

PARASITOID ASSEMBLAGES ISOLATED FROM EXTERNALLY FEEDING LEPIDOPTERANS AND CODLING MOTH (*CYDIA POMONELLA* L., TORTRICIDAE) IN A YOUNG APPLE ORCHARD IN WEST BULGARIA

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Abstract

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During the period 2005 – 2009, a gradual increase in the rate of insect parasitism was observed on externally feeding Lepidoptera developing on buds, flowers, leaves and fruits of young apple trees in an orchard located in West Bulgaria. The aim of the present study was to investigate the biodiversity of parasitoids associated with the lepidopteran complex and the codling moth in this orchard. The role of natural enemies for suppressing pest populations was estimated as well. During the five-year survey, 19 parasitoid species belonging to 6 families were identified. Species of family Ichneumonidae were dominant (42.1%), followed by Braconidae (31.6%). Three other families, Pteromalidae, Chalcidae, and Encyrtidae, were represented by one species each and accounted for 5.3 % of the abundance of the established parasitoid complex. Two tachinid flies were isolated, corresponding to 10.5 % of the complex. *Dibrachys cavus* (Walker, 1835) was the first parasitoid observed during the investigation and was found as cocoons on the leaves. *Scirtetes robustus* (Woldstedt, 1877) parasitized *Orthosia cerasi* (Fabricius, 1775) in the second year after planting of the orchard.

Aporia crataegi (Linnaeus, 1758), *Hedya nubiferana* (Haworth, 1811) and *Operophtera brumata* (Linnaeus, 1758) exhibited the highest rates of parasitoid infestation within the complex of externally feeding lepidopteran larvae. The percentages of parasitism reached to 25% for the first two pests and 22 % for *O. brumata*.

The rate of parasitism of codling moth collected in the young orchard was low: 5.3% in 2008 and 0.9% in 2009. *Liotryphon caudatus* (Ratzeburg, 1848) and *Ascogaster quadridentata* (Wesmael, 1835) were the first species established to infest the larvae of the pest.

The ichneumonid wasp *Glypta pictipes* (Taschenberg, 1863) is a new species for the Bulgarian fauna. *Pimpla arcadica* (Kasparyan, 1973), isolated from codling moth, is a new distributional record for the parasitoid, and four new parasitoid - host relationships were established for the country: *Diadegma armillata* (Gravenhorst 1829) was reared of *Coleophora hemerobiella* (Scopoli 1763), *Compsilura concinnata* (Meigen 1824) of *Aporia crataegi* (Linnaeus 1758), *Protapanteles vitripennis* (Curtis 1830) of *Yponomeuta malinellus* (Zeler 1938), and *Lissonota dubia* (Holgren 1856) of *Choristoneura hebenstreitella* (Muler 1764).

Key words: parasitoid complex, lepidopteran larvae, codling moth, young apple orchard

Introduction

The tremendous biodiversity of parasitoids in apple orchards is well known and extensive researches has been performed on their isolation, identification and significance as biological control agents both in Bulgaria and worldwide (Tschorbadjieff, 1933; Nikolova, 1962; Evenhuis and Vlug, 1983; Ivanov and Slavov, 1982; Angelova, 1983; Zerova et al., 1989; Atanassov, 1986; Lecheva and Zaprianov, 1996; Atanassov et al., 1997; Balaz, 1997; Balevski, 2009; CMISS 2009; Pluciennik and Olszak, 2010.) Surprisingly, there have been no attempts to follow appearance of natural enemies and estimated their role for suppression the pest lepidopteran populations in new planted apple orchards.

In the present study, we investigate the formation of the parasitoid complex of externally feeding lepidopterans in a recently planted apple orchard located in West Bulgaria. The devastating, internally feeding pest of apples codling moth *Cydia pomonella* (Linnaeus 1758) appeared immediately after formation of the first fruits and its parasitoids were isolated and listed as well. The significance of the parasitoid complex as an ecological factor for reduction of lepidopteran pest populations and its role for pest species mortality were also assessed.

Material and Methods

In 2005 - 2009, weekly observations and collection of lepidopteran larvae were conducted in a young apple orchard planted in 2004, not treated with insecticides. The orchard covers 1.5 ha and is located in the experimental field of Plant Protection Institute near the city of Sofia - 541h a. s. l., 42°48' N and 23°12' E. The collected larvae were reared individually on apple buds and leaves under laboratory conditions. Pupation and emergence of moths and parasitoids were recorded daily. The relative density of leaf - feeding Lepidoptera was established by random sampling of five apple trees in the orchard.

Codling moth larvae were collected during summer and autumn with corrugated bands and from damaged fruit in the young apple orchard in the period 2007 – 2009, starting at the time of formation of the first fruit.

For comparison of parasitoid species composition of codling moth larvae, bands were also put on aged untreated apple trees near the experimental field. In the course of the investigation, a total of 1628 codling moth larvae and 405 externally feeding lepidopteran larvae were collected.

Results and Discussion

Dibrachys cavus (Walker 1835) was the first established parasitoid (collected on 29. 07.2005), present as cocoons on the leaves in the crowns of the apple trees. This cosmopolitan species is a wide generalist and it was difficult to suppose which hosts it parasitized.

Hedya nubiferana (Haworth 1811), *Operophtera brumata* (Linnaeus 1758) and *Coleophora hemerobiella* (Scopuli 1763) were permanent species within the complex of externally feeding Lepidoptera in the young orchard for the period of the investigation (Velcheva, 2009). *H. nubiferana* was the dominant species in 2005 and 2006 and the collected specimens constituted 16.8% of the abundance of all reared externally feeding lepidopteran larvae. Only two parasitoid species were isolated from the pest (Table 1), while in a previous investigation (Velcheva, unpublished) twelve parasitoid species (one of them secondary) were reared from *H. nubiferana* larvae collected from untreated apple trees and an abandoned apple orchard in the same region. In general, the percentage of parasitism is linked to the relative density of *H. nubiferana* (Figure 1). The most extensive established rate of infestation of the larvae registered in 2008 and 2009 was 25%.

Operophtera brumata L prevailed in 2007 and 2008 (Velcheva, 2009). For the whole period, this pest comprised 14.6% of the studied externally feeding lepidopteran complex. The established percentage of parasitism gradually increased during the years of observations (Figure 2). In 2009, the larvae of *O. brumata* were most extensively parasitized (22.0% of collected specimens). Nevertheless, only one braconid wasp, *Protapanteles immunis* (Haliday 1834), was reared from the larvae of the winter moth. Other parasitoids announced to infest *O. brumata* in Bulgaria were: *Diadegma armillata* (Gravenhorst, 1829) (Ichneumonidae, Campopleginae) reported by Lecheva and Zaprianov

Table 1
Taxonomic list of the hosts and reared parasitoids

Lepidopteran larvae and pupae		Parasitoids	
Family, subfamily, tribe	Species	Family, subfamily, tribe	Species
Tortricidae Olethreutinae Grapholitini	<i>Cydia pomonella</i> (Linnaeus, 1758)	Ychneumonidae Pimplinae, Ephialtini Pimplinae, Pimplini Braconidae Cheloninae Pteromalidae Pteromalinae Tachinidae Exoristinae	<i>Liotryphon caudatus</i> (Ratzeburg, 1848) *** <i>Pimpla arcadica</i> (Kasparyan, 1973) <i>Ascogaster quadridentata</i> (Wesmael, 1835) <i>Dibrachys cavus</i> (Walker, 1835) <i>Elodia morio</i> (Fallen, 1820)
Tortricidae Olethreutinae Olethreutini	<i>Hedya nubeferana</i> (Haworth, 1811)	Braconidae Microgasterinae Chalcidae Chalcidinae	<i>Apanteles candidatus</i> (Haliday, 1834) <i>Brachymeria tibialis</i> (Walker, 1834)
Tortricidae Tortricinae Archipini	<i>Archips xylosteana</i> (Linnaeus, 1758)	Ychneumonidae Pimplinae, Pimplini	<i>Itoplectis maculator</i> (Fabricius, 1775)
Tortricidae Tortricinae Archipini	<i>Archips crataegana</i> (Hubner, 1799)	Braconidae Macrocentinae	<i>Macrocentrus linearis</i> (Nees, 1811)
Tortricidae Tortricinae Archipini	<i>Choristoneura hebenstreitella</i> (Muller, 1764)	Ychneumonidae Banchinae Atrophini	** <i>Lissonota dubia</i> (Holgren, 1856)
Geometridae Larentiinae Operophterini	<i>Operophtera brumata</i> (Linnaeus, 1758)	Braconidae Microgasterinae	<i>Protapanteles immunis</i> (Haliday, 1834)
Noctuidae Noctuinae Orthosiini	<i>Orthosia cerasi</i> (Fabricius, 1775)	Ychneumonidae Campopleginae	<i>Scirtetes robustus</i> (Woldstedt 1877)
Noctuidae Noctuinae Xylenini	<i>Cosmia trapezina</i> (Linnaeus, 1758)	Ychneumonidae Campopleginae	<i>Scirtetes robustus</i> (Woldstedt, 1877)
Pieridae Pierinae Pierini	<i>Aporia crataegi</i> (Linnaeus, 1758****)	Braconidae Microgastrinae Tachinidae Exoristinae	<i>Cotesia glomerata</i> (Linnaeus, 1758) ** <i>Compsilura concinnata</i> (Meigen, 1824)
Yponomeutidae Yponomeutinae	<i>Yponomeuta malinellus</i> (Zeller, 1938) **** <i>Swamerdamia pyrella pyrella</i> (Villers 1789)	Ychneumonidae Campopleginae Braconidae Microgasterinae Chalcidoidea Encyrtidae Encyrtinae Ychneumonidae Ciptinae Phygadeuontini	<i>Diadegma armillata</i> (Gravenhorst, 1829) ** <i>Protapanteles vitripennis</i> (Curtis, 1830) = <i>Apanteles vitripennis</i> (Curtis, 1830) = <i>Glyptapanteles vitripennis</i> (Curtis, 1830) <i>Ageniaspis fuscicollis</i> (Dalman, 1820) <i>Gelis</i> sp. secondary parasitoid
Gelechiidae	Not identified to species	Ychneumonidae Banchinae Glyptini	* <i>Glypta pictipes</i> (Taschenberg, 1863)
Coleophoridae	<i>Coleophora hemerobiella</i> (Scopoli, 1763)	Ychneumonidae Campopleginae	** <i>Diadegma armillata</i> (Gravenhorst, 1829)

*New species for Bulgarian fauna;

**New host parasitoid relation;

***New distributional record for Bulgaria;

****The number of the larvae of these pests was not included in the sum of found because of gregarious manner of feeding in nests.

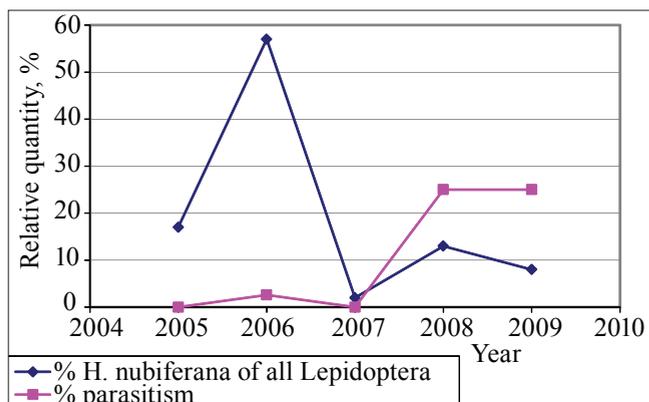


Fig. 1. Relative quantity of *H. nubiferana* in the complex of leaf eating Lepidoptera and percentage of parasitism

(1996), *Protapanteles immunis* (Haliday, 1834) (Braconidae, Mikrogastrinae), *Eulophus larvarum* (Linnaeus, 1758) (Eulophidae, Eulophinae) and *Lypha dubia* (Fallen, 1810) (Tachinidae Tachininae) found by Peeva (2009)) - *Apanteles sodalis* (= *A. ater*) (Haliday, 1834), *A. xantostigma* (Haliday 1834), *Cotesia jucunda* (Marshall, 1885) and *Cotesia praepotens* (Haliday, 1834) (Braconidae) were listed by Balevski (2009).

The other species of externally feeding lepidopterans, identified during the investigation, were parasitized only during one year of the observations. The average rate of parasitism, established for collected late instars of solitary living larvae of *Aporia crataegi* (Linnaeus, 1758) was rather high, reaching 25% of all collected individuals. The rate of parasitism in individual nests ranged from 0 to 36.7%. *Cotesia glomerata* (Linnaeus, 1758), a gregarious braconid wasp and *Compsilura concinnata* (Meigen 1824), a polyphagous parasitoid tachinid fly, were isolated from the pest (Table 1). This is the first report of *A. crataegi* as a host of *C. concinnata* for Bulgaria. The emerged tachinid fly was previously reported as a parasitoid of twelve other lepidopteran species in the country (Hubenov, 1985, 2001). This species parasitizes over 100 different hosts in the Palearctic Region, at least 161 in North America, and 67 in Japan (Ichiki and Nakamura, 2007). In Italy *A. crataegi* L was established as a host of two more tachinid flies - *Phryxe vulgaris*, *Exorista segregate* (Cerretti and Tschorsnig, 2010).

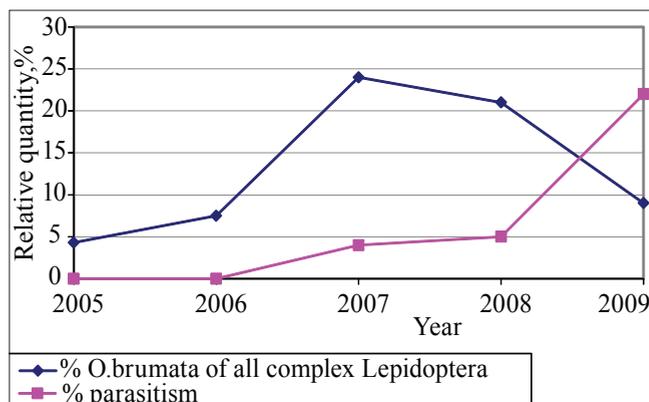


Fig. 2. Relative quantity of *O. brumata* in the complex Lepidoptera and percentage of parasitism

In 2005 there was an outbreak of *Yponomeuta mallinellus* (Zeller, 1938) and its nests were found on 34.92% of the apple trees. Three species of parasitoids were isolated from the pest and one new trophic host - parasitoid relationship was established for Bulgaria (Table 1). Tschorbadjieff (1933) listed fourteen parasitoids isolated from *Y. mallinellus*: *Z.-Herpestomus brunnicornis* (Gravenhorst, 1829), *Dirophanes fulvitaris* (Wesmael, 1845), *Exochus gravipes* (Gravenhorst, 1820), *Chorinaeus cristator* (Gravenhorst, 1829), *Mesochorus vittator* (Zetterstedt, 1838), *Itopectis alternans* (Gravenhorst, 1829), *Diadegma armillata* (Gravenhorst, 1829), *Campoplex* sp., *Elasmus* sp. (Ichneumonidae), *Tetrastichus crassinervis* (Thompson, 1878), *Pteromalus variabilis* (Ratzeburg, 1844), *Ageniaspis fuscicollis* (Dalman 1820), *Baryscapus evonimellae* (Bouché, 1834) (Chalcidoidea) and *Eurysthaea scutellaris* (Robineau - Desvoidy, 1848) [*Roeselia*, *Erythroceras*, *Discochaeta (h)yponomeutae* Rondani, *evonymellae* Ratzeburg] (Tachinidae). Jacentkovsky (1936) reported *Actia crassicornis* (Meigen, 1824) as a new species for the Bulgarian fauna. Consequently, Trenchev (1980) reported *Y. mallinellus* as a host of *A. crassicornis*. The author also isolated two other parasitic tachinid flies from the pest, *Bessa parallela* (Meigen 1824) (= *B. fugas* Rondani, 1861) and *Peribaea tibialis* (Robineau-Desvoidy, 1851) (= *Strobilomyia tibialis* Robineau-Desvoidy), which were new for the Bulgarian fauna. Popov (1956) reported *Bessa selecta* (Meigen, 1824)

and *Bactromyia aurulenta* (Meigen, 1824) as parasitoids of *Y. mallinellus* in Bulgaria. Hence, the list of insects known to parasitize *Y. mallinellus* in Bulgaria includes 27 species.

The oligophagous ichneuomonid wasp *Scirtetes robustus* (Woldstedt), which develops in noctuid larvae, was one of the first parasitoids to appear in the young apple orchard. One infested larva of *Orthosia cerasi* (Fabricius, 1775) was found on 3.05.2007, and *S. robustus* emerged on 17.03.2008.

The species which first parasitized the codling moth were *Liotryphon caudatus* (Ratzeburg 1848) and *Ascogaster quadridentata* (Wesmael, 1835). The first found parasitoid in young orchard *D. cavus* emerged of codling moth larvae on 27.07.2009.

In total, five parasitoids were isolated from codling moth larvae, collected in the young apple orchard. Two of them belong to family Ychneuomonidae, one to Braconidae, and one to Pteromalidae (Table 1). *A. quadridentata* was the only egg-larval parasitoid. The list of parasitoid species isolated from codling moth developing in the fruits of aged untreated apple trees in the vicinity of the investigated experimental orchard comprised two more representatives of family Ychneuomonidae – the solitary larval endoparasitoid *Pristomerus vulnerator* (Panzer, 1799) (Cremastinae) and the solitary pupal endoparasitoid *Pimpla turionellae* (Linnaeus, 1758) (Pimplinae), as well as one more species from family Braconidae – *Bassus (Microdus) rufipes* (Nees, 1812). The list did not include *Pimpla arcadica* (Kasparyan, 1973) which was isolated from codling moth in the young orchard. According to Kolarov (1997), *P. arcadica* is very rare and was found by the author in April only in Strandzha mountain in our country. The species was first described from The Crimea by Kaparjan (1973). Consequently, it was reported for Sweden (Yu et al., 2005), Norway (Hansen et al., 2010) and the North-East Anatolian Region of Turkey (Coruh, 2010). Obviously, *P. arcadica* Kas. develops two generations per year - in Turkey it was collected in June and August (Coruh, 2010), while our specimen emerged on 12.08.2009.

The parasitoid tachinid fly *Elodia morio* (Fallen, 1820) emerged from codling moth larvae collected with bands from both the young and the aged apple

trees. The parasitoid was registered for the first time in 2008, four years after planting of the orchard. Ten wasps of the family Ichneuomonidae were listed as larval and pupal parasitoids of *C. pomonella* in Bulgaria by Atanassov (1986) and eleven braconids were listed by Balevski (2009). *Aleiodes bicolor* (Spinola, 1808) and *Microgaster meridiana* (Haliday, 1834) were reported as parasitoids of codling moth by Peeva et al. (2010) and *P. arcadica* (Kasparyan, 1973) by Kolarov (1997). We also isolated *Exorista fasciata* (Fallen, 1820) from codling moth larvae collected in an organic orchard in Plovdiv region. This is a new report of host - parasitoid relationship, contributing to the extensive host list compiled by Hubenov (1985, 2001). With the addition of *Elodia morio* (Fallen, 1820) first announced by Trenchev (1980), the Bulgarian faunistic list of parasitoids of the codling moth now comprises 26 parasitoids. So, 19.2% of all parasitoid species known to occur in our country were attracted immediately after the appearance of their host in the young apple orchard. According to Lloyd (by Laurence et al., 2005), there are over 100 insect species parasitizing the codling moth worldwide.

The rates of parasitism on codling moth established in our study and presented in Table 2 were very low. There are similar reports of considerably low rates of parasitism on *C. pomonella*. For example, Monteiro et al. (2010) reported mean rates of infestation of 3.7%, 2.3% and 0.8% in organic and conventional with and without mating disruption in France. The authors did not find parasitized diapausing codling moth larvae in 79% and 83.6% of the observed apple orchards.

In the period 1994 – 1995, the reported mean rate of infestation of the codling moth in Switzerland (La

Table 2
Relative density of codling moth, externally leaving lepidopteran larvae and mean rate of parasitism

Years	Mean number of diapausing codling moth larvae/band	Rate of parasitism, %	Relative density of larvae on 100 organs	Rate of parasitism, %
2005	0	0	6.7	0
2006	0	0	8.8	3
2007	3.1	0	16.7	5
2008	11.9	5.3	13.9	8.9
2009	7.8	0.9	8.6	9

Côte) was 3% and in Göttingen, South Germany – 4.9% (Atanassov et al., 1997). Subinprasert (1987) established a rather different rate of parasitism in South Sweden, ranging from 22.9% to 61.1% during the period 1981-1984, or mean - 38.3%. Perhaps, the reason was that his investigations were performed in very old apple orchards, planted about 50 years ago. Tkachev (1986) also reported a high percentage of parasitism, reaching up to 27%, from Ukraine.

Conclusions

Nineteen parasitoid species belonging to six families were reared from the lepidopteran pests collected from the young apple orchard. Species of families Ichneumonidae were dominant (42.1%), following by Braconidae (31.6%). Parasitoid flies from family Tachinidae had a relative abundance of 10.5%, and three families - Pteromalidae, Chalcidae, and Encyrtidae were represented by one species each, accounting for 5.3 % of established parasitoid complex. It can be supposed, that the first reared parasitoid species generally have a very high searching ability and detect their hosts earlier than others, or that they have found better living conditions in the crowns of the young trees, compared with other environments.

Dibrachys cavus (Walker, 1835) was the first parasitoid to appear in the young apple orchard. It was found as cocoons on the leaves in the crowns of the apple trees.

Scirtetes robustus (Woldstedt, 1877) parasitized larvae of *Orthosia cerasi* (Fabricius, 1775) in the second year after planting of the orchard, in 2006. The ichneumonid wasp emerged in 2007.

Two parasitoids were isolated from one of the dominant pests - *Hedya nubiferana* (Haworth, 1811). The rate of infestation of its larvae was high in the fourth and the fifth year after planting of the orchard. The established rates of parasitism were related to the density of the pest.

The rates of parasitism of the other of dominant species in the complex of externally feeding Lepidoptera, *Operophtera brumata* (Linnaeus, 1758), gradually increased during the years of investigation and reached 22% in 2009.

The phytophagous insect *Aporia crataegi* Linnaeus (1758) exhibited a high rate of parasitoid infestation reaching 25%.

Liotryphon caudatus (Ratzeburg, 1848) and *Ascogaster quadridentata* (Wesmael, 1835) were the first parasitoid species reared from codling moth. Five parasitoids were isolated altogether. The rate of infestation of codling moth was too low to play a significant role in decreasing the populations of the pest.

The ichneumonid wasp *Glypta pictipes* (Taschenberg, 1863) is a new species for the Bulgarian fauna.

Pimpla arcadica (Kasparyan, 1973) reared from codling moth is a new distributional record for the parasitoid.

Four new parasitoid - host relationships were established for the country: *Diadegma armillata* (Gravenhorst, 1829) - *Coleophora hemerobiella* (Scopoli, 1763), *Compsilura concinnata* (Meigen, 1824) - *Aporia crataegi* (Linnaeus, 1758), *Protapanteles vitripennis* (Curtis, 1830) - *Yponomeuta malinellus* (Zeler, 1938), and *Lissonota dubia* (Holgren, 1856) - *Choristoneura hebenstreitella* (Muler, 1764).

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