatment sprayed with spinosad (T4) (ANOVA: df = 5, 63; F = 5.512; P < 100204 0.001; n = 12). In particular, the first application of spinosad (T4) maintained infestation levels at 205 the lowest values recorded in all treatments (Table 2). On July 22 (S4), at the end of the growing 206 season, thrips populations generally reached maximum values. In treatment T2, after the fourth 207 application of lambda-cyhalothrin, mean numbers of *T. tabaci* per plant beating were significantly 208 higher than in all other treatments, except T5 where acibenzolar-S-methyl was applied for the fourth 209 time (ANOVA: df = 5, 63; F = 14.438; P < 0.001; n = 12). Conversely, both the single application 210 of lambda-cyhalothrin (T3) and the second application of spinosad, after acibenzolar-S-methyl (T6), 211 maintained thrips populations at the lowest values recorded in all treatments, reducing infestation 212 levels observed on the previous sampling date (Table 2).

213 Side effects on predatory thrips Aeolothrips intermedius Bagnall (Thysanoptera: Aeolothripidae) 214 was the dominant zoophagous species collected on onions, with populations composed of both 215 adults (females and males) and larval stages. Overall, 1,492 adult predatory thrips were sampled by 216 plant beating, and all the identified adults (n = 230) belonged to this species. Larval stages were 6% 217 of the total predatory thrips sampled by plant beating (n = 1,595); among the treatments, 218 percentages of Aeolothripidae larvae varied in the treatments from 3.7% (T1) to 9.5% (T2). 219 Moreover, average percentages were variable throughout the growing season: 2.5%, 3.3%, 23.0%, 220 13.0%, and 3.7% of total specimens sampled on June 15, June 23, July 4, July 13 and July 22,

respectively.

222 The mean numbers of A. intermedius (adults plus larvae) collected in the plots of each 223 treatment, on five sampling dates, are reported in Table 3. On June 15 (Pre-S), before the first 224 chemical application, mean numbers per plant beating ranged between 1.4 and 1.9 predatory thrips 225 without any significant differences between the treatments (ANOVA: df = 5, 63; F = 0.644; P =226 0.667; n = 12). On June 23 (S1), the predatory thrips population increased and reached maximum 227 levels in the unsprayed plots (i.e., T1, T3, T4); the maximum mean number per plant beating (2.3 228 thrips) was observed in treatment T1 (Table 3). In contrast, after the first chemical application of 229 lambda-cyhalothrin (T2) and acibenzolar-S-methyl (T5 and T6), the mean numbers of A. 230 *intermedius* per plant beating decreased significantly (ANOVA: df = 5, 63; F = 47.541; P < 0.001; n231 = 12), as previously observed for onion thrips. This side effect was confirmed in particular for 232 lambda-cyhalothrin; in fact, significantly lower numbers of predatory thrips per plant beating were 233 observed in T2 after the second application on July 4 (ANOVA: df = 5, 63; F = 5.234; P = 0.001; n234 = 12), and after the fourth application on July 22 (ANOVA: df = 5, 63; F = 11.500; P < 0.001; n =235 12), and also in T3 on July 22, after the single application at the action threshold (Table 3). By 236 contrast, on July 13 (ANOVA: df = 5, 63; F = 2.367; P = 0.049; n = 12) and on July 22, after both 237 applications of spinosad (T4), the numbers of predatory thrips per plant beating were lower but not 238 significantly different from the untreated control (Table 3).

239 *Predator/prev ratio* Population abundances of A. intermedius and T. tabaci, and therefore the 240 predator/prey ratios, were variable during the field surveys (Fig. 1). On June 15 (Pre-S), predatory 241 thrips were more abundant than onion thrips in all treatments (>1.1 predator/prey). On June 23 (S1), 242 the ratio exceeded 1.3 in T1, T3 and T4, where no chemicals were applied. By contrast, ratios drastically decreased in treatment T2, and especially in T5 and T6, where lambda-cyhalothrin (T2) 243 244 and acibenzolar-S-methyl (T5, T6) were sprayed for the first time, with mean values significantly different from those recorded in the unsprayed plots (ANOVA: df = 5, 66; F = 14.145; P < 0.0001;245 246 n = 12). After S1, overall ratios started to decline, due to both the increase of *T. tabaci* and the 247 decrease of A. intermedius. In treatment T2, where lambda-cyhalothrin was applied four times, T. 248 tabaci increased more distinctly, whereas A. intermedius decreased almost to the point of 249 disappearance. Consequently, the lowest predator/prey ratios were recorded in T2 and T3 on July 250 22, after the first application of lambda-cyhalothrin. On the last sampling date (S4), the ratios 251 recorded in T2 were significantly lower than those in the untreated control (T1) and plots sprayed 252 with spinosad (T4) (ANOVA: df = 5, 66; F = 6.978; P < 0.0001; n = 12). After the first application 253 of acibenzolar-S-methyl (S1) in both T5 and T6, predator/prey ratios were drastically reduced; 254 however, after the second application (S2), the ratios were similar to those observed in the other 255 treatments (except the lambda-cyhalothrin treatment), and the same trend was recorded in T5 after 256 the third and fourth applications (Fig. 1).

#### 257 <u>Laboratory bioassays</u>

The percentages of onion thrips and predatory thrips alive in the vial bioassays after 24 h and 48 h are shown in Figure 2. Significant differences between the treatments were found for onion thrips after both 24 h (Kruskal-Wallis: df = 3;  $\chi^2 = 52.329$ ; P < 0.001; n = 7) and 48 h (Kruskal-Wallis: df= 3;  $\chi^2 = 22.979$ ; P < 0.001; n = 7), and for predatory thrips after both 24 h (Kruskal-Wallis: df = 3;  $\chi^2 = 23.007$ ; P < 0.001; n = 15) and 48 h (Kruskal-Wallis: df = 3;  $\chi^2 = 48.498$ ; P < 0.001; n = 15). After 24 h, adult mortality of onion thrips and predatory thrips in the untreated control never reached 5%. Spinosad was the most effective active ingredient; in fact, there were no adults of *T*. *tabaci* or of *A. intermedius* alive after only 24 h. With lambda-cyhalothrin, 10% of onion thrips and
1% of predatory thrips survived, but these percentages were not statistically different from those
with spinosad. On the contrary, with acibenzolar-S-methyl, the percentages of live adults of onion
thrips and predatory thrips were statistically the same as the control (Fig. 2).

After 48 h, survival of *T. tabaci* and *A. intermedius* adults was statistically the same in acibenzolar-S-methyl and control treatments. In the lambda-cyhalothrin treatment, although 6% of onion thrips survived, there were no statistically significant differences between this active ingredient and spinosad for either onion thrips or predatory thrips (Fig. 2).

273 Statistically significant differences were found between the survival data of onion thrips and 274 predatory thrips in certain treatments. A. intermedius adults were negatively affected by the TIBS 275 method more than T. tabaci adults. Even in the absence of any treatment exposure (i.e., control), 276 after 48 h of isolation in the vials, under the same experimental conditions, mortality of predatory 277 thrips (40%) was significantly higher than mortality of onion thrips (7%) (ANOVA: df = 1, 20; F =278 24.288; P < 0.0001; n = 15, 7), showing a greater sensitivity of the former species compared with 279 the latter, at least at the adopted experimental conditions. Exposure to lambda-cyhalothrin resulted 280 in significantly higher mortality of A. intermedius than T. tabaci after both 24 h and 48 h (ANOVA: 281 df = 1, 20; F = 11.738; 9.679; P = 0.003; 0.006; n = 15, 7). No differences between mortality of 282 predatory and onion thrips were recorded with spinosad (where no thrips survived in any of the 283 cases) or acibenzolar-S-methyl (ANOVA: df = 1, 20; F = 0.028; 1.074; P = 0.868; 0.312; n = 15, 7).

284

## 285 **Discussion**

During field surveys, two thrips species were dominant on onion, the phytophagous *T. tabaci* and the zoophagous *A. intermedius*. In fact, the most numerous species was *T. tabaci*, independent of the treatments, with population levels increasing from late June–early July, as observed in other countries (Larentzaki *et al.* 2008; Torres-Vila *et al.* 1994). Nevertheless, population levels were very low throughout the season, ranging from 0.3 to 4.7 thrips per plant by plant beating, probably 291 due to unfavorable climatic conditions. In 2011, temperatures in July were on average lower than in 2010 (i.e., maximum, minimum and mean of 4.3°, 1.6° and 2.7 °C, respectively). Additionally, 292 293 rainfall in June (38.4 mm) and in July (30.0 mm) was more abundant than in 2010. It is well known 294 that heavy T. tabaci infestations occur mainly under hot and dry conditions (Theunissen & 295 Schelling 1997; Torres-Vila et al. 1994; Trdan et al. 2005b); thus the weather conditions probably 296 played an important role in the low population levels, compared with those observed in the growing 297 season of 2010 (Mautino et al. 2012). Moreover, during June, the frequent rainfalls (13 rainy days) 298 delayed the first chemical application, and consequently the low chemical pressure allowed 299 predatory thrips to migrate and establish in the field. Using the regression equation previously 300 developed to adjust the number of thrips per plant recorded with the beating method into the visual 301 method (Mautino et al. 2012), a mean seasonal value of around five thrips per plant (corresponding 302 to 1.5 thrips per plant by beating) overall was detected, and on average around seven thrips per 303 plant (corresponding to 2.3 thrips per plant by beating) were observed on the last sampling date 304 (July 22).

305 Among zoophagous thrips belonging to the family Aeolothripidae that feed on 306 phytophagous thrips (Bournier et al. 1979; Yano 2004;, Zegula et al. 2003), A. intermedius is 307 considered to be a potentially important autochthonous facultative predator in Europe (Bournier et 308 al. 1978; Franco et al. 1999; Torres-Vila et al. 1994; Trdan et al. 2005a). The coexistence of A. 309 intermedius with the onion thrips, and also with F. intonsa, has already been observed in Italy 310 (Bournier et al. 1978, 1979; Conti 2009), but it has never been investigated thoroughly. The highest 311 predator population on the onion crop was detected in mid-late June, similar to populations in 312 France and Tuscany (central Italy) (Bournier et al. 1978; Conti 2009), and ranged from 0.03 to 2.3 313 predatory thrips per plant in relation to the treatment; these values were higher than those observed 314 on leek in Piedmont, where *Aeolothrips* sp. numbered on average 0.1 and 0.2 predatory thrips per 315 plant (Bosco & Tavella 2010).

316 During the field experiments in this study, the untreated plots did not exhibit the highest 317 onion thrips levels, as had been observed previously (Mautino et al. 2012). In this instance of low 318 thrips infestation, the untreated plots presented the best solution; this strongly supports the 319 importance of a supervised control based on pest monitoring before spray applications. On the 320 contrary, lambda-cyhalothrin applications were followed by the highest infestation levels of T. 321 tabaci. Resistance of onion thrips to pyrethroids (including lambda-cyhalothrin) has been reported 322 worldwide (Foster et al. 2010; Herron et al. 2008; MacIntyre Allen et al. 2005; Martin et al. 2003), 323 and in laboratory bioassays performed with the TIBS method (Rueda & Shelton 2003; Shelton et al. 324 2006). Nevertheless, in our laboratory bioassays by TIBS the efficacy of lambda-cyhalothrin was 325 high, especially after 48 h of exposure.

326 In the field, the failure of repeated lambda-cyhalothrin applications against onion thrips has 327 previously been observed (Nault & Shelton 2010). In our field experiments, failure was more likely 328 linked to factors other than the inefficacy of this active ingredient on the onion thrips population 329 itself, for example a side effect on autochthonous predators. Little information is available on the 330 direct (i.e., physiological or behavioral) and indirect (e.g. habitat destruction, oviposition, resting, 331 mating sites) effects of this chemical on non-target organisms, including predatory thrips (Desneux 332 et al. 2007; Li et al. 2006; Mori & Gotoh 2001). In the field experiments, the lowest population 333 levels of A. intermedius were generally observed in the treatment with four applications of lambda-334 cyhalothrin. Additionally, commencing from the second consecutive application, the lowest 335 predator/prey ratio was also found in this treatment, confirming that T. tabaci increased noticeably 336 whereas A. intermedius decreased almost to its disappearance. Moreover, in the laboratory 337 bioassays the sensitivity of A. intermedius to lambda-cyhalothrin was significantly higher than that 338 registered for onion thrips both after 24 h and 48 h of exposure (even if after 48 h the TIBS method 339 in itself negatively affected predatory thrips). Until now, no specific data existed regarding A. 340 intermedius sensitivity to pyrethroids used in IPM programs against T. tabaci (Bosco & Tavella 341 2010; Harper 1978), and the result obtained may be corroborated by further laboratory research

with *T. tabaci*, and its predatory thrips, under different experimental conditions and concentrationsof lambda-cyhalothrin.

344 Spinosad is well known to be one of the most effective insecticides against T. tabaci (Gent 345 et al. 2006; Shelton et al. 2006; Yarahmadi et al. 2009), and a reduced-risk insecticide for many 346 useful arthropods, if used properly (Funderburk et al. 2000; Jones et al. 2005; Ludwig & Oetting 347 2001; Workman & Martin 2002). In our study, the efficacy of spinosad against T. tabaci was 348 confirmed both under field and laboratory conditions, where it was the most insect-toxic among the 349 tested products. The same toxicity of this product was recorded in A. intermedius in the laboratory 350 bioassays; onion thrips and predatory thrips did not survive beyond 24 h of exposure to the active 351 ingredient at field concentrations. However, in the field experiments spinosad was less insect-toxic 352 than lambda-cyhalothrin to predatory thrips, as already observed on leek in northwestern Italy 353 (Bosco & Tavella 2010). The predator/prey ratios recorded after spinosad applications were similar 354 to ratios recorded in the other treatments, including the untreated control, unlike the observations 355 for lambda-cyhalothrin. Spinosad applications likely equally affected both thrips populations 356 without heavily impacting the predator/prey balance, thereby enabling the re-establishment of 357 predator activity and colonization of the crop. Thus, for the Aeolothrips genus, spinosad could 358 represent a reduced threat when used at the recommended timing and number of applications; 359 otherwise the active ingredient could prevent the development of predatory thrips (Workman & 360 Martin 2002) and other natural enemies useful for onion thrips control (Biondi et al. 2012). 361 The potential value of SAR compounds against several insect pests Alcantra et al. 2010; 362 (Correa et al. 2005; Costa et al. 2007; Tomquelski et al. 2007), and more specifically against 363 tospovirus and in thrips vector control (Gent et al. 2006; Momol et al. 2004; Pappu et al. 2000), 364 has been shown. Thrips feeding induces the expression of gene markers for the jasmonic and 365 salicylic acid pathways (JA and SA, respectively) involved in the basic plant defense response (Abe 366 et al. 2008). Acibenzolar-S-methyl is the synthetic functional analog of SA inducing the 367 corresponding response pathway (Gorlach et al. 1996). Moreover, a substantial co-regulation or

368 cross-talk among the salicylate and jasmonate plant defense pathways has been demonstrated 369 (Schenk et al. 2000); however, it is not fully understood how this signal interaction affects plant 370 response to thrips damage (Abe et al. 2012; Thaler et al. 2002). In the field experiments, 371 acibenzolar-S-methyl ensured low infestation levels of thrips on June 23 and July 4, as already 372 observed with high infestation levels (Mautino et al. 2012); nonetheless, acibenzolar-S-methyl was 373 unable adequately to control the *T. tabaci* population at the following sampling dates when plants 374 started to wither. Therefore, its effects on herbivores could be mediated by plant phenology. On the 375 other hand, where the SAR activator was sprayed only two times, followed by spinosad (T6), onion 376 thrips was effectively controlled. Unlike antiherbivore effects on insect pests, until now no 377 information was available about the effect of acibenzolar-S-methyl on A. intermedius. Acibenzolar-378 S-methyl showed a similar side effect on predatory thrips, when it was applied singly and associated 379 with spinosad. In the laboratory bioassays, acibenzolar-S-methyl showed no effect on either T. 380 tabaci or A. intermedius. Adult survival in vials treated with this active ingredient was not 381 significantly different from that observed in the untreated control. Therefore, in the absence of the 382 plant, there is no side effect of acibenzolar-S-methyl on thrips, as previously observed in the vial 383 bioassay under the same experimental conditions (Mautino et al. 2012).

384 Spinosad appears to be the most effective control strategy against onion thrips, and the most 385 low-risk insecticide for A. intermedius if applied at the economic threshold, limiting the number of 386 applications, and in alternation with pesticides of a different mode of action (Biondi et al. 2012). As 387 an alternative control strategy, the addition of acibenzolar-S-methyl at the beginning of the growing 388 season should be considered also based on its minor impact on the overall predator/prey balance. 389 An environmentally friendly control strategy can maintain populations of the autochthonous A. 390 *intermedius* at reasonable levels in the open field. This species is naturally able to reduce T. tabaci 391 populations; therefore, its potential economic importance in IPM programs for onion is relevant. 392 Although in the field experiments densities of onion thrips were probably too low to comprehend 393 fully the direct effects of A. *intermedius* predatory activity, predator/prey ratios starting from

approximately 0.2 seem to be adequate to contain infestation levels of *T. tabaci*. Moreover,

395 supplemental techniques that are useful in improving thrips control are crucial and thus require

396 further investigation. In particular, methods to increase colonization by autochthonous predators in

397 the onion fields, such as programmed releases of combined beneficial arthropods (Fathi *et al.* 2008)

398 and intercropping with forage plants, should be developed (Bán *et al.* 2010; Theunissen & Schelling

399 1997; Trdan et al. 2006).

400

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407

#### 408 **References**

#### 'et al.' should be in italics

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- 577 *Pflanzen*, 55, 169-174 (German, with English summary).
- 578

579 Table 1. Active ingredients, rates, number and timing of applications of the products sprayed in the

| Treatment       | Active ingredient    | Rate $(l ha^{-1})$ | No. <sup>z</sup> | Timing                               |
|-----------------|----------------------|--------------------|------------------|--------------------------------------|
| T1 <sup>y</sup> | -                    | -                  | -                | -                                    |
| T2              | Lambda-cyhalothrin   | 1.3                | 4                | 19 June; 29 June;<br>9 July; 19 July |
| T3              | Lambda-cyhalothrin   | 1.3                | $1^{x}$          | 19 July                              |
| T4              | Spinosad             | 0.8                | 2                | 9 July; 19 July                      |
| T5              | Acibenzolar-S-methyl | 0.2                | 4                | 19 June; 29 June;<br>9 July; 19 July |
| T6              | Acibenzolar-S-methyl | 0.2                | 2                | 19 June; 29 June                     |
|                 | +Spinosad            | 0.8                | 2                | 9 July; 19 July                      |

580 experimental plots of the onion field during the 2011 growing season

- 581 <sup>z</sup>Number of applications
- <sup>y</sup>Untreated control
- <sup>x</sup>Pyrethroid application at the action threshold of two thrips per plant by plant beating
- 584

585 Table 2. Mean numbers (± SE) of *Thrips tabaci* (adults plus larvae) per plant sampled by plant

586 beating in the six tested treatments during field surveys in 2011(Statistical analyses were performed on

587 log-transformed data which are not shown)

| Treatment        | Pre-S <sup>z</sup> 15 June | S1 <sup>z</sup> 23 June  | S2 <sup>z</sup> 04 July | S3 <sup>z</sup> 13 July | S4 <sup>z</sup> 22 July |
|------------------|----------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| T1               | $1.03 \pm 0.14$            | 1.60±0.14 a <sup>y</sup> | 1.13±0.23               | 1.38±0.25 bc            | 2.52±0.40 b             |
| T2               | $1.07 \pm 0.11$            | 0.27±0.10 b              | $1.22\pm0.19$           | 2.68±0.46 ab            | 4.70±0.63 a             |
| T3               | $0.98 \pm 0.15$            | 1.20±0.15 a              | $0.98 \pm 0.29$         | 2.05±0.31 abc           | 1.08±0.25 c             |
| T4               | $1.45 \pm 0.17$            | 1.52±1.27 a              | $1.35 \pm 0.27$         | 1.25±0.20 c             | 1.67±0.20 bc            |
| T5               | $1.02 \pm 0.14$            | 0.30±0.09 b              | $0.87 \pm 0.18$         | 3.23±0.33 a             | 2.87±0.42 ab            |
| T6               | $1.13 \pm 0.17$            | $0.47{\pm}0.08~{\rm b}$  | $0.67 \pm 0.14$         | 1.92±0.26 abc           | 1.10±0.18 c             |
| Р                | 0.327                      | < 0.001                  | 0.256                   | < 0.001                 | < 0.001                 |
| $F_{5,63}$       | 1.182                      | 20.438                   | 1.347                   | 5.512                   | 14.438                  |
| SED <sup>x</sup> | 0.101                      | 0.103                    | 0.152                   | 0.147                   | 0.146                   |

588

<sup>z</sup>Pre-S represents the sampling before chemical applications; S1, S2, S3, and S4 represent sampling after the

- 590  $1^{\text{st}}$ ,  $2^{\text{nd}}$ ,  $3^{\text{rd}}$  and  $4^{\text{th}}$  applications, respectively
- 591 <sup>y</sup>Within columns, means followed by a common letter do not differ significantly (P < 0.05, Tukey's test
- following ANOVA). ANOVA results (P and F values, df = 5, 63, n = 12) are reported
- 593 <sup>x</sup>Standard errors of the difference values

595 Table 3. Mean numbers (± SE) of *Aeolothrips intermedius* (adults plus larvae) per plant sampled by

596 plant beating in the six tested treatments during field surveys in 2011 (Statistical analyses were

| Treatment         | Pre-S <sup>z</sup> 15 June | S1 <sup>z</sup> 23 June  | S2 <sup>z</sup> 04 July | S3 <sup>z</sup> 13 July    | S4 <sup>z</sup> 22 July |
|-------------------|----------------------------|--------------------------|-------------------------|----------------------------|-------------------------|
| T1                | 1.63±0.24                  | 2.32±0.25 a <sup>y</sup> | 0.65±0.10 a             | 0.25±0.09 ab               | 0.87±0.15 a             |
| T2                | $1.78 \pm 0.21$            | 0.18±0.13 b              | 0.15±0.06 b             | $0.07 \pm 0.04 \text{ b}$  | 0.10±0.04 c             |
| T3                | $1.80\pm0.23$              | 1.88±0.28 a              | 0.73±0.18 a             | 0.28±0.09 ab               | $0.10{\pm}0.06~{\rm c}$ |
| T4                | $1.63 \pm 0.28$            | 2.10±0.30 a              | 0.78±0.15 a             | $0.17 \pm 0.05 \text{ ab}$ | 0.48±0.08 ab            |
| T5                | $1.87 \pm 0.20$            | 0.03±0.02 b              | 0.45±0.13 ab            | 0.40±0.07 a                | 0.47±0.13 abc           |
| T6                | $1.43\pm0.21$              | 0.12±0.05 b              | 0.35±0.11 ab            | 0.28±0.14 ab               | 0.15±0.03 bc            |
| Р                 | 0.667                      | < 0.001                  | 0.001                   | 0.049                      | < 0.001                 |
| F <sub>5,63</sub> | 0.644                      | 47.541                   | 5.234                   | 2.367                      | 11.500                  |
| SED <sup>x</sup>  | 0.120                      | 0.113                    | 0.099                   | 0.081                      | 0.085                   |

597 performed on log-transformed data which are not shown)

598

<sup>2</sup>Pre-S represents the sampling before chemical applications; S1, S2, S3, and S4 represent sampling after the

 $1^{\text{st}}$ ,  $2^{\text{nd}}$ ,  $3^{\text{rd}}$  and  $4^{\text{th}}$  applications, respectively

601 <sup>y</sup>Within columns, means followed by a common letter do not differ significantly (P < 0.05, Tukey's test

following ANOVA). ANOVA results (*P* and *F* values, df = 5, 63, n = 12) are reported

603 <sup>x</sup>Standard errors of the difference values

## 605 **Figure captions**

| 606 | Fig. 1 Mean | ratios of predate | or/prey (Aeo | lothrips interm | iedius/Thrips | <i>tabaci</i> ) per | <sup>,</sup> plant in th | ne six |
|-----|-------------|-------------------|--------------|-----------------|---------------|---------------------|--------------------------|--------|
|-----|-------------|-------------------|--------------|-----------------|---------------|---------------------|--------------------------|--------|

- 607 tested treatments during field surveys. Within the same sampling date, bars labeled with different
- 608 letters are significantly different (Tukey's test following ANOVA, P < 0.01). Statistical analyses
- 609 were performed on log-transformed data which are not shown. Pre-S represents the sampling before
- 610 chemical applications; S1, S2, S3, and S4 represent sampling after the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> applications,
- 611 respectively

612

- 613
- 614 **Fig. 2** Mean (± SE) survival percentages of adult *Thrips tabaci* (**a**) and *Aeolothrips intermedius* (**b**)
- 615 after 24 h and 48 h in vials treated with the tested products at field concentrations. Within thrips

treatments, bars labeled with different letters (lower case and capital, for survivor thrips at 24 h and

617 48 h, respectively) are significantly different (P < 0.05, Kruskal-Wallis, df = 3)



Fig. 1



