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

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Abstract

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

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

Introduction

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

Pesticide impact on human and environmental health begs for alternative pest management approaches, such as the use of straw mulch (Gent *et al.* 2006; Larentzaki *et al.* 2008; Momol *et al.* 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 volatiles (Koschier *et al.* 2002) against *T. tabaci*. Moreover, activators of natural systemic acquired resistance (SAR), such as acibenzolar-S-methyl, appear promising for reducing the use of conventional insecticides (Mautino *et al.* 2012). Acibenzolar-S-methyl was used as an effective 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).

Furthermore, the conservation and augmentation of predators feeding on thrips are important 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* (Hemiptera: Anthocoridae) (Bosco *et al.* 2008; Funderburk *et al.* 2000; Riudavets 1995), mirids of 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 comparatively few data on the predatory efficiency of the autochthonous thrips species belonging to the genera *Aeolothrips*, *Haplothrips* and *Franklinothrips* (Thysanoptera: Aeolothripidae), that are potential biological control agents of *T. tabaci* (Cox *et al.* 2006; Fathi *et al.* 2008; Kakimoto *et al.* 2006; Trdan *et al.* 2005a).

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

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

Materials and methods

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" N, 8°34'41" E, 105 m a.s.l.). The experimental site was flanked on all sides by at least 2 m of insecticide-free onions within the grower's field. Experimental plots (10.5 m^2 each) consisted of six onion rows 7 m in length, with rows spaced every 0.2 m. Plots were separated within rows by 1.0 m and spaced every 1.5 m. Onions of the golden onion cultivar 'Derek' were seeded in April; diseases and weeds were controlled using products recommended for onion production (pendimethanil, ioxynil, metalaxyl-M + copper, dimethomorph + pyraclostrobin, cyprodinil + fludioxonil).

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

Commencing in May, onion plots were surveyed weekly for the presence of *T. tabaci* and their predators. At the first occurrence of onion thrips infestation on the crop (pre-sampling, *i.e.*,

pre-S, June 15), insecticide applications were sprayed, in relation to the treatment, on June 19, June 29, July 9 and July 19. Sampling was carried out 3 days after each cluster of sprays (*i.e.*, S1, June 23; S2, July 4; S3, July 13; S4, July 22). *Thrips tabaci*, thrips predators including anthocorid, mirid bugs (Hemiptera: Anthocoridae, Miridae), and predatory thrips (Thysanoptera: Aeolothripidae) were detected during field surveys. Plant beating was chosen as the sampling method for its high feasibility, and because a strong relationship between this method and visual inspection has previously been observed, and also for the larval population, generally underestimated with the beating sampling method (Mautino *et al.* 2012). Five plants were randomly selected at three points in each plot (15 plants per plot), and these were beaten over a plastic tray (350 × 250 mm). Thrips adults and larvae, and their predators, were counted, collected with a mouth aspirator and transferred to the laboratory. Subsequently, adult onion thrips were observed under a 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 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 160× magnification and attributed to the family Aeolothripidae or Thripidae, according to Vierbergen *et al.* (2010).

Data on local weather conditions during field experiments were provided by Rete Agrometeorologica, Regione Piemonte, Settore Fitosanitario. In particular, the following average 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, 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. Rainfall was 34.2, 102.6 and 33.8 mm in May, June and July, respectively.

Laboratory bioassays Adults of *T. tabaci* and predatory thrips collected in untreated plots on 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 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 ± 1°C, 65 ± 5% r.h. and a 16h:8h L:D cycle (Tedeschi *et al.* 2001).

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

normality (Shapiro-Wilk), and analyzed by Univariate Analysis of Variance (ANOVA) for randomized blocks (treatments and blocks were the factors).

To describe the effect of treatments on the relationship between phytophagous and predatory 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 (Levene) and normality (Shapiro-Wilk), and analyzed by ANOVA and Tukey's *post hoc* test.

Percentage survival data obtained in the laboratory bioassays for predatory thrips and *T. tabaci* were separately transformed to arcsine square-root values before analysis; the non-parametric Kruskal-Wallis was chosen since data were non-homogeneous, and means were compared using the Mann-Whitney U test. For each treatment, differences between the survival data of *T. tabaci* and predatory thrips were analyzed by ANOVA after tests of homogeneity of variance (Levene) and normality (Shapiro-Wilk).

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

Results

Field experiments

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

The mean numbers of total (adults plus larvae) onion thrips collected in the plots of each treatment are reported in Table 2. On June 15 (Pre-S), before the first chemical application, mean 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 the first chemical application of lambda-cyhalothrin (T2) and acibenzolar-S-methyl (T5 and T6), independently of the product, numbers of *T. tabaci* per plant in the sprayed plots (*i.e.*, T2, T5, T6) were significantly lower than in the unsprayed plots (*i.e*, T1, T3, T4) (ANOVA: df = 5, 63; F = 20.438; $P < 0.001$; n = 12). On July 4 (S2), thrips populations naturally decreased in the untreated control (T1) and in all of the unsprayed plots (T3, T4), whereas in the sprayed plots (T2, T5, T6) populations increased despite the second chemical application of lambda-cyhalothrin (T2) and acibenzolar-S-methyl (T5 and T6). Nevertheless, on this sampling date no significant differences between the treatments were recorded (ANOVA: df = 5, 63; F = 1.347; P = 0.256; n = 12) (Table 2). On July 13 (S3) in treatments T3, T5 and T6, and on July 22 (S4) in the other treatments, thrips populations started to increase and reached maximum infestation levels. On July 13 (S3), the maximum numbers of *T. tabaci* per plant beating were recorded after the third application of acibenzolar-S-methyl in treatment T5; mean numbers were significantly higher than in the untreated control (T1), and in the treatment sprayed with spinosad (T4) (ANOVA: df = 5, 63; F = 5.512; $P < 0.001$; n = 12). In particular, the first application of spinosad (T4) maintained infestation levels at the lowest values recorded in all treatments (Table 2). On July 22 (S4), at the end of the growing season, thrips populations generally reached maximum values. In treatment T2, after the fourth application of lambda-cyhalothrin, mean numbers of *T. tabaci* per plant beating were significantly higher than in all other treatments, except T5 where acibenzolar-S-methyl was applied for the fourth time (ANOVA: df = 5, 63; F = 14.438; $P < 0.001$; n = 12). Conversely, both the single application of lambda-cyhalothrin (T3) and the second application of spinosad, after acibenzolar-S-methyl (T6), maintained thrips populations at the lowest values recorded in all treatments, reducing infestation levels observed on the previous sampling date (Table 2).

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

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

Predator/prey ratio Population abundances of *A. intermedius* and *T. tabaci*, and therefore the predator/prey ratios, were variable during the field surveys (Fig. 1). On June 15 (Pre-S), predatory thrips were more abundant than onion thrips in all treatments (>1.1 predator/prey). On June 23 (S1), 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) 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: $df = 5, 66$; $F = 14.145$; $P < 0.0001$; n = 12). After S1, overall ratios started to decline, due to both the increase of *T. tabaci* and the decrease of *A. intermedius*. In treatment T2, where lambda-cyhalothrin was applied four times, *T. tabaci* increased more distinctly, whereas *A. intermedius* decreased almost to the point of disappearance. Consequently, the lowest predator/prey ratios were recorded in T2 and T3 on July 22, after the first application of lambda-cyhalothrin. On the last sampling date (S4), the ratios recorded in T2 were significantly lower than those in the untreated control (T1) and plots sprayed with spinosad (T4) (ANOVA: $df = 5, 66$; $F = 6.978$; $P < 0.0001$; n = 12). After the first application of acibenzolar-S-methyl (S1) in both T5 and T6, predator/prey ratios were drastically reduced; however, after the second application (S2), the ratios were similar to those observed in the other treatments (except the lambda-cyhalothrin treatment), and the same trend was recorded in T5 after the third and fourth applications (Fig. 1).

Laboratory bioassays

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).

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

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
292 2010 (*i.e.*, maximum, minimum and mean of 4.3°, 1.6° and 2.7 °C, respectively). Additionally,
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

342 with *T. tabaci*, and its predatory thrips, under different experimental conditions and concentrations
343 of lambda-cyhalothrin.
344 Spinosad is well known to be one of the most effective insecticides against *T. taba*ci (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

394 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
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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

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

Treatment	Active ingredient	Rate (l ha^{-1})	No.[z]	Timing
T1[y]	-	-	-	-
T2	Lambda-cyhalothrin	1.3	4	19 June; 29 June; 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; 9 July; 19 July
T6	Acibenzolar-S-methyl	0.2	2	19 June; 29 June
	+Spinosad	0.8	2	9 July; 19 July

581 [z]Number of applications

582 [y]Untreated control

583 [x]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[z] 15 June	S1[z] 23 June	S2[z] 04 July	S3[z] 13 July	S4[z] 22 July
T1	1.03±0.14	1.60±0.14 a[y]	1.13±0.23	1.38±0.25 bc	2.52±0.40 b
T2	1.07±0.11	0.27±0.10 b	1.22±0.19	2.68±0.46 ab	4.70±0.63 a
T3	0.98±0.15	1.20±0.15 a	0.98±0.29	2.05±0.31 abc	1.08±0.25 c
T4	1.45±0.17	1.52±1.27 a	1.35±0.27	1.25±0.20 c	1.67±0.20 bc
T5	1.02±0.14	0.30±0.09 b	0.87±0.18	3.23±0.33 a	2.87±0.42 ab
T6	1.13±0.17	0.47±0.08 b	0.67±0.14	1.92±0.26 abc	1.10±0.18 c
P	0.327	< 0.001	0.256	< 0.001	< 0.001
$F_{5,63}$	1.182	20.438	1.347	5.512	14.438
SED[x]	0.101	0.103	0.152	0.147	0.146

588

589 [z]Pre-S represents the sampling before chemical applications; S1, S2, S3, and S4 represent sampling after the

590 1st, 2nd, 3rd and 4th applications, respectively

591 [y]Within columns, means followed by a common letter do not differ significantly ($P < 0.05$, Tukey's test

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

593 [x]Standard errors of the difference values

594

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

Treatment	Pre-S[z] 15 June	S1[z] 23 June	S2[z] 04 July	S3[z] 13 July	S4[z] 22 July
T1	1.63±0.24	2.32±0.25 a[y]	0.65±0.10 a	0.25±0.09 ab	0.87±0.15 a
T2	1.78±0.21	0.18±0.13 b	0.15±0.06 b	0.07±0.04 b	0.10±0.04 c
T3	1.80±0.23	1.88±0.28 a	0.73±0.18 a	0.28±0.09 ab	0.10±0.06 c
T4	1.63±0.28	2.10±0.30 a	0.78±0.15 a	0.17±0.05 ab	0.48±0.08 ab
T5	1.87±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±0.21	0.12±0.05 b	0.35±0.11 ab	0.28±0.14 ab	0.15±0.03 bc
P	0.667	< 0.001	0.001	0.049	< 0.001
$F_{5,63}$	0.644	47.541	5.234	2.367	11.500
SED[x]	0.120	0.113	0.099	0.081	0.085

598
599 [z]Pre-S represents the sampling before chemical applications; S1, S2, S3, and S4 represent sampling after the
600 1st, 2nd, 3rd and 4th applications, respectively
601 [y]Within columns, means followed by a common letter do not differ significantly ($P < 0.05$, Tukey's test
602 following ANOVA). ANOVA results (P and F values, df = 5, 63, n = 12) are reported
603 [x]Standard errors of the difference values
604

Figure captions

Fig. 1 Mean ratios of predator/prey (*Aeolothrips intermedius*/*Thrips tabaci*) per plant in the six tested treatments during field surveys. Within the same sampling date, bars labeled with different letters are significantly different (Tukey's test following ANOVA, $P < 0.01$). Statistical analyses were performed on log-transformed data which are not shown. Pre-S represents the sampling before chemical applications; S1, S2, S3, and S4 represent sampling after the 1st, 2nd, 3rd and 4th applications, respectively

Fig. 2 Mean (± SE) survival percentages of adult *Thrips tabaci* (**a**) and *Aeolothrips intermedius* (**b**) 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 48 h, respectively) are significantly different ($P < 0.05$, Kruskal-Wallis, $df = 3$)

line figure
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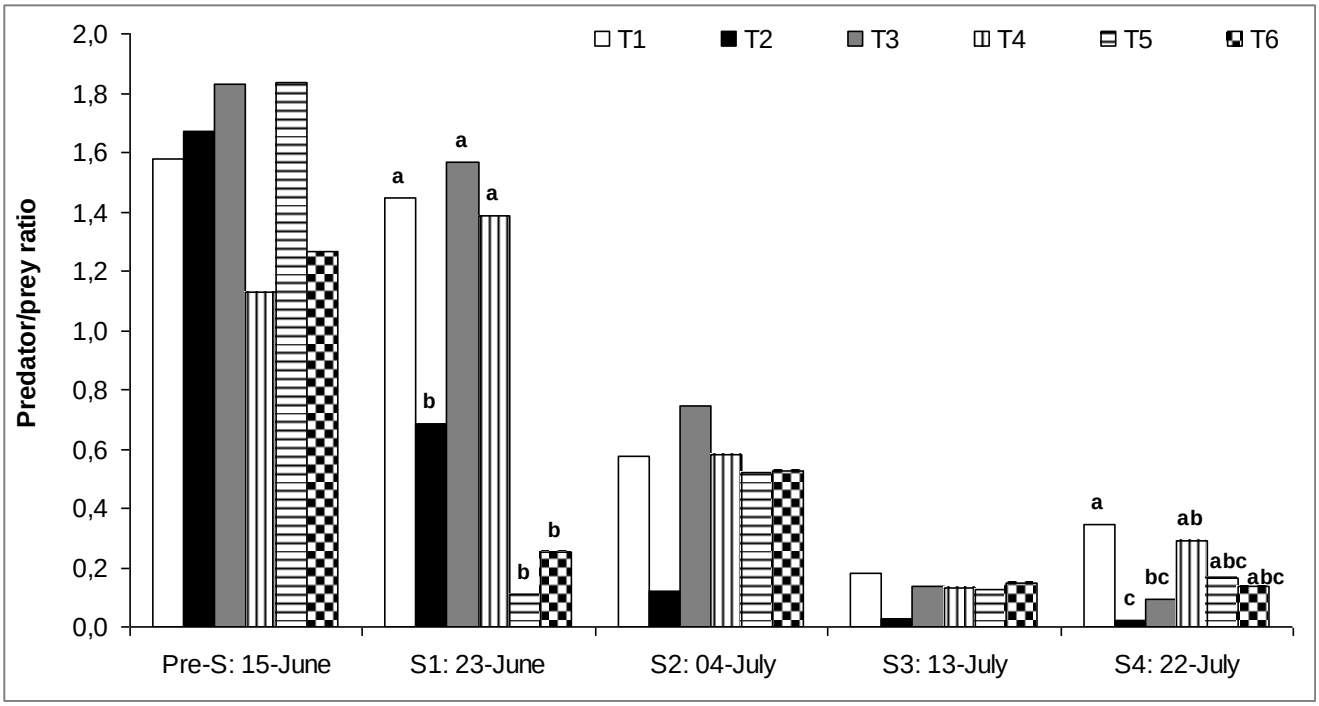

Fig. 1

line figure
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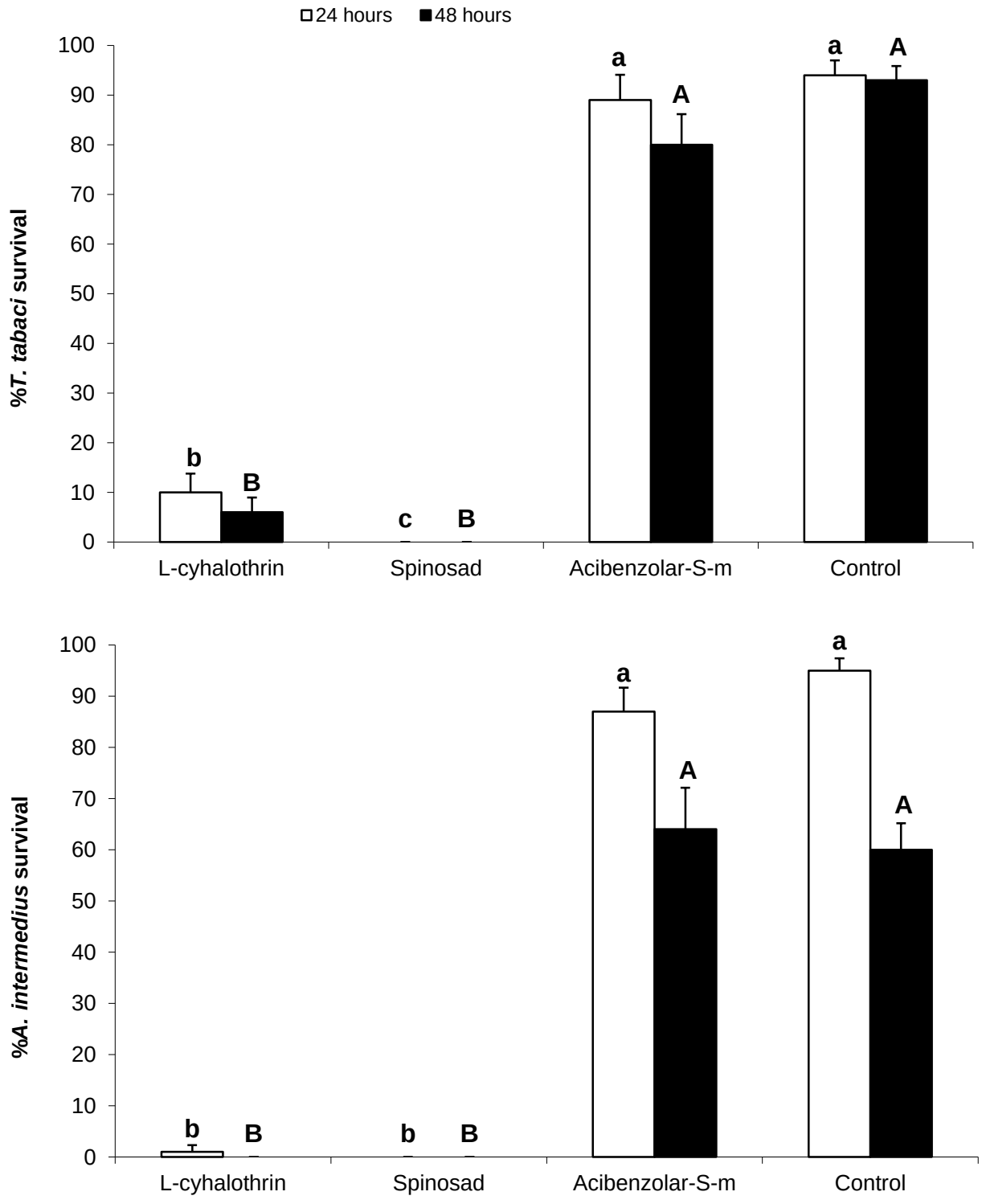

Figure 2