

# A preliminary report on the effects of the rootstocks on the postembryonic development of *Capnodis tenebrionis* on semi-artificial substrates

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## Abstract

*Capnodis tenebrionis* (L.) (Coleoptera Buprestidae) is one of the major pests of apricot and other stone-fruit trees in the Mediterranean basin, central and southern Europe, North Africa, and around the Black and Caspian Seas areas. This study was aimed at evaluating the influence of the rootstocks on the postembryonic development of the pest and emerged adults. Larvae were reared on semi-artificial substrates from the egg hatching to the pupa occurrence. The substrates differed only for the flour obtained from the bark of eight rootstocks (Adesoto, Cab6P, Colt, Garnem, GF677, MaxMa60, Montclar, Myrabolan 29C), selected among those more commonly used. For each treatment, weight increase of each larva, their survival, appearance time of pupae and adults' size were recorded. Larval survival resulted the highest for GF677 treatments and the lowest for Colt and Adesoto. The biggest adults emerged from the substrate containing Montclar flour, whereas the smallest emerged from Adesoto and MaxMa60 treatments. Current results suggest that Adesoto, Colt and MaxMa60 may affect negatively the postembryonic development of this beetle more than the other assayed rootstocks. This different susceptibility might be used in an integrate management of this pest.

**Key words:** peach flat headed root borer, semi-artificial diet, no-choice assays, larvae, ontogenetic development, adult size.

## Introduction

The peach flat headed root borer, *Capnodis tenebrionis* (L.) (Coleoptera Buprestidae), is widespread in the Mediterranean basin, central and southern Europe, North Africa, and around the Black and Caspian Seas areas where it is a major threat to stone-fruit orchards (Garrido *et al.*, 1984; Ben-Yehuda *et al.*, 2000; Mendel *et al.*, 2003; Bari *et al.*, 2019). Adults devour petioles, defoliate plants, destroy buds and consume bark of tender or weakly lignified twigs causing damages in nurseries and young plantations. They can live for more than one year and a female can lay more than 1000 eggs, placed in dry ground, inserted in cracks or under stones, usually near the base of trees (Rivnay, 1946; Garrido, 1984; Gindin *et al.*, 2009). The neonate larvae can locate roots from 60 cm distance, at most, and penetrate them (Rivnay, 1946). Endophytic larvae dig winding and girdling galleries in the roots and at the base of the trunk between bark and wood. The postembryonic development lasts usually one year and one generation can be biannual (Garrido, 1984). Severe larval infestations considerably affect plant growth up to the death of trees (Ben-Yehuda *et al.*, 2000).

Efficient and largely used early monitoring protocols and devices are lacking, even though adults can be detected through a visual inspection, but larval infestation can be recognized only with the plant suffering and collapse. The chemical control could not be always well timed in relation to the infestation and the integrated control of this pest is currently not enough to keep the pest under control (Bari *et al.*, 2004). Efficient specific natural enemies of this pest are poorly known (Marannino and de Lillo, 2007). Entomopathogenic nematodes (EPNs) have been used successfully against many soil-inhabiting and

burrowing insects (Klein, 1990), and research confirmed the susceptibility of *C. tenebrionis* to them (Lobatón *et al.*, 1998; Marannino *et al.*, 2004; del Mar Martínez de Altube *et al.*, 2008; Morton and García del Pino, 2008). Further biological control means, like entomopathogenic fungi, are still under investigation (Marannino *et al.*, 2006; 2008; 2010; Dana Ment, personal communication). Finally, the use of genetic resistance in plant material would be a promising eco-friendly and complementary approach within the integrated control strategy of *Capnodis* spp. (Salazar *et al.*, 1991). Plant roots produce a multitude of compounds (Uren, 2000), which can influence below-ground herbivores by affecting directly or indirectly their behaviour and development (Vetter, 2000; Hiltbold and Turlings, 2012). Similarly, the high or low concentration of the compounds present in the rootstock tissues of stone-fruit trees is supposed to affect growth and viability of *C. tenebrionis* larvae living into the roots, but data are poor, still inconclusive and require further experimental evidences. Larval rearing on artificial substrate could help in increasing data.

Several semi-artificial diets have been developed for rearing coleopteran wood pests, but little information exists in this regard for Buprestidae. Gould *et al.* (2005) succeeded in rearing emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera Buprestidae), adding ash phloem powder in a substrate previously used with success for larvae of *Hylobius transversovittatus* (Goeze) (Coleoptera Curculionidae) (Blossey *et al.*, 2000). Attempts in rearing *C. tenebrionis* larvae were carried out by Mourikis and Vasilaina-Alexopoulou (1975), and Marannino and Germinara (2005), but only Gindin *et al.* (2009) were able to standardize the diet adding root tissue flour of the host plant into the substrate.

The current research was aimed at evaluating the effects of the rootstocks on the larval viability and growth, and on the adult emergence of *C. tenebrionis*. The larvae were reared following Gindin *et al.* (2009) protocol in which the root flour of 8 rootstocks was used as the sole difference. Current information could have practical implications in the selection of less susceptible rootstocks, assisting growers to manage this pest.

## Materials and methods

### Collection and rearing of *C. tenebrionis* adults

Adults were collected from infested apricot orchards in the District of Bari and Matera (Southern Italy) from March to June 2017, by hand or entomological net and transported in the laboratory. Active and healthy adults were selected and held in metal net cages (30 × 30 × 30 cm) (5-15 beetles of both sexes per cage). Beetles were maintained at room temperature and fed on fresh apricot twigs (Bari *et al.*, 2019). Cages were inspected every 5-7 days in order to replace dried twigs with fresh ones, and remove feces and dead individuals. Adults were left to mate.

### Bark flour and substrate preparation

The bark flour included into the substrates was obtained from 2-3 years-old stone-fruit plants provided by Battistini Vivai (Cesena, Italy) and Vivai Fortunato (Sam-michele di Bari, Bari, Italy) in May 2017. The rootstocks were selected among those more commonly used by growers: Adesoto (*Prunus domestica* subsp. *insititia* (L.) Bonnier et Layens), CAB6P (*P. cerasus* L.), Colt (*P. avium* L. × *P. pseudocerasus* Lindl.), Garnem (*P. persica* (L.) Batsch × *P. dulcis* Webb), GF 677 (*P. persica* (L.) Batsch × *P. dulcis* Webb), MaxMa 60 (*P. mahaleb* L. × *P. avium* L.), Montclar (*P. persica* (L.) Batsch) and Myrabolan 29C (*P. cerasifera* Ehrh.). Plants were up-rooted, roots were washed under running water in order to remove soil particles, then left for 2 hours in 10% commercial bleach solution (Blossey *et al.*, 2000) and rinsed again under running water. The bark was removed from the larger roots by means of a knife. Bark and small roots were both put in an oven at 70 °C for about 24 hours (Galina Gindin, personal communication) until their complete drying, in order to facilitate the mechanical

breaking. These materials were chopped with shears, shredded in a grinder for obtaining a homogenous flour and stored in PVC jars at -20 °C (Gindin *et al.*, 2009).

The substrate was prepared according to Gindin *et al.* (2009) using 5% of bark flour of each rootstock (each of them represent an experimental treatment). The fresh prepared substrate of each treatment was stored in PVC containers at -20 °C and warmed at room temperature before its use.

### Rearing and measuring of larvae and adults

Egg-laying arenas were introduced into the rearing cages (reported above). The egg-laying arenas consisted of a Petri dish bottom (10 cm of diameter) containing a transparent cellulose disc (10 cm of diameter) covered by a thin layer of dried and fine soil passed through a 20-mesh sieve (openings = 850 µm). Eggs laid on the cellulose discs were incubated at 27 ± 2 °C, relative humidity of 60 ± 10%, in darkness in a thermostatic refrigerator chamber (DAS 37000, Intercontinental®, Roma, Italy) for 8-15 days until hatching. All treatments of the trial started at the same time using larvae, maximum 24 hours old, which were selected randomly among the most active and healthy ones. It was not possible to relate the eggs to a specific female. They were transferred by a fine brush onto the substrate reported above (Gindin *et al.*, 2009) contained in a Petri dish (3.5 cm of diameter). Each larva was coded to allow the data recording of the same individual from hatching to the adult stage. After one week, the dishes were inspected and survived larvae were weighed and transferred onto fresh substrate in Petri dishes (5 cm of diameter). Each treatment of the trial consisted of at least 32 larvae, which were reared up to the adulthood (table 1). The number of initial reared larvae takes into account the loose of individuals occurred during the inspections as consequence of accidental mechanical injuries and few cases of fungi infection. These events did not allow us having the same number of cases for each treatment. Larvae were kept in a dark chamber with controlled temperature at 27 ± 1 °C. The substrate was replaced every two weeks from July 2017 to July 2019 up to pupation. The weight of each larva and its survival were recorded every two weeks (when the substrate was replaced) starting from the first inspection. Appearance of pupae was recorded for each treatment. Pupae were moved to clean Petri dishes (5 cm of diameter)

**Table 1.** Survival of *C. tenebrionis* larvae on substrates containing the bark flour of eight stone-fruit rootstocks.

Rootstocks	number larvae at the beginning of the trial	number larvae survived at the 5 <sup>th</sup> week	larval survival from beginning to the 5 <sup>th</sup> week (%)	number larvae become adults	larval survival from the beginning to the adulthood (%)
Adesoto	50	23	46.0 a	18	36.0 a
CAB6P	32	28	87.5 b	22	68.7 b
Colt	50	20	40.0 a	12	24.0 a
Garnem	45	19	42.2 a	17	37.7 a
GF677	34	31	91.2 b	27	79.4 b
MaxMa60	37	30	81.1 b	23	62.2 b
Montclar	38	25	65.8 ab	22	57.9 ab
Myrabolan 29C	45	30	66.7 ab	22	48.9 ab

Values followed by the same letter within a column do not differ significantly (Yates corrected  $\chi^2$  test  $P \leq 0.05$ ).

**Table 2.** Larval development and pupae appearance of *C. tenebrionis* on substrates containing the bark flour of eight stone-fruit rootstocks. Mature larvae were considered those just before their pupation.

Rootstocks	1 <sup>st</sup> pupa at day	last pupa at day	mean weight $\pm$ SD (mg) of mature larvae
Adesoto	218	491	468 $\pm$ 105 b
CAB6P	232	505	531 $\pm$ 95 ab
Colt	274	386	528 $\pm$ 84 ab
Garnem	218	592	491 $\pm$ 119 ab
GF677	232	521	524 $\pm$ 112 ab
MaxMa60	218	372	464 $\pm$ 91 b
Montclar	232	344	551 $\pm$ 92 a
Myrabolan 29C	218	386	505 $\pm$ 107 ab

Means  $\pm$  SD followed by the same letter within a column do not differ significantly (Duncan test:  $P \leq 0.05$ ).

without substrate (one pupa per each dish) and left at  $27 \pm 1$  °C up to the emergence of the adults. Adults were separated based on the sex. Their body length and pronotum width were measured by a caliper.

The exact number of larvae at the beginning of the trial, those survived at the 5<sup>th</sup> week and those reaching the adult stage were recorded in order to obtain the survival rate. The 5<sup>th</sup> week was chosen because the larval survival appeared to be more variable among the substrates during these first period of life (survival ranged from 40 to 91.2%) compared to the next one (adults were from 60 to 89% of the larvae at the 5<sup>th</sup> week) and could give much more discriminations among the different rootstocks.

#### Data analysis

Since the weight of mature larvae, the body length and pronotum width of the adults resulted normally distributed (according to Shapiro-Wilk test), the factorial ANOVA analysis followed by Duncan post-hoc comparison test ( $P \leq 0.05$ ) were applied to the data using Statistica 10 software (StatSoft, 2010). Larval survival was analyzed by non-parametric Yates corrected  $\chi^2$  test, using Statistica 10 software.

#### Results

The highest larval survival at the end of the trial was observed on GF677 treatment (79.4%) and the lowest on Colt, Adesoto and Garnem treatment (24.0, 36.0% and 37.7%, respectively) (table 1). Analysing the rough data and dividing the trial in two periods of time (i.e., from the beginning to the fifth week and from the fifth week to adulthood), distinct survival results were observed among treatments. Larvae reared on Colt, Garnem and Adesoto treatments had a lower survival in the first five weeks (40.0, 42.2 and 46.0%, respectively), whereas GF677 and Cab6P treatments showed the highest survival (91.2, 87.5%, respectively) during the same period (table 1). A few larvae were still alive on 18 July 2019 after 1 year and 49 weeks on CAB6P (4 larvae), Garnem (2 larvae) and GF677 (1 larva) treatments and subsequently died (these larvae were not included in the current statistical analysis on the survival and larval growth).

The first pupae were observed on the 218<sup>th</sup> day

(31<sup>st</sup> week) of rearing for Adesoto, Garnem, MaxMa60 and Myrabolan 29C treatments, on the 232<sup>nd</sup> day (33<sup>rd</sup> week) for Cab6P, GF677 and Montclar, and on 274<sup>th</sup> day (39<sup>th</sup> week) for Colt. The time range during which larvae became pupae was longer for Garnem (374 days) and shorter for Colt and Montclar treatments (112 days), but any statistical difference was observed among the treatments (table 2). No correlation was found between larval weight and duration of pupal formation.

The mean weight of larvae recorded in the last inspection before their pupation varied around 500 mg, it was significantly higher for Montclar (551  $\pm$  92 mg) ( $F_{(1,7)} = 2.188$ ,  $P = 0.039$ ) and lower for MaxMa60 (464  $\pm$  91 mg) and Adesoto (468  $\pm$  105 mg) (table 2).

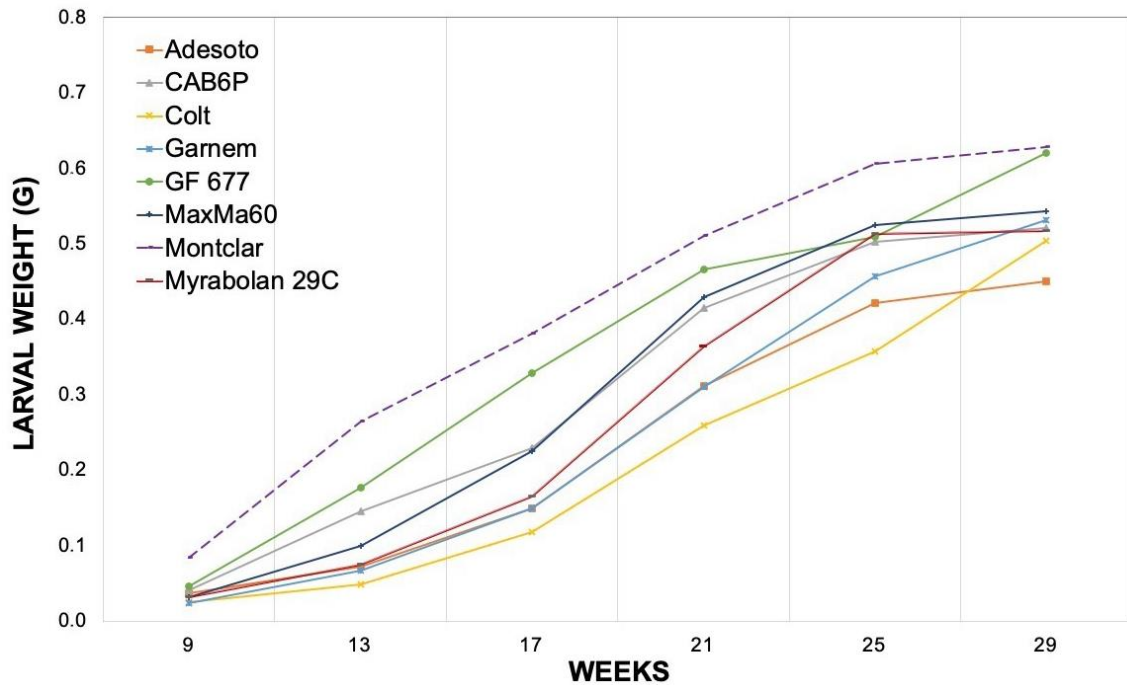
The mean weight of mature larvae producing females (530 mg  $\pm$  115 mg) was significantly higher ( $F_{(1,1)} = 8.971$ ,  $P = 0.003$ ) than that producing males (486  $\pm$  87 mg).

The larval weight increase was compared among the treatments until the 29<sup>th</sup> week, when the first pupae appeared. In the first 7 weeks no significant differences were recorded among the treatments, whereas from the 9<sup>th</sup> to 13<sup>th</sup> week, larvae developed on Montclar substrate had a mean weight significantly ( $F_{(1,7)} = 3.21$ ,  $P < 0.01$ ) higher than the other treatments (figure 1; table 3). From 17<sup>th</sup> to 29<sup>th</sup> week, larvae for Montclar and GF677 treatments increased their weight significantly more than Adesoto, Colt and Garnem (figure 1; table 3).

At the 29<sup>th</sup> week, the larvae grown on Adesoto substrate showed a mean weight of 450 mg, significantly lower ( $F_{(1,7)} = 3.21$ ,  $P < 0.01$ ) than larvae on GF677 and Montclar treatments, with a mean weight of 621 and 628 mg respectively (figure 1; table 3).

New adults emerged after 3 weeks of pupation and no statistical differences were found among the treatments.

The size of the adults emerged from the substrate containing Montclar bark flour was significantly bigger ( $F_{(1,7)} = 2.36$ ,  $P = 0.02$  for the length;  $F_{(1,7)} = 2.24$ ,  $P = 0.034$  for the width) than that of adults emerged from substrates with Adesoto and MaxMa60, which resulted the smallest (table 4). The size of adult females (length  $19.58 \pm 0.17$  mm; width  $7.34 \pm 0.07$ ) obtained from the rearing was significantly greater ( $F_{(1,1)} = 11.88$ ,  $P = 0.0008$  for the length;  $F_{(1,1)} = 7.35$ ,  $P = 0.008$  for the width) than the one of males (length  $18.95 \pm 1.16$  mm; width  $7.13 \pm 0.54$  mm) (table 5).



**Figure 1.** Weight increase of *C. tenebrionis* larvae in substrates containing bark flour of eight stone-fruit rootstocks. The lines represent the mean weight of the larvae until the first pupa appearance (at the 29<sup>th</sup> week for Adesoto, Garnem, MaxMa60 and Myrabolan 29C).

**Table 3.** *C. tenebrionis* larval weight in substrates containing bark flour of eight stone-fruit rootstocks. The mean weight of the larvae from the 9<sup>th</sup> week up to the first pupa appearance time (at the 29<sup>th</sup> week for Adesoto, Garnem, MaxMa60 and Myrabolan 29C).

Rootstocks	mean larval weight $\pm$ SD (mg)					
	9 <sup>th</sup> week	13 <sup>th</sup> week	17 <sup>th</sup> week	21 <sup>st</sup> week	25 <sup>th</sup> week	29 <sup>th</sup> week
Adesoto	37 $\pm$ 19 bcd	73 $\pm$ 36 cd	149 $\pm$ 134 c	311 $\pm$ 221 bc	421 $\pm$ 195 bc	450 $\pm$ 185 b
CAB 6P	41 $\pm$ 16 bc	145 $\pm$ 132 bc	229 $\pm$ 169 bc	416 $\pm$ 198 ab	502 $\pm$ 177 b	521 $\pm$ 165 b
Colt	26 $\pm$ 6 cd	49 $\pm$ 10 d	118 $\pm$ 48 c	260 $\pm$ 162 c	357 $\pm$ 154 c	504 $\pm$ 156 b
Garnem	24 $\pm$ 15 d	67 $\pm$ 38 d	150 $\pm$ 129 c	310 $\pm$ 180 bc	458 $\pm$ 152 b	532 $\pm$ 111 b
GF677	46 $\pm$ 14 b	176 $\pm$ 126 b	329 $\pm$ 210 ab	467 $\pm$ 190 a	510 $\pm$ 155 ab	621 $\pm$ 125 a
MaxMa60	32 $\pm$ 13 bcd	100 $\pm$ 34 cd	225 $\pm$ 144 bc	430 $\pm$ 171 ab	525 $\pm$ 92 ab	543 $\pm$ 108 ab
Montclar	84 $\pm$ 46 a	264 $\pm$ 215 a	381 $\pm$ 240 a	511 $\pm$ 198 a	606 $\pm$ 128 a	628 $\pm$ 98 a
Myrabolan 29C	32 $\pm$ 18 bcd	75 $\pm$ 34 cd	165 $\pm$ 124 c	364 $\pm$ 156 abc	513 $\pm$ 121 ab	517 $\pm$ 107 b

Means  $\pm$  SD followed by the same letter within a column do not differ significantly (Duncan test:  $P \leq 0.05$ ).

**Table 4.** Size of new emerged adults of *C. tenebrionis* obtained by larvae reared on substrates containing the bark flour of eight stone-fruit rootstocks.

Rootstocks	mean length	mean width
	of the body $\pm$ SD (mm)	of the body $\pm$ SD (mm)
Adesoto	18.76 $\pm$ 1.19 b	7.02 $\pm$ 0.59 b
CAB6P	19.36 $\pm$ 1.26 ab	7.34 $\pm$ 0.55 ab
Colt	19.28 $\pm$ 0.89 ab	7.21 $\pm$ 0.44 ab
Garnem	18.90 $\pm$ 1.38 ab	7.21 $\pm$ 0.58 ab
GF677	19.54 $\pm$ 1.48 ab	7.37 $\pm$ 0.63 ab
MaxMa60	18.70 $\pm$ 1.32 b	7.00 $\pm$ 0.52 b
Montclar	19.79 $\pm$ 1.11 a	7.51 $\pm$ 0.48 a
Myrabolan 29C	19.53 $\pm$ 1.29 ab	7.17 $\pm$ 0.64 ab

Means  $\pm$  SD followed by the same letter within a column do not differ significantly (Duncan test:  $P \leq 0.05$ ).

## Discussion and conclusions

Only one study is available on the development and growth of *C. tenebrionis* larvae on semi-artificial diets containing bark flour of rootstocks (Gindin *et al.*, 2009). A diet containing chopped apricot stems was also successfully used but data are scanty (Marannino and Germinara, 2005). In the current study, the post-embryonic development of *C. tenebrionis* was carried out on semi-artificial substrates using Gindin *et al.* (2009) protocol. Diets differed only for the origin of the bark flour which was taken from eight rootstocks among those more commonly used for the stone-fruit cultivations, and obtained from plants at the same physiological stage. The first pupae per each substrate occurred between the 31<sup>st</sup> and 39<sup>th</sup> week of rearing. Most of the new adults emerged 9-10 months after the larval hatching, at  $27 \pm 1$  °C. Pupation

**Table 5.** Size of new emerged adults of *C. tenebrionis* divided by sex, obtained by larvae reared on substrates containing the bark flour of eight stone-fruit rootstocks.

Rootstocks	mean length of the body $\pm$ SD (mm)		mean width of the body $\pm$ SD (mm)	
	Male	Female	Male	Female
Adesoto	18.20 $\pm$ 0.82	19.04 $\pm$ 1.28	6.78 $\pm$ 0.68	7.14 $\pm$ 0.54
CAB6P	19.06 $\pm$ 1.25	19.81 $\pm$ 1.22	7.26 $\pm$ 0.54	7.46 $\pm$ 0.57
Colt	19.01 $\pm$ 0.98	19.70 $\pm$ 0.94	7.07 $\pm$ 0.45	7.43 $\pm$ 0.37
Garnem	18.08 $\pm$ 1.13	19.85 $\pm$ 1.02	6.90 $\pm$ 0.51	7.58 $\pm$ 0.44
GF677	18.98 $\pm$ 0.99	20.21 $\pm$ 1.74	7.18 $\pm$ 0.47	7.61 $\pm$ 0.74
MaxMa60	18.82 $\pm$ 1.16	18.57 $\pm$ 1.53	7.14 $\pm$ 0.52	6.83 $\pm$ 0.50
Montclar	19.57 $\pm$ 1.04	19.97 $\pm$ 1.18	7.43 $\pm$ 0.43	7.57 $\pm$ 0.53
Myrabolan 29C	19.32 $\pm$ 1.33	19.87 $\pm$ 1.22	7.07 $\pm$ 0.67	7.33 $\pm$ 0.60
Overall mean	18.95 $\pm$ 1.16 a	19.58 $\pm$ 1.39 b	7.12 $\pm$ 0.54 a	7.34 $\pm$ 0.60 b

Overall mean  $\pm$  SD followed by the same letter do not differ significantly (Duncan test:  $P \leq 0.05$ ).

in the current trial was reached also after more than one year of rearing (about 20 months) for the substrate containing Garnem bark flour. In a previous investigation, the complete larval development in a semi-artificial diet was obtained in about 4 months at  $26 \pm 1$  °C (Mourikis and Vasilaina-Alexopoulou, 1975). In Gindin *et al.* (2009) trials, 80% of larvae became pupae in about 3 months at 28 °C and the larval development required 10-12 months at about 24 °C. The biotype of *C. tenebrionis* used in the current trial might differ biologically from those used by Gindin *et al.* (2009) in Israel. In fact, the molecular characterization of *C. tenebrionis* adults showed a certain distance between the Israeli and the Apulian biotypes (Magal, 2017). The differences between our data and those by Gindin *et al.* (2009) might depend also on the different providers of the chemicals.

Field data on the length of the postembryonic development appear to be close to the current results (10-23 months by Chrestian, 1955; 13 months by Hmimina *et al.*, 1988; 13-14 months by Kaitazov, 1958) but the comparison is quite hard for the different temperature trend. It should be mentioned that Chrestian (1955) advanced the hypothesis of the existence of a race with a short postembryonic development (one year) and a race with a long one (two years).

In an attempt to summarize the results of the current trials, the substrates containing bark flour from Adesoto, Colt, Garnem and MaxMa60 appear to affect the larval fitness. The lowest survival of larvae was observed for Adesoto, Garnem and Colt treatments, meanwhile the lowest mean weight of the mature larvae as well as the smallest size of the adults were ascertained for MaxMa60 and Adesoto treatments. The mean weight of larvae reared on Colt, Adesoto and Garnem substrates was significantly lower than the other treatments. These data suggest an effect of these latter rootstocks in reducing the success of *C. tenebrionis* larvae. On the contrary, the fitness of *C. tenebrionis* larvae was better performed when they were reared on substrates containing GF677 and Montclar bark flour. In fact, the larval survival was the highest on GF677 treatment, whereas Montclar showed the highest mean increase of larval weight and the biggest size of the adults.

Malagón and Garrido (1990), and Mulas (1994) suggested that plant susceptibility to *C. tenebrionis* was directly related to the cyanide content of roots and hypothesized that prunasin might be involved in this action. Later,

Ben-Yehuda *et al.* (2001) and Mendel *et al.* (2003) experimentally demonstrated that the larval infestation of *Prunus* spp. was more relevant in roots with the highest concentration of prunasin. Other few studies on the same issue pointed out the inverse relationship even though a few genotypes were considered less susceptible to *C. tenebrionis* (Dicenta *et al.*, 2002; Soler *et al.*, 2014). All these investigations were carried out in the field on rootstock saplings, providing eggs or neonate larvae to the soil (in open field or in pots) close to the base of young (1-2 years) saplings and evaluating the infestation rate after weeks or months. Concentration of the cyanogenic compounds in different species, hybrids and cultivars appears to be variable (Vetter, 2000) and not well studied. Similarly, the translocation of this compounds on the same plant is not well known (Negri *et al.*, 2008). According to Mfarrej and Sharaf (2011), bitter and sweet almonds have significantly higher prunasin contents in their vegetative parts and roots than apricots and plums, whereas peaches have the least content. Conversely, Montclar and GF677 rootstocks derives from peach and we can speculate that this might explain the higher success obtained by the larvae on the diets containing their bark flour.

The current results are quite promising and they come from a first attempt to apply a more standardized method for analysing rootstock susceptibility to *C. tenebrionis*. These results have to be further validated with supplementary trials in the laboratory and field, and they have to be compared along with the biochemical composition of the rootstocks.

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## References

- BARI G., DE CRISTOFARO A., DE LILLO E., GERMINARA G. S., 2004.- Studio preliminare sulle interazioni intraspecifiche ed interspecifiche in *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae), pp. 725-731. In: *Atti del XIX Congresso Nazionale Italiano di Entomologia*, 10-15 June 2002 Catania, Italy.
- BARI G., SCALA A., GARZONE V., SALVIA R., YALCIN C., VERNILE P., ARESTA A. M., FACINI O., BARALDI R., BUFO S. A., VOGEL H., DE LILLO E., RAPPARINI F., FALABELLA P., 2019.- Chemical ecology of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae): behavioral and biochemical strategies for intraspecific and host interactions.- *Frontiers in Physiology*, 10 (604): 1-21.
- BEN YEHUDA S., ASSAEL F., MENDEL Z., 2000.- Improved chemical control of *Capnodis tenebrionis* L. and *C. carbonaria* Klug (Coleoptera: Buprestidae) in stone-fruit plantations in Israel.- *Phytoparasitica*, 28 (1): 27-41.
- BEN YEHUDA S., ASSAEL F., MENDEL Z., 2001.- Infestation of roots of stone-fruit rootstocks by larvae of two *Capnodis* spp. (Buprestidae) and its relation to level of cyanogenic compounds.- *IOBC/wprs Bulletin*, 24 (5): 91-95.
- BLOSSEY B., EBERTS D., MORRISON E., HUNT T. R., 2000.- Mass rearing the weevil *Hylobius transversovittatus* (Coleoptera: Curculionidae), biological control agent of *Lythrum salicaria*, on semiartificial diet.- *Journal of Economic Entomology*, 93: 1644-1656.
- CHRISTIAN P., 1955.- *Le Capnode noir des Rosacées*. Travaux originaux n. 6.- Protectorat de la République Française au Maroc, Service de la Défense des Végétaux, Rabat, Morocco.
- DEL MAR MARTINEZ DE ALTUBE M., STRAUCH O., FERNANDEZ DE CASTRO G., MARTINEZ PEÑA A., 2008.- Control of the flat-headed root borer *Capnodis tenebrionis* (Linné) (Coleoptera: Buprestidae) with the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae) in a chitosan formulation in apricot orchards.- *Bio-control*, 53: 531-539.
- DICENTA F., MARTINEZ-GÓMEZ P., GRANÉ N., LEÓN A., CÁNOVAS J. A., BERENGUER V., 2002.- Relationship between cyanogenic compounds in kernels, leaves, and roots of sweet and bitter kernelled almonds.- *Journal of Agricultural and Food Chemistry*, 50: 2149-2152.
- GARRIDO A., 1984.- Bioecología de *Capnodis tenebrionis* L. (Coleop.: Buprestidae) y orientaciones para su control.- *Boletín de Sanidad Vegetal. Plagas*, 10: 205-221.
- GINDIN G., KUZNETSOVA T., PROTASOV A., BEN YEHUDA S., MENDEL Z., 2009.- Artificial diet for two flat-headed borers, *Capnodis* spp. (Coleoptera: Buprestidae).- *European Journal of Entomology*, 106: 573-581.
- GOULD J., TANNER J., WINOGRAD D., LANE S., 2005.- Initial studies on the laboratory rearing of emerald ash borer and foreign exploration for natural enemies, pp. 73-74. In: *Proceedings emerald ash borer research and technology development meeting*. Romulus, Michigan, October 5-6, 2004. USDA Forest Service Publication FHTET-2004-15.
- HILTPOLD I., TURLINGS T. J., 2012.- Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield.- *Journal of Chemical Ecology*, 38: 641-650.
- HIMMINA M., SEKKAT A., LAHFA L., HIDIANE M., 1988.- Cycle biologique de *Capnodis tenebrionis* L. (Coleoptera, Buprestidae) dans la région de Meknès.- *Actes de l'Institut Agronomique et Vétérinaire Hassan II*, 8 (1-2): 41-49.
- KAITAZOV A., 1958.- *Capnodis tenebrionis* L. bionomics and measure for control.- *Zashita Rastenii*, 1: 159-187 (In Bulgarian).
- KLEIN M. G., 1990.- Efficacy against soil-inhabiting insect pests, pp. 195-214. In: *Entomopathogenic nematodes in biological control* (GAUGLER R., KAYA H.K., Eds).- CRC Press, Boca Raton, Florida, USA.
- LOBATÓN C. S., VELA J. R. G., LÓPEZ M. P. L., ROCA A. C., 1998.- Ensayos de laboratorio con una población de *Steinernema carpocapsae* (Filipjev) detectada en larvas de *Capnodis tenebrionis* L.- *Boletín de Sanidad Vegetal. Plagas*, 24: 679-686.
- MAGAL N. S., 2017.- Molecular characterization of wood-boring beetles and moths in Israel, and identification of essential and unique genes in *Capnodis tenebrionis* (Coleoptera, Buprestidae). *MS Thesis*, Department of Zoology, Tel-Aviv University - George S. Wise Faculty of Life Sciences - Graduate School, Israel.
- MALAGÓN J., GARRIDO A., 1990.- Relación entre el contenido de glicósidos cianogénicos y la resistencia a *Capnodis tenebrionis* L. en frutales de hueso.- *Boletín de Sanidad Vegetal. Plagas*, 16: 499-503.
- MARANNINO P., DE LILLO E., 2007.- The peach flatheaded root borer, *Capnodis tenebrionis* (L.), and its enemies.- *IOBC/wprs Bulletin*, 30 (1): 197-200.
- MARANNINO P., GERMINARA S., 2005.- Dati preliminari sull'allevamento continuo in laboratorio di *Capnodis tenebrionis* (L.) (Coleoptera Buprestidae), p. 236. In: *Atti XX congresso nazionale italiano di entomologia*, 13-18 June 2005, Perugia-Assisi, Italy.
- MARANNINO P., TARASCO E., DE LILLO E., 2004.- Biological notes on larval hatching in *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) and evaluating entomopathogenic nematodes against neonate larvae.- *Redia*, 86: 101-106.
- MARANNINO P., SANTIAGO-ALVAREZ C., DE LILLO E., QUESADA-MORAGA E., 2006.- A new bioassay method reveals pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against early stages of *Capnodis tenebrionis* (Coleoptera: Buprestidae).- *Journal of Invertebrate Pathology*, 93: 210-213.
- MARANNINO P., SANTIAGO ALVAREZ C., DE LILLO E., QUESADA-MORAGA E., 2008.- Evaluation of *Metarhizium anisopliae* (Metsch) Sorok. to target larvae and adults of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae) in soil and fiber band applications.- *Journal of Invertebrate Pathology*, 97: 237-244.
- MARANNINO P., TARASCO E., TRIGGIANI O., 2010.- Laboratory evaluation of *Beauveria bassiana* (Bals. - Criv.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin Mediterranean fungi isolates against adults of *Capnodis tenebrionis* (L.) (Coleoptera: Buprestidae).- *Redia*, 93: 15-18.
- MENDEL Z., ASSAEL F., BEN YEHUDA S., 2003.- Host selection and root colonization of cyanogenic stone-fruit species by *Capnodis* spp. (Coleoptera: Buprestidae).- *Annals of the Entomological Society of America*, 96: 127-134.
- MFARREJ M. F. B., SHARAF N. S., 2011.- Host selection of Peach Rootborer *Capnodis tenebrionis* L. (Coleoptera: Buprestidae) to stone-fruit trees in Jordan.- *Jordan Journal of Agricultural Sciences*, 4: 682-699.
- MORTON A., GARCÍA DEL PINO F., 2008.- Effectiveness of different species of entomopathogenic nematodes for biocontrol of the Mediterranean flat headed root borer, *Capnodis tenebrionis* (Linné) (Coleoptera: Buprestidae) in potted peach tree.- *Journal of Invertebrate Pathology*, 97: 128-133.
- MOURIKIS P. A., VASILAINA-ALEXOPOULOU P., 1975.- Über die Laborzucht und Entwicklung des Pfirsich-Prachtkäfers, *Capnodis tenebrionis* L. (Col., Buprestidae).- *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz*, 48: 75-77.
- MULAS M., 1994.- Almond genetic resources and resistance to *Capnodis tenebrionis*.- *Acta Horticulturae*, 373: 41-48.

- NEGRI P., BASSI D., MAGNANINI E., RIZZO M., BARTOLOZZI F., 2008.- Bitterness inheritance in apricot (*P. armeniaca* L) seeds.- *Tree Genetics & Genomes*, 4 (4): 767-776.
- RIVNAY E., 1946.- Physiological and ecological studies on the species of *Capnodis* in Palestine (Col., Buprestidae). II. - Studies on the larvae.- *Bulletin of Entomological Research*, 36 (1): 103-119.
- SALAZAR D. M., MIRO M., GARCÍA S., 1991.- Rootstocks for dry region apricot tree faced with *Capnodis tenebrionis* L.- *Acta Horticulturae*, 293: 401-404.
- SOLER A., TORRENTS J., DICENTA F., 2014.- Resistance to *Capnodis tenebrionis* in new *Prunus* rootstocks.- *Acta Horticulturae*, 1028: 201-204.
- STAT-SOFT, 2010.- *Statistica 10.0*.- Stat-Soft inc., Tulsa, USA.
- UREN N. C., 2000.- Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, pp. 19-40. In: *The rhizosphere: biochemistry and organic substances at the soil-plant interface* (PINTON R., VARRANINI Z., NANNIPIERO P., Eds).- Marcel Dekker, New York, USA.
- VETTER J., 2000.- Plant cyanogenic glycosides.- *Toxicon*, 38 (1): 11-36.

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