First record of Braconidae as parasitoids of *Capnodis tenebrionis* (Linnaeus) (Coleoptera: Buprestidae), with notes on the ecology of *Spathius erythrocephalus* Wesmael (Hymenoptera: Braconidae)

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Key words: biological control; *Capnodis tenebrionis*; Coleoptera; Buprestidae; *Spathius erythrocephalus*; Hymenoptera; Braconidae; gregarious; ectoparasitoid; plum; apricot; host location; host finding.

For the first time an ichneumonoid parasitoid is reported from the peach flatheaded rootborer *Capnodis tenebrionis* (Linnaeus, 1758) (Coleoptera: Buprestidae) in a heavily infested plum and apricot orchard in south-western Sicily. In 2005 and 2006 the ecology of the ectoparasitoid *Spathius erythrocephalus* Wesmael, 1838, (Hymenoptera: Braconidae) was studied. The larvae are ectophagous and gregarious; obviously the parasitoid search for the large buprestid larval (6.0 ± 0.4 cm). The adult parasitoid appeared in both cultivations in August and/or September and selected only the mature larvae for oviposition. In 2006 35% of the rootborer larvae on plum were parasitized. Significant differences were found between the mean depth in the soil in which parasitized and non-parasitized larvae were found, although *C. tenebrionis* larvae closest to the level of the soil surface are the easier to reach. The parasitoid is able to search for and parasitize host larvae up to a soil depth of 20 cm. No significant difference was found in the distribution of the parasitoid with respect to the compass orientation within the tree. *Spathius erythrocephalus* is a new promising antagonist for the biological control of the noxious peach flatheaded rootborer.

Introduction

The peach flatheaded rootborer *Capnodis tenebrionis* (Linnaeus, 1758) (Coleoptera: Buprestidae; figs 4, 8) is highly destructive in orchards of drupe fruit trees, in particular of plum (Garrido, 1984; Martin et al., 1998; Ben Yehuda et al., 2000). It is widespread in the Mediterranean region and has serious effects in organic orchards where trees may be rapidly killed by the destructive action of its larva. They attack the neck and roots of the trees forming large, sinuous galleries. The adult beetle is diurnally active and flies during warm days (Bonsignore & Bellamy, 2007). It feeds on the young bark of the shoots, buds and above all at the base of the leafstalks; the leaves drop off and form a layer under the tree revealing the presence of the adults (fig. 1; Bonsignore et al., 2008).

At present, control of this insect pest relies on the use of chemicals, primarily aimed at controlling newly emerged larvae and adults, which are the most susceptible (Sekkat et al., 1997; Sanna-Passino & Delrio, 2001). Up to now the use of alternative control methods is confined to enclosed environments (Garcia del Pino & Morton, 2005; Marannino et al., 2006) and at the moment does not provide a suitable alternative due to both the limited availability of means and their poor efficacy. Only the application of the entomopathogenic nematode *Steinernema carpocapsae* Weiser, 1955, seems to cause high

Figs 1-10, *Capnodis tenebrionis* (Linnaeus). 1, Dying plum tree because of attack by *C. tenebrionis* larvae; 2, young larvae; 3, full grown larva; 4, 8, adult beetle; 5, pupa; 6, emergence holes; 7, 9, larvae of *S. erythrocephalus* Wesmael on full grown larva; 10, adult *S. erythrocephalus* Wesmael. Scale bar is 1 mm.

mortality of the larvae (Del Mar Martinez de Altube et al., 2007). The antagonist biocoenosis does not provide a significant contribution to mortality, with only a modest number of species in the Mediterranean area. The known natural enemies, *Pheidole pallidula* (Nylander, 1849) (Pussard, 1934; Hymenoptera: Formicidae), *Avetianella capnodiobia* Trjapitzin, 1968 (Alexeev, 1984; Hymenoptera: Encyrtidae), *Sclerodermus cereicollis* Kieffer, 1904 (Marannino & De Lillo, 2005, 2007; Hymenoptera: Bethylidae), *Billaea adelpha* (Loew, 1873) and *B. subrotundata* (Rondani, 1862) (D'Aguilar & Feron, 1949; Diptera: Tachinidae), *Sarcophila latifrons* (Fallén, 1817) (Rivnay, 1947; Diptera: Sarcophagidae) and *Steinernema carpocapsae* (Santos Lobatón et al., 1998; Rhabditida: Steinernematidae) are unable to exert, either individually or together, a satisfactory control of the *C. tenebrionis* populations.

During 2005 the widespread West Palaearctic *Spathius erythrocephalus* Wesmael, 1838 (Hymenoptera: Braconidae; fig. 10) (Fauna Europaea Web Service, 2004) was found to be parasitizing *C. tenebrionis* in Sicily. Recently, this species has been reported from the cerambycid *Anoplophora chinensis* (Forster, 1771) (Hérard et al., 2005). For the identification the second author used his forthcoming overview of the European members of the genus *Spathius* Nees, 1819; in this paper the full synonymy will be given. The mature larvae of the parasitoids spin their cocoons within the host's feeding site and adjacent to the host remains. Individual cocoons are hard to count because they are adpressed in a close mass. The study was undertaken to find and characterise new biological agents against *C. tenebrionis*.

Materials and methods

Study site and sampling of *Capnodis tenebrionis*.

The study was carried out in the countryside (latitude 37°25′52″N, and 13°52′57″E at 500 m above sea level) in Sicily (Italy) in an apricot and a plum orchard, both of approximately 1.0 ha, during the period April-October in 2005 and 2006. The area specializes in the production of peaches, grapes, apricots, etc. The orchards had been biologically cultivated for 11 years without any chemical treatment and are heavily attacked by *C. tenebrionis* (fig. 1). The apricot and plum plants were grafted onto the Mirabolano (*Prunus cerasifera* Ehrh.) rootstock with the trees 4 m apart.

Every fifteen days from April 2005 two trees of each species displaying clear signs of the buprestid attack were randomly selected from both cultivations for a detailed analysis of the extent of damage to the neck and the primary and secondary roots (in part). The four trees were uprooted and the bark covering the roots and neck was carefully lifted to count the number of dead and alive larvae and pupae of *C. tenebrionis* (figs 2, 3, 5) and to note their position in relation to the surface level of the soil and the compass orientation in the root (north-east 0-90°, south-east 91-180°, south-west 181-270° and north-west 271-360°).

The larvae found with parasitoids were placed individually, together with the roots or the neck (without discarding the bark cover) in plastic containers (20 cm in diameter by 12 cm in height) containing filter paper soaked in distilled water (added every 4 days) closed with tulle and placed in an environment with a temperature ranging from 20 to 25°C until adult parasitoids emerged. For a small number of buprestid larvae the number of larvae of the parasitoid could be counted (figs 7, 9) and the length of the parasitized larvae was measured. The measurements of the average depth (relative to the surface level of the soil) of parasitized and non-parasitized larvae during the period the parasitoid was present are compared by using the *t*-test for two independent samples. The homogeneity of variances was examined using the f test before the data were analysed.

The χ^2 test with degrees of freedom (k-1) was used for the distribution of parasitized larvae compared to the different sectors of exposition of the tree, with k being the number of sectors of compass orientation (NE-SE-SW-NW) and it was possible to test whether the distribution of parasitized larvae was random or orientated. Before the test, verifications on density and distribution of host larvae did not underline a measurable effects on parasitoid distribution. Yates' correction for continuity was used to improve the reliability of the test.

Biology of host

Capnodis tenebrionis adults are sexually dimorphic. Females are larger and heavier than males (mean female length = 2.426 ± 0.053 cm; prothorax width = 0.973 ± 0.024 cm, weight = 0.812 ± 0.045 g: male length = 2.268 ± 0.039 cm; prothorax width = 0.904 ± 0.018 cm, weight = 0.606 ± 0.026 g; unpublished data from a hibernating population collected in 2005). Furthermore, the last visible abdominal segment is rounded in females and flat in males.

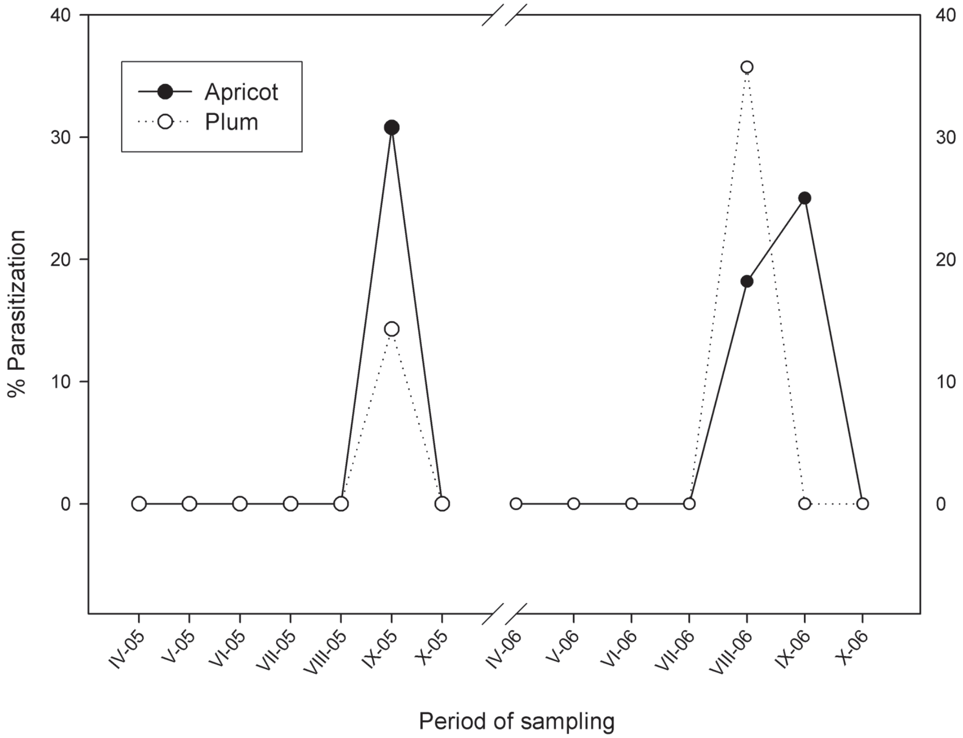

Fig. 11. Percentage of parasitized host larvae (compared to all host larvae) during the research period.

The emergence of the adults start in spring when the ambient temperature rises (Bonsignore et al., 2007). They can live for more than one year and few hibernate twice. The adults of the subsequent generation emerge at the end of summer and reach sexual maturity the following spring (Bonsignore, unpublished). Males of *Capnodis tenebrionis* on apricot do not show signs of parental care and aggregate on the host tree at the same time as the females. In the reproductive phase the insect chooses plants that are weakened, generally as a consequence of previous or actual larval infestation of the roots or neck. In the area of study the egg-laying in proximity at the neck begins in June and continues for the rest of the season. The larvae spend at least two winters in the tree, depending on the temperature and the food supply.

Results

In 2005 the first parasitized larvae were found in both host trees in September, while in 2006 they appeared in August on plum and in August and September on apricot (fig. 11). Prepupae found earlier in the year were not attacked by the parasitoid. In cases where the bark of the trees examined was removed and the parasitized larvae were exposed, no subsequent emergence of the parasitoid was observed. Reared adults of *Spathius erythrocephalus* emerged 1-2 months later at 20-25°C. The percentage of parasitization was up to 35% of the total larval population examined, with a difference between apricot and plum

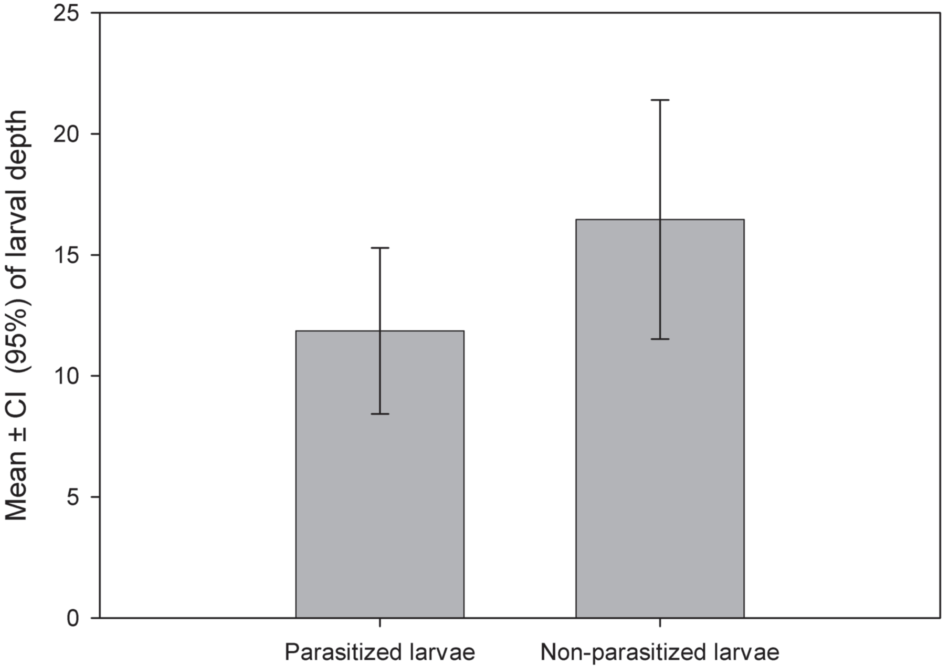

Fig. 12. Mean ± CI (95%) depth (cm below soil level) of parasitized and non-parasitized larvae. Note. Numbers are rather small and, therefore, may not reveal a possible bias. Value of *t*- test = 4.15; n=45; P<0.05

that is difficult to interpret. In plum on average 14.3% and 35.7% was parasitized in 2005 and 2006, respectively, for apricot it was 30.8% and 25%, respectively.

The average length of parasitized buprestid larvae was 6.0 cm ± 0.4 (n = 14; P = 0.05; figs 7, 9). Larvae measuring less than 5.5 cm were not parasitized (fig. 2), which is likely the result of the host-searching mechanism of the gregarious parasitoid. Most likely sound, or e.g. semiochemicals associated with prepupal changes, enables the parasitoid to focus on big hosts (with a high efficiency for the parasitoid). Otherwise, the parasitoid should adjust brood size (as is normal for gregarious idiobionts) according to host size (M.R. Shaw, pers. comm). Simultaneously, the available beetle larvae range from 1-7 cm, indicating an overlap of larval instars. The mean depth of the parasitized larvae (in relation to the level of the soil surface; fig. 12) was 11.9 cm ± 3.4 (n = 14; P = 0.05), while for non-parasitized larvae the mean depth was 16.5 ± 5.0 (n = 37; P = 0.05). The **t** value for the comparison between the mean depths of parasitized and non-parasitized larvae revealed a significant difference between the two values (fig. 12), but the rather small sample size allows only a provisional conclusion. Although *C. tenebrionis* larvae closest to the level of the soil surface are the easiest to reach, the parasitoid is able to search for and reach the host in the tree up to a soil depth of 20 cm.

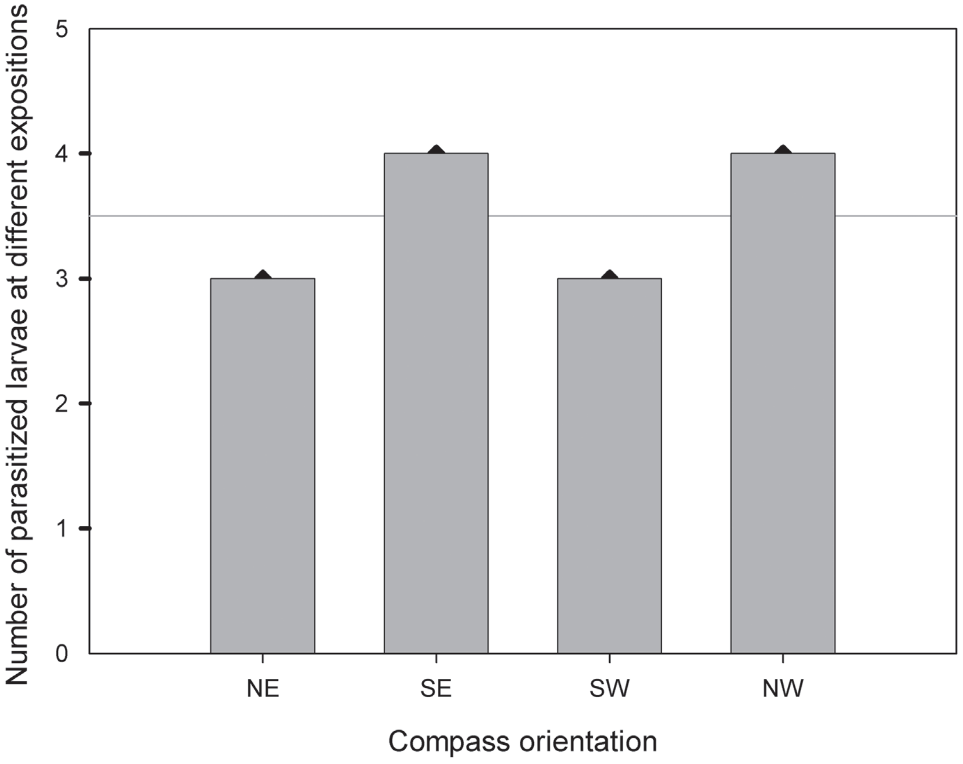

Fig. 13. Number of parasitized larvae found at different compass orientations in the tree; the χ^2 value was 0.57 (P>0.05). The grey solid line represents the number of parasitized larvae expected for each exposition. Note. Numbers are small and, therefore, may not reveal a possible bias.

The χ^2 test showed a uniform distribution of parasitized larvae in the different sectors of the tree (NE - SE - SW - NW) plants (fig. 13). The χ^2 value was 0.57 ($P>0.05$). Deviations from expected values were not significant and the data obtained confirms the random distribution parasitized larvae (fig. 13). The mean number of the ectophagous and gregarious larvae on the parasitized buprestid larva was equal to 9.26 ± 1.27 ($n = 5$; $P = 0.05$).

Discussion

The research conducted in 2005 and 2006 on the ecology of the peach flatheaded rootborer in fruit orchards of southern Sicily underlined the scarcity of natural enemies of the pest. The most important mortality factors are probably attributable to other causes, such as entomopathogenic fungi, dehydration and starvation of the larvae in the roots of too heavily attacked or dead trees. It makes the presence of *S. erythrocephalus* especially important and indicates the need to understand its potential effect on the pest. The appearance of *S. erythrocephalus* towards the end of summer, before and during the formation of the pupae by *C. tenebrionis*, mirrors the behaviour of bark beetle parasitoids that develop in considerable numbers when the preferred host is at the final larval instar or the pupal phase (Camors & Payne, 1973; Dixon & Payne, 1979; Berisford et al., 1995). The attack on the rootborer larvae was observed almost at the same time as the formation of the pupae, which, in the site examined, occurs mainly in August (Bonsignore, unpublished data). However, at the time of attack the host is present in different biological stages, as a result of overlap of generations. Members of the genus *Spathius* Nees, 1819, have probably rather narrow host ranges (M.R. Shaw, pers. comm.), being in similar environments parasitoids of several families of Coleoptera: Buprestidae, Cerambycidae, Curculionidae and Scolytidae (Fischer, 1966; Matthews, 1970; Shaw, 1988; Moraal & Hilszczanski, 2000; Moraal & van Achterberg, 2001; Lozan & Zelený, 2002; Alexander, 2002; Bauer et al., 2005 and Yang et al., 2005). Although a very wide range of hosts is recorded for many species, most records have been made uncritically and have questionable reliability (cf. Shaw, 1994) and in fact we have a poor idea of the real host ranges of most *Spathius* species. The records of lepidopteran larvae as hosts (Watanabe, 1964, Tanwar & Varma, 1997 and Saxena, 1992) are unlikely and need to be treated with particular suspicion. Most likely *S. erythrocephalus* will use also other coleopteran hosts as *C. tenebrionis*, if available, since the geographical range of the parasitoid is much larger than that of the host. Presumably the parasitoid used also a different host at the study site earlier in the year. The presence of *S. erythrocephalus* during a very short period may be an adaptation to the buprestid host when no other hosts in the preferable stadium are present in the field.

S. erythrocephalus (fig. 10) is highly proficient at searching for a host and is able to locate it also at considerable soil depth. In this respect, it is necessary to understand the mechanisms it uses to interact with the buprestid larvae. Considering the soil depth reached by the parasitoid, it is probable that they use cracks in the soil around the trunk of the trees. It is not known what is governing its searching behaviour; probably chemical mediators are involved, but also sound (vibrations by the large digging buprestid larvae) should be considered. In a similar species, the Nearctic *S. pallidus* Ashmead, 1893, it has been ascertained that the localization of the larvae of the southern pine beetle *Dendroctonus frontalis* Zimmermann, 1868, is mediated by a combination of different semiochemicals (Sullivan et al., 1997). It is obvious, that much more research on the subject is necessary before this parasitoid can be manipulated for biological control of *C. tenebrionis*.

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