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Influence of *Ostrinia nubilalis* larval density and location in the maize plant on the tachinid fly *Lydella thompsoni*

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Abstract

A three years research (2008-2010) was carried out in the Po valley (northern Italy) on *Lydella thompsoni* Herting (Diptera Tachinidae), an important parasitoid of ECB (European corn borer - *Ostrinia nubilalis* Hubner, Lepidoptera Crambidae). Several factors can affect the action of *L. thompsoni* against ECB. This case of study was focused on the influence of ECB larval density and position in maize plant organs. Wintering ECB larvae and parasitoid puparia were sampled from maize plants and reared. *O. nubilalis* density ranged from 0.2 to 3.2 larvae/plant. *L. thompsoni* was the only parasitoid regularly reared from ECB larvae. Parasitisation rates were 17.9, 16 and 11% in 2008, 2009 and 2010 respectively. It was noticed that ECB larvae distribution in the maize plants was related to infestation degree. ECB larvae preferably colonized ears; however, when their density increased, their proportion in the stalk did, as well. The density of *L. thompsoni* was higher in stalk tissues, below and above the ear. Among the range of ECB densities recorded as a result of this study, it was not observed a density dependent relation between ECB and *L. thompsoni.*

Key words: *Lydella thompsoni*, *Ostrinia nubilalis*, *Zea mays*, parasitisation, host density dependent.

Introduction

Lydella thompsoni Herting **(**Diptera Tachinidae) is an endophagous parasitoid of the European corn borer (ECB) *Ostrinia nubilalis* Hubner (Lepidoptera Crambidae) larvae (Kuske *et al*., 2004; Pélissié *et al*., 2010). ECB is the most important pest of maize (*Zea mays* L.) in the northern hemisphere (Europe, and North America) where it is responsible for significant yield losses (Cagán *et al*., 1999; Bourguet *et al*., 2002; Leniaud *et al*., 2006). The distribution of *L. thompsoni* ranges from Spain (Eizaguirre *et al*., 1990; Monetti *et al*., 2003), France (Galichet *et al*., 1985; Folcher *et al*., 2011), Italy (Platia and Maini, 1975; Cerretti and Tschorsnig, 2010), Slovakia (Perniš and Cagán, 2011), southern Russia, Tadzhikistan, Mongolia (Herting, 1984) to Japan (Shima, 2006). In Asia the fly is attacking the Asian corn borer, *Ostrinia furnacalis* (Guenee), earlier confused as *O. nubilalis* (Wang, 1982).

L. thompsoni, together with many other species of natural enemies, was introduced into the USA from 1920 to 1938 by USDA after the invasion of corn cultivations by ECB (Thompson and Parker, 1928; Baker *et al*., 1949). *L. thompsoni* colonized many areas where it was not released, but a decline of this species was recorded in North America in the early 1960s. Thus, in the mid 70s, a new stock of this natural enemy was introduced to reinforce the populations (Mason *et al*., 1991). Actually it is one of the 6 species which could establish in the United States successfully (Brindley and Dicke, 1963; Burbutis *et al*., 1960; 1981; Mason *et al*., 1994).

Females are ovolarviparous in the sense of Herting (1960). After mating, females fly actively in search for host larvae stimulated by ECB frass (Mason *et al*., 1991). Firstly, *L. thompsoni* lay eggs with larvae (of the planidium type) ready to hatch next to the holes made by ECB in maize stalks (Galichet *et al*., 1985). Then the planidium moves into the tunnel and finds its way to the host larva. Once in the host, the maggot feeds first on hemolymph, then on the fatty tissues and internal organs, until its full development. It finally leaves the host remains and pupates in the tunnel nearby (Thompson and Parker, 1928). In areas like the Po river floodplain (northern Italy), where ECB usually develops two generations, *L. thompsoni* can overwinter after pupation or within the body of the dead larva. In the first case, its puparia can be found by dissecting maize plants at the end of summer. These overwintering puparia usually fail to let an adult emerge during the following spring (Camerini and Maini, unpublished data). *L. thompsoni* usually behaves as a solitary parasitoid (Platia and Maini, 1975).

Parasitisation levels, estimated by means of larvae rearing, vary among the distribution range; the percentage ranges from negligible values up to 75% (Jarvis and Guthrie, 1987). The extent of parasitisation is influenced by several factors, such as ECB density (Sandlan *et al*., 1983), landscape structure (Maini, 1974; Platia and Maini, 1975; Landis and Haas, 1992) or availability of alternative hosts (Galichet *et al*., 1985). The use of molecular markers as a new approach for the evaluation of parasitoidism revealed an underestimation coming from the traditional rearing protocols (Agustì *et al*., 2005). Plantevin and Grenier (1990) have shown that the life cycle of the host and parasitoid is synchronized. *L. thompsoni* may be defined, therefore, as koinobiont-like *sensu* Belshaw (1994) and Dindo (2011). An important factor affecting host parasitoid synchronization is ECB life cycle, which tends to change according to latitude. In the Po valley, the main Italian agricultural area, a first peak of ECB flight is recorded between the last decade of May and the beginning of June, due to the emergence of moths coming from the overwintering generation. A second flight peak is observed during the second half of August (Camerini *et al.*, 2015). In response to favourable weather conditions, a partial third generation can be recorded (Alma and Lessio, 2005). The complex of ECB parasitoids identified between 1926 and 1931 in the Po valley included 15 species (Goidanich, 1931), but only five of them have been found as a result of studies carried out in Emilia Romagna (Maini, 1972; 1974; Platia and Maini, 1975; Maini and Burgio, 1990), Lombardy (Camerini, 1995), Friuli Venezia Giulia (Barbattini, 1989) and across the whole Po valley (Manachini, 2000; 2003). All those investigations demonstrated that in the Po river plain *L. thompsoni* is the most effective ECB parasitoid, together with the oophagous *Trichogramma brassicae* Bezdenko (Hymenoptera Trichogrammatidae). This paper reports the results of a three years research on *L. thompsoni* and *O. nubilalis* which was carried out in maize fields of 9 farms located in the province of Pavia (central western Po valley). Aim of the research was to investigate the trends of ECB parasitisation by *L. thompsoni* in relation to ECB larval density and position in the maize plant organs.

Materials and methods

The research was carried out between 2008 and 2010 by collecting ECB larvae from maize fields located in the agroecosystems near Pavia. Maize, wheat and horticultural crops are the main cultivations in this rural district, where the principles of crop rotation are widely used.

Samples were taken from maize fields with 7.4 plants per m^2, same sowing time (first half of April) and final destination of the crop (grain maize) in order to minimize the influence of such parameters on ECB abundance (Landi and Maini, 1982).

The number of sampled plants was planned in relation to field size. In fields smaller than 2 hectares, 40 maize plants were taken both from the central core and margins. When margins were similar (i.e. weed strip border) the 40 plants margin sample was made of 20 plants coming from both margins. When margins had a different structure (i.e weed strip/wooded margin) 40 plants were collected from each of them

By following the same criteria, the number of sampled maize plants was doubled when the field area was larger than 2 hectares. In order to make the identification of margins and central core easier, fields having a rectangular shape were selected for research purposes. The central core and margins were marked by means of a beam fixed to a maize plant on which a red ribbon was tied. The margin point of reference was located in the middle of the minor sides, at a distance of 8 meters from field margin.

Samples (40 plants) were also taken from three different plots of maize cultivars (600 plants) on a farm included in the study area (Bastida Pancarana).

Sampling sites located in the study area included: Barbianello 45°03'56"N 9°10'47"E; 45°04'31"N 9°11'17"E; Bastida Pancarana 45°05'24"N 9°04'59"E; 45°06'08"N 9°05'32"E; Bressana Bottarone 45°05'19"N 9°06'18"E; 45°04'44"N 9°05'57"E; Castelletto di Branduzzo 45°04'31"N 9°06'37"E; Corana 45°03'53"N 8°56'35"E; 45°03'24"N 8°57'09"E; Lungavilla 45°02'21"N 9°04'09"E; Silvano Pietra 45°03'32"N 8°57'42"E; Voghera 45°02'48"N 9°01'19"E.

Just before crop harvest (September-October) maize plants were sampled from fields by cutting the whole plant to the ground. Second generation ECB larvae were promptly removed from tunnels by dissecting maize stalks and ears. *L. thompsoni* puparia were also extracted and preserved for rearing in test tubes. ECB density (larvae/plant) was calculated as follows:

$$D = [(NE+NL)/NP] \times 100$$

where *NE* is the number of ECB specimens, *NL* the number of *L. thompsoni* puparia and *NP* the number of dissected plants.

The position of ECB larvae and *L. thompsoni* puparia inside each maize plant was recorded. Three categories were identified: larvae inside ear and ear shank; larvae in stalk below ear; larvae in stalk above ear.

No insecticide was sprayed in sampled maize fields. The ECB larvae extracted from dissected plants were kept in plastic boxes provided with a net-cover until emergence. Since not all larvae extracted from stalk dissection were mature and ready to pupate, pieces of maize, as a food, were added. They were then fixed into wet foam bases laid on the bottom of boxes, together with test tubes containing parasitoids and cardboard rolls usable by mature ECB larvae as a refuge. Larvae from each sample were reared and kept separate in a rearing box. All breeding boxes were kept outdoors during winter inside a big cage under shelter. A layer of sand (10 cm thickness) was put at the bottom of the cage and periodically wet in order to keep it moist.

The percentage of wintering ECB larvae parasitised by *L. thompsoni* (P) was calculated as follows:

$$P = [NL/ (NE+NL)] \times 100$$

NE is the number of adult ECB adults and *NL* is the number of *L. thompsoni* adults emerged from both puparia and wintering ECB larvae. The *L. thompsoni* puparia that did not hatch were not computed in the formula.

All the moths and parasitoids emerged from overwintering larvae were then fixed, pinned and identified by means of a stereoscopic microscope. Specimens are stored in the entomological collection of Dipartimento di Scienze della Terra e dell'Ambiente (University of Pavia). Since overwintering *L. thompsoni* puparia do not usually eclose, it was necessary to analyze the puparia found during dissection by means of identification keys provided by Grenier and Nardon (1983).

Data were statistically elaborated using Biostat software. Data sets were first analyzed to test their normal distribution according to five specific tests: Kolmogorov-Smirnov/Lilliefor, Shapiro-Wilibuitk, D'Agostino Skewness, D’Agostino kurtosis, D'Agostino Omnibus.

Since data sets distribution did not agree with a normal pattern, non-parametric tests were applied. The Mann-Whitney U test was used to compare differences between two independent groups of data. Contingency tables were used to record and analyze the relation between two or more categorical variables. The statistical dependence between two variables was analyzed by means of Spearman's rank correlation coefficient.

Results and discussion

The spring emergence trends of both ECB and *L. thompsoni* adults (overwintering generation) reared from 2008 and 2010 samples are shown in figures 1 and 2. Due to low numbers of tachinid flies reared from overwintering larvae, it was not possible to achieve a clear pattern of emergence for 2009 season.

L. thompsoni was the only parasitoid regularly reared from ECB larvae. Only a few specimens (n = 8) of *Sinophorus turionum* (Ratzeburg) (Hymenoptera Ichneumonidae) eclosed from overwintering ECB larvae. This result was in agreement with previous investigations on ECB parasitoids in Northern Italy (Maini, 1974; Platia and Maini, 1975; Maini and Burgio, 1990).

L. thompsoni adults (figures 1 and 2) emerged from the end of April to the first decade of June, a little bit earlier than ECB adults and later than available ECB larvae. This finding is consistent with observations coming from other studies on *L. thompsoni* in Europe (Maini 1972; 1974; Galichet *et al.*, 1985; Barbattini, 1989; Cagán *et al.*, 1999).

L. thompsoni puparia extracted from maize plants hatched in September-October, while no eclosion was observed in the next spring; this trend is consistent with data achieved in the past in the same study area (Camerini and Maini, unpublished data).

Sex ratio of ECB adults was year by year in favour of females: 53.5% (N = 380) in 2008, 53.1% (N = 113) in 2009 and 55.8% (N = 210) in 2010.

ECB density was on average 2.41 larvae/plant in 2008, while parasitoidism ranged from 4.2 to 44% (table 1).

ECB larval density and parasitisation rates were positively related, but such a correlation was not significant (Rs = 0.33; p = 0.25).

Results coming from 2009 samples were deeply affected by ECB larvae low densities (table 2). Larval density was generally lower than 1 specimen/plant. Since the number of emerged moths and parasitoids was very small, it was not possible to assess the correlation between parasitisation rates and larval density.

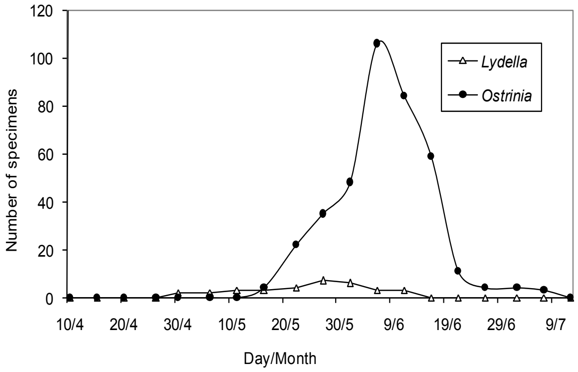

Figure 1. *O. nubilalis* and *L. thompsoni* emergence trend (overwintering generation - year 2008).

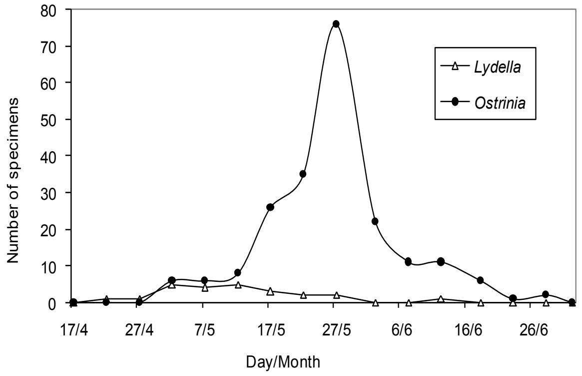

Figure 2. *O. nubilalis* and *L. thompsoni* emergence trend (overwintering generation - year 2010).

In 2010 ECB larval density was similar to the one recorded in 2008, being on average 2.63 larvae/plant, while parasitoidism percentage was on average 11% (table 3). ECB larval density and parasitisation were not significantly related (Rs = −0.35; p = 0.27).

The position of samples within fields did not seem to affect ECB larval density. Results of Mann-Whitney U

Table 1. List of samples. ECB density and parasitisation rates (year 2008).

Sampling site	Sample	Dissected plants	Larvae/plant	Reared larvae	*Ostrinia*	*Lydella*	% Parasitisation
Bastida Pancarana	Plot 1	40	1.95	56	20	10	33.3
Bastida Pancarana	Plot 2	40	2.45	61	30	12	28.6
Bastida Pancarana	Plot 3	40	2.63	81	47	13	21.7
Barbianello	Core	40	2.5	73	10	4	28.6
Barbianello	Margin	40	3	81	14	11	44.0
Bressana Bottarone	Core	40	2.55	70	37	4	9.8
Bressana Bottarone	Margin	40	2.56	50	18	2	10.0
Silvano Pietra	Core	40	2.38	64	24	6	20.0
Silvano Pietra	Margin	40	3.2	55	26	5	16.1
Bastida Pancarana	Core	40	2.43	69	11	3	21.4
Bastida Pancarana	Margin	40	1.85	51	9	1	10.0
Castelletto di Branduzzo	Core	80	2.25	121	59	8	11.9
Castelletto di Branduzzo	Wooded Margin	40	1.88	54	29	2	6.5
Castelletto di Branduzzo	Opposite margin	40	2.3	55	46	2	4.2
Total/Mean		600	2.41	941	380	83	17.9

Table 2. List of samples. ECB density and parasitisation rates (year 2009).

Sampling site	Sample	Dissected plants	Larvae/plant	Reared larvae	*Ostrinia*	*Lydella*	% Parasitisation
Bastida Pancarana	Plot 1	40	0.42	20	8	1	11.1
Bastida Pancarana	Plot 2	40	0.3	20	5	0	0
Bastida Pancarana	Plot 3	40	0.46	21	5	0	0
Bastida Pancarana	Core	80	0.78	31	10	1	9.1
Bastida Pancarana	Margin	80	0.28	27	6	0	0
Bressana Bottarone	Core	80	0.91	47	11	2	15.4
Bressana Bottarone	Wooded margin	40	0.25	22	7	1	12.5
Bressana Bottarone	Opposite margin	40	0.85	27	6	0	0
Corana	Core	40	0.2	21	7	0	0
Corana	Margin	40	0.35	25	7	1	12.5
Lungavilla	Core	40	0.69	44	10	6	37.5
Lungavilla	Wooded margin	40	1.35	41	16	6	27.3
Lungavilla	Opposite margin	40	1.58	39	12	3	20
Total/Mean		640	0.64	385	113	21	16

Table 3. List of samples. ECB density and parasitisation rates (year 2010).

Sampling site	Sample	Dissected plants	Larvae/plant	Reared larvae	*Ostrinia*	*Lydella*	% Parasitisation
Bastida Pancarana	Plot 1	40	2.3	30	12	1	7.69
Bastida Pancarana	Plot 2	40	2.23	28	16	4	20.00
Bastida Pancarana	Plot 3	40	3.13	71	36	2	5.26
Bastida Pancarana	Core	40	3.08	81	12	1	7.69
Bastida Pancarana	Margin	40	3.13	77	20	1	4.76
Barbianello	Core	40	2.55	54	17	5	22.73
Barbianello	Margin	40	2.68	46	13	3	18.75
Voghera	Core	80	2.73	87	16	1	5.88
Voghera	Wooded margin	40	2.03	32	14	2	12.50
Voghera	Opposite margin	40	2.18	47	27	1	3.57
Corana	Core	40	2.33	31	15	4	21.05
Corana	Margin	40	3.05	67	12	1	7.69
Total/Mean		520	2.63	651	210	26	11

test, which compared densities recorded between 2008 and 2010 in margins and core of fields, did not show a significant difference ($N = 26$; $p = 0.63$).

Also the proximity of maize fields to woods did not condition ECB density. Both the comparisons with opposite, not wooded margins ($N = 8$; $p = 0.39$ - Mann-Whitney U test) and core samples ($N = 8$; $p = 0.56$) did not show a significant difference.

On the contrary, parasitisation rates by *L. thompsoni* were higher in core samples than in margin ones, as shown by the contingency table ($N = 549$; d.f. = 1; $\chi^2 = 6.7$; $p < 0.05$).

ECB larvae distribution within maize plants was affected by infestation degree (figure 3). Ears were the favourite location of ECB borers when density was low. As density increased, the percentage of larvae located in the shanks and ears decreased. According to Spearman's rank analysis, such a negative correlation was statistically significant ($N = 39$; $Rs = -0.79$; $p < 0.01$).

Figure 4 displays the distribution of both ECB and *L. thompsoni* in maize plants. The contingency table analysis demonstrated that such a distribution was described by distinct patterns ($\chi^2 = 31.9$; d.f. = 2; $p < 0.01$). ECB larvae tended to feed on ears preferably, while the proportion of the parasitoids was higher in stalk tissues below and above the ear.

Figure 5 reports the frequency distribution of *L. thompsoni* puparia in relation to ECB infestation degree (number of larvae/plant). Parasitoid frequency raised significantly together with hosts density ($\chi^2 = 75.9$; d.f. = 6; $p < 0.01$).

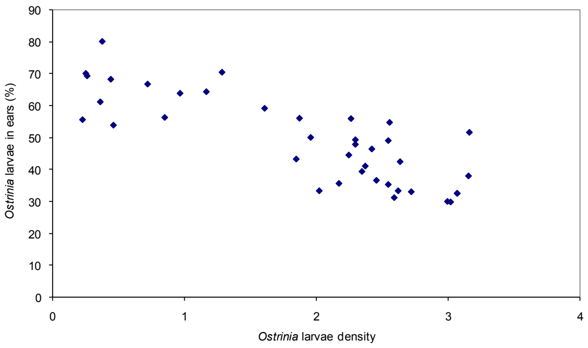

Figure 3. *O. nubilalis* larvae distribution in maize ears in relation to density (larvae/plant).

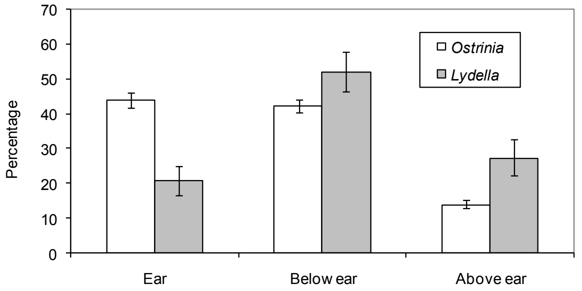

Figure 4. Frequency distribution of *O. nubilalis* larvae (N = 3212) and *L. thompsoni* (N = 121) (years 2008-2010). S.E. is shown.

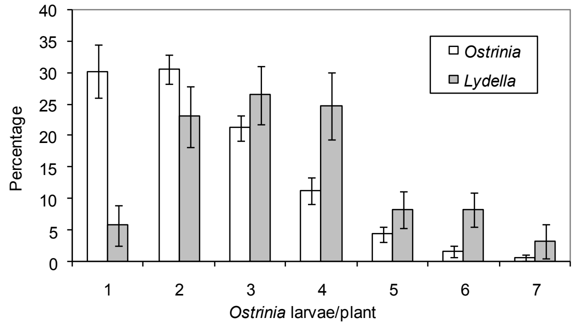

Figure 5. Frequency distribution of *L. thompsoni* puparia (N = 121) and ECB larvae (N = 3212) in relation to *O. nubilalis* infestation (larvae/plant). S.E. is shown.

Conclusions

Our investigations confirm that, in the study area, *L. thompsoni* is the only ECB larval parasitoid which may give a contribution to ECB natural control. *L. thompsoni* adults that emerge in September/October tend to attack ECB larvae and overwinter in them; a similar pattern was observed in Slovakia (Perniš and Cagán, 2011). In spring, on the other hand, *L. thompsoni* cycle is not synchronized with the one of ECB, but it is reasonable to suppose that the flies emerged between May and the first decade of June can survive until young ECB larvae are available as a host.

A factor involved in modulating the choice of the host by the parasitoid could be the adult longevity. In the Po valley the most active and abundant *L. thompsoni* populations were also the ones showing the greatest longevity (Manachini, 2000). Furthermore, *L. thompsoni* is not a monophagous parasitoid. In southern Europe, where ECB is bivoltine, it can survive thanks to hosts other than ECB. In Rhone delta (Southern France) larvae of the spring *L. thompsoni* generation can develop in the noctuid larvae *Archanara geminipuncta* (Haworth) and *Archanara dissoluta* (Treitschke), which feed on reeds (*Phragmites communis* Trinius) (Galichet *et al.*, 1985).

Also *Sesamia nonagrioides* Lefebvre (Lepidoptera Noctuidae) and *Sesamia cretica* (Lederer) are recorded as hosts of *L. thompsoni* (Eizaguirre *et al.*, 1990; Riolo *et al.*, 2001; Monetti *et al.*, 2003). In the United States a similar role as alternative host is played by *Papaipema nebris* Guenee (Lepidoptera Noctuidae), a quite common stalk borer that feeds on weeds (York *et al.*, 1955).

ECB larvae parasitisation rates were widely variable and no significant difference between ECB density in margins and interiors of the fields was found. This observation suggests that the distribution of ECB egg masses and larvae throughout the field was essentially random (Shelton *et al.*, 1986).

ECB larvae of the second generation tended preferably to bore into ear tissues, where the sap flow rate is maximum, but as ECB density tended to grow, the proportion of larvae hosted by stalks also raised. Ears were the optimal micro habitat for ECB larvae, but not for *L. thompsoni*, suggesting that the host is more susceptible to *L. thompsoni* attack when it tunnels stalk tissues rather than the inside of ears.

Finally, among the range of ECB densities recorded as a result of this study, it was not observed a density dependent relation between ECB and *L. thompsoni*.

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