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Artificial diet for two flat-headed borers, *Capnodis* spp. (Coleoptera: Buprestidae)

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Abstract. The main objective was to develop an artificial diet for two flat-headed borers, *Capnodis tenebrionis* L. and *C. carbonaria* Klug. (Coleoptera: Buprestidae), which are severe pests of stonefruit plantations in the Mediterranean basin. The effect of proteins from various sources, percentage of cortex tissue in the diet and diet structure on larval growth and survival were investigated. The most successful diet contained 2.8% casein and 4.6% dry brewer's yeast as the protein source. For complete larval development and successful pupation it is essential to include cortex tissue from the host plant in the diet. Mean larval development time was shortened by 10–12 days when reared on a diet containing 20% cortex tissue compared with rearing on diet containing 10% cortex tissue. Two different diet structures were required, a viscous matrix for the first and second instar larvae and drier crumbly diet, which allows the larvae to move within the diet, for older larvae. At 28°C on the artificial diet *C. tenebrionis* and *C. carbonaria* completed their development in 2–2.5 months compared to the 6–11 months recorded in Israeli orchards. *C. tenebrionis* successfully completed two generations on the artificial diet.

INTRODUCTION

The flat-headed borers, *Capnodis tenebrionis* L. and *C. carbonaria* Klug. are severe pests of stone fruit (*Prunus* spp., Rosaceae) plantations in the Mediterranean basin and southern Europe (Garrido, 1984; Mahhou & Dennis, 1992; Colasurdo et al., 1997; Mendel et al., 2003). The adult borers become active when temperatures exceed 28°C.

Like many other species of Buprestidae, the young adults feed on the cortex (live bark, including phloem, parenchyma, cambium and young xylem) of twigs and young branches prior to mating and oviposition (Haak & Slansky, 1987). The adults may live for more than 1 year (Rivnay, 1946) and one female may lay more than 1000 eggs (Rivnay, 1944). The eggs are placed in dry ground, imbedded in the soil, inserted in cracks or under stones, usually near the base of stone fruit trees (Rivnay, 1944; Garrido, 1984). The neonate larvae of these *Capnodis* spp. penetrate and feed on the cortex of roots; they can locate a root from a distance of 60 cm (Rivnay, 1945). Depending on the ambient temperature and rootstock, larval development takes 6–18 months in Israel (Rivnay, 1945), whereas in cooler zones development may take 2 years (Martin, 1951). The larvae excavate typical long galleries, which are initially narrow but then widen, and leave behind them compressed masticated frass (Rivnay, 1945).

Naturally occurring arthropod enemies of these *Capnodis* spp. are rare (Avidov & Harpaz, 1969; Marannino & de Lillo, 2007a). These beetles are mainly controlled by the application of synthetic insecticides (Garrido et al., 1990; Sekkat et al., 1997; Ben-Yehuda et al., 2000; Sanna-Passino & Delrio, 2001). Recently nematodes were used to control *C. tenebrionis* (Marannino et al., 2003; Martinez de Altube et al., 2008; Morton & García-del-Pino, 2008) and the efficacy of entomopathogenic fungi as biocontrol agents tested (Marannino et al., 2006, 2008).

One environmentally friendly way of replacing the heavy dependence on insecticides for controlling *Capnodis* spp. would be to breed a resistant rootstock (Salazar et al., 1991). Mendel et al. (2003) examined the relative resistance of the roots of nine major rootstock taxa of stonefruits to colonization by larvae of these borers and show that even the most resistant root stock is colonized. Malagón & Garrido (1990) and Mulas (1994) suggest that resistance to *C. tenebrionis* is directly related to the cyanide content of the roots and hypothesize that prunasin is probably involved in the resistance. Mendel et al. (2003) present a different picture. These authors show that the resistance is inversely proportional to the prunasin content, significantly correlated with the indices of susceptibility of the rootstocks to *C. tenebrionis* and *C. carbonaria*.

Among the potential environmental benefits derived from genetically modified trees is resistance to insect pests. It is suggested that enhancing resistance against *Capnodis* by using transgenic *Prunus* spp. offers great potential for developing rootstocks that are immune to attack by neonates and even by advanced larval stages. Such rootstocks might avoid the need for the frequent use of insecticides in stonefruit plantations. Therefore, the main objective of the present study was to develop an

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artificial diet for *C. tenebrionis* and *C. carbonaria*, which could be used to test the efficacy of toxins with a known genetic basis that might then be introduced into new stonefruit rootstocks.

Several semi-artificial diets developed for rearing coleopteran pests contain the same array of ingredients, often with the addition of dried and milled host plant material. This type of diet is reported for several pests: two weevils (Coleoptera: Curculionidae), the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Shimoji & Yanagishi, 2004) and the purple loosestrife root weevil *Hylobius transversovittatus* Goeze (Blossey et al., 2000); the Asian longhorned beetle, *Anoplophora glabripennis* (ALB) (Coleoptera: Cerambycidae) (Dubois et al., 2002); the western corn rootworm, *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) (Pleau et al., 2002); and two leaf beetles (Coleoptera: Chrysomelidae), *Gastrophysa atrocyanea* Motschulsky (Ojima et al., 2005) and the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Gelman et al., 2001). There is little information on artificial diets for rearing Buprestidae: Gould et al. (2004) report some success in rearing the larvae of the emerald ash borer, *Agrilus planipennis* Fairmaire and Mourikis & Vasilaina-Alexopoulou (1975) report an artificial diet that allowed some development of *C. tenebrionis*. However, the latter was tested in the present study and found to be useless for rearing *Capnodis* larvae (see below).

The present paper summarizes a series of trials that formed part of an attempt to produce an artificial diet on which *C. tenebrionis* and *C. carbonaria* could complete their life cycle. The roles of different sources of proteins and host cortex were investigated. We also examined the effects of the concentration of cortex and matrix structure of the diet on the growth and survival of these borers. Rearing them in Petri dishes also allowed us to observe some previously unrecorded behavioural traits of *Capnodis* larvae.

MATERIAL AND METHODS

Collection of adult *Capnodis* and production of neonates

Adults of *C. tenebrionis* were collected in apricot and plum orchards in the Hula Valley, and those of *C. carbonaria* in almond plantations in the Bet Shean Valley. In the laboratory the beetles were kept in 3–4 ventilated Plexiglas cages (about 100 adults per cage) (80 × 60 × 50 cm) at 28–30°C under natural photoperiod from October to April after which they were kept in a greenhouse at 25–35°C (daily range) from May to September. The adults were fed with fresh 6- to 12-year-old branches of apricot or peach, which were put in 1-L jars with water and placed in the center of the cage. The branches were replaced every 2–3 days. The freshly cut branches were kept at 10°C until used, but not for more than 2 weeks. Beetles laid eggs in a Petri dish whose bottom was lined with paper and covered with a 2 cm layer of sandy soil that had been sterilized for 1 h at 127°C. The females laid their eggs in the sand and glued them to the paper, which was replaced every 2–3 days. The eggs collected from the rearing cages every 2–3 days were covered with about 1.0–1.5 cm of dry sterile sand and incubated at 28°C in darkness for about 8–10 days until they hatched. The Petri dishes were checked daily for newly hatched larvae, which were immediately transferred to the test diets. Most of our tests were done using *C. tenebrionis*. A diet suitable for rearing of *C. carbonaria* was evaluated in the last part of the study after the development of a suitable diet for the former species.

Scheme of diet development

The general approach was to modify existing diets for insects with similar feeding habits (Cohen, 2004). Our first choice was to rear the *C. tenebrionis* larvae on the diet proposed by Mourikis & Vasilaina-Alexopoulou (1975). None of the neonates survived for more than a week on that diet or several modifications of it. In light of the report that the larvae of the emerald ash borer (Gould et al., 2004) can survive for at least 4 weeks on a modified *H. transversovittatus* diet (Blossey et al., 2000) we chose this diet as the starting model for our *Capnodis* diet. In the first phase the survival of larvae on about 50 variations of the diet was determined. These diets differed in the source of the protein and carbohydrates, the concentration of antimicrobial compounds and presence of several specific compounds such as choline chloride or cholesterol (data not presented). Based on information accumulated in the first phase, the survival of *C. tenebrionis* on 10 representative diets were tested in the second phase. The composition of these 10 diets is presented in Table 1. All diets were prepared simultaneously. Each diet (about 50 g) was divided among 8 Petri dishes, (diam. 5.0 cm, with about 6 g diet per dish). Five neonates, 1–12 hours old, were placed in each Petri dish (a total of 40 neonates per diet). The neonates were reared at 28°C for a week and their survival recorded.

In the third phase diet no 10 (Table 1) was modified by incorporating cortex tissues from different sources and then tested: (1) plum (*Prunus domestica*) vs peach (*Prunus persica*), (2) cortex from peach roots vs stems, (3) fresh peach root cortex, vs dry, (4) dry peach root cortex added to the diet before vs after sterilization. The preparation details of these diets are presented in section "Cortex preparation". In a similar design of test to that used previously, 40 neonates were reared on each of the diets and their survival recorded after a week.

In the fourth phase we tested the effect of incorporating different percentages of cortex tissue (5, 10 and 20%) into the diet on several developmental parameters of *C. tenebrionis*. In these tests we used improved diet no 10, to which was added dry peach cortex (a blend of stem and root) prior to autoclaving the diet (Table 2). Diets were prepared in two formulations: as a dense paste-like substance or crumb-like substance, which was prepared using a fine grater. Details of the diet preparation and the rearing of larvae are described below.

To generate the most suitable diet structure, various inert substrates, such as plum (host) tree or pine tree sawdust, palm bark, straw or bran (all non-hosts) were partially (50%) or completely substituted for cellulose.

Cortex preparation

The effect of adding fresh, dried roots, stem cortex, or small roots together with their cortex to the diet were evaluated. The cortex tissue added to the diets was obtained from 2-yr-old peach or plum trees that grew in the experimental farm at the Volcani Center (Bet Dagan, Israel). Trees were uprooted and sawn into sections, after which the main roots, stem and main branches were separated. The sections were washed with water to remove soil particles and their surfaces sterilized by immersion for 2 h in 10% commercial bleach solution (Blossey et al., 2000) and then rinsed in running water. Cortex together with bark was removed from moist tree logs and dried for several hours at room temperature. For the preparation of fresh cortex, moist cortex was cut into small pieces (1–2 cm), shredded in a

TABLE 1. Modification of diets for *C. tenebrionis* rearing by substitution of protein sources and addition of root cortex.

Compound (%)	Diet									
	1	2	3	4	5	6	7	8	9	10
Protein soure										
Casein (Bio-serv, 1100)	2.8	–	2.8	–	–	–	2.8	2.8	2.8	2.8
Casein hydrolysate (Fluka, 22090)	–	2.8	–	–	–	–	–	–	–	–
Wheat germ dry (Bio-serv, G1661)				2.8	–	–				
Boiled soy (supermarket)	–	–	–	–	2.8	2.8		–	–	–
Yeast extract (Difco, 212750)	1.8	1.8	1.8	1.8	1.8	1.8	–	–	–	–
Brewer's yeast hydrolysate (Bio-serv, 17100)	–	–	–	–	–	–	1.8	–	–	–
Brewer's yeast dry (MP biomedicals, 101400)	–	–	–	–	–	–	–	1.8	4.6	4.6
Other compounds										
Sucrose (Bio-serv, 3900)	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Vanderzant vitamin mix (Bio-serv, F8045)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Wesson's salt mix (Bio-serv, F8680)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Sorbic acid (MP biomedicals, 102937)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Methyl paraben (MP biomedicals, 102341)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Bactoagar (Difco, 214010)	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Cellulose (Bio-serv, 3425)	24.3	24.3	20.3	24.3	24.3	24.3	24.3	24.3	21.5	20.3
Peach cortex	–	–	4.0	–	–	4.0	–	–	–	4.0
Water	64.7	64.7	64.7	64.7	64.7	60.7	64.7	64.7	64.7	61.9

commercial HGBSS blender, (Waring Products, Torrington, CT 06790) and immediately used for diet preparation. For the preparation of dry cortex powder, cortex from logs was dried at 80°C for 24–36 h and shredded in a mechanical grinder (Retsch, SM 100 Comfort SMF, Haan, Germany). Dry cortex powder or pieces were stored in plastic boxes at –20°C for up to 6 months.

Diet preparation

The diets were prepared in three parts. The first consisted of assembling the major nutritional ingredients: yeast products, sucrose, salt mix and agar (Table 2). The soybeans were previously boiled in an autoclave at 121°C for 40 min and homogenized in a HGBSS blender. These ingredients were added to about three-quarters of the final volume of water, stirred and autoclaved for 15 min at 121°C in a stainless steel vessel with tight-fitting lid to prevent water loss and then cooled to about 60–70°C. The second part of the diet, containing casein, vitamins and antimicrobial compounds, was added to the remaining quarter of the final volume of water, stirred and then added to the autoclaved first part. This mixture was blended for 2–3 min in the blender and then added to the third part of the diet, which contained cellulose and/or various inert compounds such as sawdust, palm bark, straw or bran. To optimize the diet preparation, the effect of sterilization on cortex quality was evaluated by adding 4% fresh cortex to the diet in one of two ways: (1) to the post-autoclaved portion, or (2) to the pre-autoclaved portion.

After mixing all the ingredients and cooling to room temperature, the paste-like diet was poured into small Petri dishes (5 cm diam.) to form a layer 3–4 mm deep (≈ 6 g of diet per dish). The neonate larvae were reared for 10–14 days on this diet and then transferred to a similar Petri dish containing the same diet but with a crumbly texture. Older larvae (about 1-month old and usually more than 5 cm long) were transferred to larger (9 cm diam.) Petri dishes containing a 4–5 mm deep layer (≈ 15 g) of the crumbly diet. Petri dishes with larvae were sealed with Parafilm and incubated at 28°C. Before use, the diets were stored in plastic boxes at –20°C, usually for no more than 1 month.

Handling and rearing of larvae

Newly hatched neonates were collected from the surface of the sand with a fine brush and transferred to Petri dishes (Ø 5 cm) containing one of the diets. Since it was unknown whether the larvae are cannibalistic or aggressive, we placed five neonates in each dish. Five small pits (1–2 mm diam.) were made in the surface of the diet with a sterile glass rod in order to facilitate neonate penetration into the diet. Each neonate was placed in an individual pit. The dishes were capped and sealed with Parafilm to prevent water loss, and placed in the dark in a temperature controlled rearing chamber at 28°C. As after two weeks some of the larvae had been eaten by the surviving larvae the survivors were the reared one per dish. For determining the duration of development the larvae were transferred to a fresh diet every 2 weeks. Pupae were kept under same conditions and the adults were transferred to oviposition cages. Larva were weighed on a BX320H electronic balance (Shimadzu, Kyoto, Japan).

Data analysis

Survival was defined as the percentage of neonates that survived for 1–3 weeks on the diets. Development time, percentage of larvae that pupated, weight of pupae and adults, and the percentage of adults that were deformed were recorded for most diets that supported complete development. The data were subjected to single-factor analysis of variance (ANOVA) using LIFETEST software (SAS 2002). When a statistically significant difference between mean values was obtained, Tukey's test was applied (at $p < 0.05$). The significant differences between mean weights of larvae reared on the diets containing three different percentages of cortex tissues were subjected to One-Way ANOVA and Multiple Range Tests for values at 95% LSD. The variance in the weight measurements was homogenized by logarithmically transforming the results. The calculations were done using the Statgraphics Plus 5 software package, version 5.0 (Manugistics Inc., Rockville, Maryland, USA).

TABLE 2. Diets with different cortex concentration for rearing of *C. tenebrionis*.

	Diet ingredients*	5% cortex	10% cortex	20% cortex
Pre-autoclaved portion	Sucrose, g	8	8	8
	Wesson's salt mix, g	2	2	2
	Brewer's yeast dry, g	15	15	15
	Bactoagar, g	6	6	6
	Cortex dry, g	15	33	66
	Water (deionized), ml	150	150	150
Post-autoclaved portion	Casein, g	8.8	8.8	8.8
	Sorbic acid, mg	500	500	500
	Vanderzant vitamin mix, g	2.4	2.4	2.4
	Methyl paraben, mg	320	320	320
	Water (deionized), ml	50	50	50
Texturizing agent	Cellulose, g	74	56	23

* Details of the main ingredients are given in Table 1.

RESULTS

Effect of protein sources on larval development

In the early tests (data not presented) larvae only survived on diets that contained casein and/or yeast products. Therefore, these ingredients were included in most diet modifications (Table 1). Larval survival on artificial diets was significantly affected ($F = 46.06$, $df = 49,9$; $P < 0.001$) by the protein source (Fig. 1). Only 16–24% of the neonates survived a week on the diets that contained casein (diet 1) or casein hydrolyzate (diet 2) and yeast extract. Substituting wheat germ for casein (diet 4; diet 3 is discussed below) or soya (diet 5) reduced larval survival to 8–12%. Adding yeast hydrolysate instead of yeast extract to casein-based diet (diet 7) completely suppressed larval development. On the other hand, addition of dry brewer's yeast (diets 8–9) resulted in significantly higher survival rates than those recorded on a similar diet containing yeast extract. Neonate survival on diet 9 (Table 1, Fig. 1), which contained 4.6% dry yeast, was higher (68.0 ± 11%) than that recorded on diet 8 (44.0 ± 8.7%), which contained only 1.8% dry yeast.

Effects of cortex tissues on larval development

The initial test demonstrated that the addition of fresh root cortex of plum or peach trees to diet 3 (Table 1) increased larval survival (72.3 and 75.5%, respectively). All the following diets were prepared using peach tree cortex. Addition of freshly milled cortex to diet containing yeast extract (diet 3) significantly improved larval survival (Table 1, Fig. 1). However, adding it to the soy-based diet (diet 6) did not increase larval survival. The effect of cortex in the diet that contained whole yeast (Diet 10) was insignificant during the first week of rearing (Table 1, Fig. 1). However, further study revealed that larvae fed on any diet lacking cortex tissue survived for a relatively short period (maximum 5–6 weeks) and never reached the pupal stage. Neonate survival on diets containing cortex tissue added after or before autoclaving the diet were similar (85.2 ± 12.0 and 87.5 ± 10.5, respectively). To reduce the risk of contamination all the diets were subsequently prepared by adding cortex tissue prior to autoclaving.

Fresh root cortex positively affected larval survival, but obtaining fresh material in sufficient quantity is a tedious task. Fig. 2 presents a comparison of the survival on diets containing a similar weight of fresh and dry cortex, and cortex from various plant parts. Neonate survival on diets with cortex from any source was significantly higher than on a diet without cortex ($F = 20.81$, $df = 24,4$; $P < 0.001$) and survival was similar on diets containing either dry or fresh cortex. Larval survival on diets containing either root or stem cortex was similar and twice that recorded on diets containing the powder obtained by shredding whole small roots.

The effect of increasing the percentage cortex in the diet was determined by substituting dry shredded cortex for cellulose (Table 2). Larvae reared on identical diets showed widely different weight gains (Fig. 3). However, most of the larvae (about 63%) reared on a diet containing 20% cortex weighed 100 to 400 mg after 4 weeks, whereas 84% of those reared on a diet containing 5% cortex weighed less than 100 mg (Fig. 3). There was a significant effect of the percentage of cortex on larval weight ($F = 64.07$, $df = 134,2$; $P < 0.001$). The average time to pupation of larvae reared on a 20% cortex diet was significantly shorter ($F = 8.86$, $df = 109,2$; $P =$

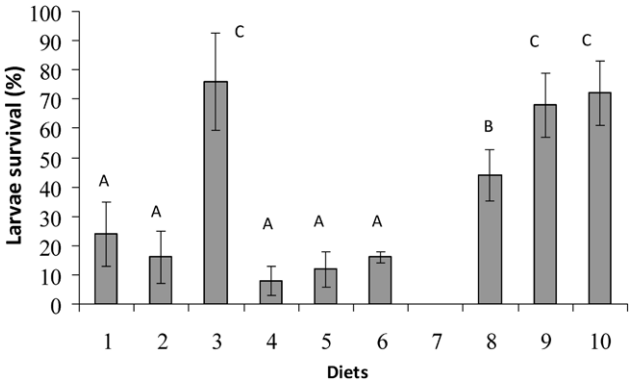

Fig. 1. Mean percentage (± SD) survival of *C. tenebrionis* larvae reared on diets containing protein from various sources and different percentages of cortex tissue. The survival was determined after one week of rearing on each diet. Compositions of the diets are presented in Table 1.

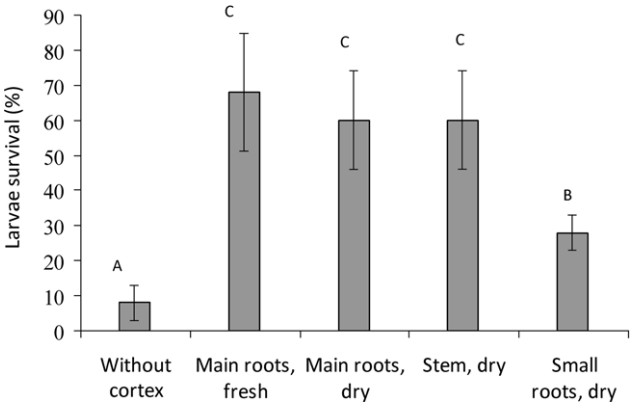

Fig. 2. Mean percentage (± SD) survival of *C. tenebrionis* larvae fed on diet no 10 (see Table 1) containing either 4% by weight of dry cortex tissue prepared from peach stem, or of fresh or dry material prepared from the main roots, or of dry material prepared from small roots. The material was shredded and added to diet in place of 4% cellulose. The survival was recorded after one week of rearing on each diet.

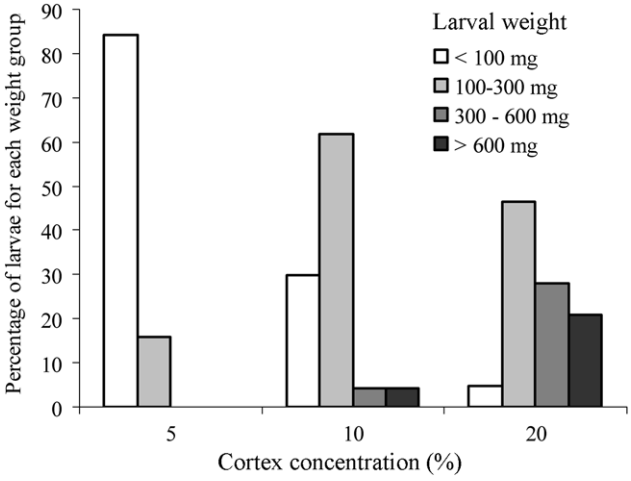

Fig. 3. Effect of the percentage of cortex tissue in the diet on the percentage of 1-month-old larvae of *C. tenebrionis* (40 individuals per diet) recorded in each of four weight groups.

0.0003) than that of those reared on diets with 5% cortex (Table 3). However the weights of pupae and adults reared on these diets did not differ significantly (Table 3).

Larval survival on diets differing in structure

All of the neonates easily penetrated the viscous diet with a paste-like structure (water content of the diet about 60–62%). Penetration into the diet took 1–10 min, after which the larvae made long tunnels in the diet matrix (Fig. 4a–b). On moister diets, with a water content of > 70%, none of the neonates penetrated into the diet and became inactive after 20–30 min. The dry crumbly diets were also unsuitable as the neonates stayed on the surface of the diet for 1–2 days, moved rapidly in and out of the diet matrix and gained only about one third (10.6 ± 5.6 mg) of the weight of those reared on the viscous diets (32.2 ± 3.8 mg).

The structure influenced not only neonate penetration into the diet but also larval survival. The neonates easily penetrated into the dense viscous diets, forming long tunnels and successfully moulting once or twice. However, 2–3 weeks after the introduction of the neonates they all died due to the physical pressure exerted by the hard tunnel wall when they attempted to increase the width of their thorax during moulting. The trials in which diet structure was changed by substituting plum (host plant) or pine (non-host plant) tree sawdust, palm bark, straw or bran for 50% or all of the cellulose did not improve larval survival as after 2–3 weeks all of them were dead. Reduction in the concentration of agar from 2.1 to 1% resulted in an increase in larval survival in the first 2-weeks from 12.0 ± 11.0 to 42.0 ± 11.4%. The highest survival, close to 100%, was obtained when 10- to 14-day-old larvae were transferred from a viscous diet, suitable for rearing neonates, to a crumbly diet with a somewhat higher concentration of cellulose (up to 25%).

Rearing of *Capnodis* spp. on optimal diets

Observations of larvae feeding on the artificial diets revealed that one of causes of larval mortality was cannibalism of both young and mature larvae. The victims of cannibalism were mainly individuals in the process of moulting. Larvae reared in groups were frequently observed biting one another and the seriously wounded died later from what seemed to be a bacterial infection. Because of the risk of cannibalism two week old larvae were reared individually on a crumbly diet (Fig. 4c). This diet dried out relatively quickly and was replaced with fresh diet every 2–3 weeks. Adopting this procedure resulted in most of the larvae successfully reaching the pupal stage (Fig. 4d–e).

The rearing parameters of the *C. tenebrionis* that developed from adults collected in the orchards were recorded.

TABLE 3. The rearing parameters of *C. tenebrionis* and *C. carbonaria*.

Capnodis source (adults)	Cortex in diet (%)	Larvae mortality[a] (%)	Pupated larvae[b] (%)	Time to pupation (days) (mean ± SD)[c]	Pupae wt (mg) (mean ± SD)[c]	Adult wt (mg) (mean ± SD)[c]
C. tenebrionis, wild	5	6.8	86.4	65.2 ± 10.7b	672.1 ± 147.4a	571.9 ± 114.2a
	10	0	77.6	63.3 ± 12.9ab	654.0 ± 118.5a	557.0 ± 104.2a
	20	15.2	79.1	53.9 ± 12.1a	651.1 ± 109.3a	561.8 ± 96.0a
C. tenebrionis, lab	5	5.3	78.9	69.0 ± 10.9b	666.3 ± 103.1a	566.9 ± 102.2a
C. carbonaria, wild	5	0	100	57.8 ± 4.5ab	1107.3 ± 208.3b	910.0 ± 164.5b

[a] Percentage from 100 neonates; [b] percentage from 40 larvae; [c] means within a column followed by same letters are not significantly different ($p > 0.05$; Tukey test).

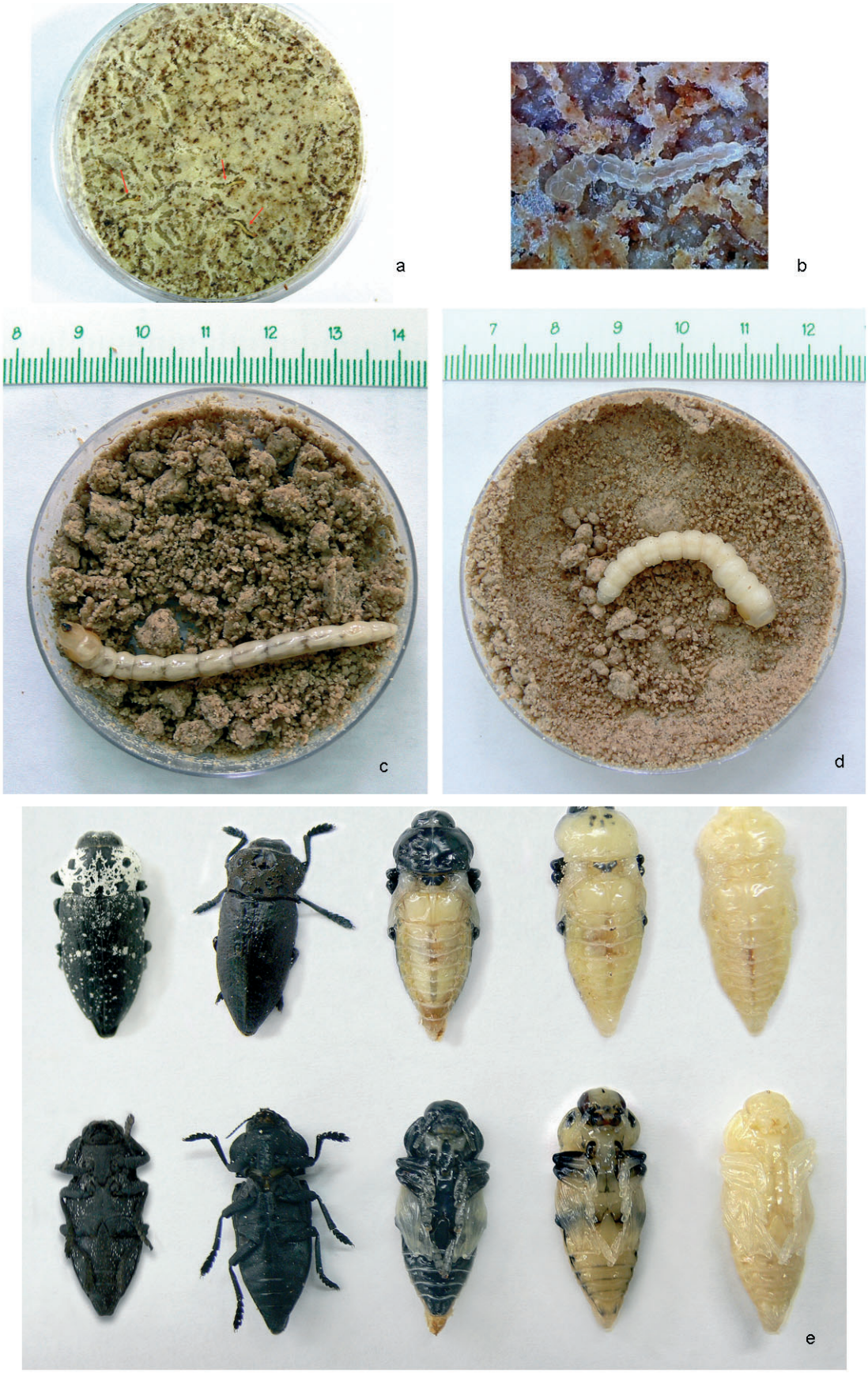

Fig. 4. Photographs of the different developmental stages of *C. tenebrionis* reared on artificial diet: first instar larvae in typical galleries in a paste-like diet (a); close-up of first instar larvae in the diet (b); mature larvae in crumbly diet (c); pre-pupa (d); mature pupae and adults (e).

Their offspring were allowed to feed on diets containing different percentages of cortex tissue; the compositions and preparation of which are as shown in Table 2. Survival of *C. tenebrionis* larvae was close to 100% on all the diets. About 80% of the larvae pupated after 54–65 days when reared at 28°C on all the diets (Table 3). A significant reduction in time to pupation was only observed when the diet contained 20% cortex tissue rather than 5% ($F = 8.86$, $df = 109,2$; $P = 0.0003$). However, the pupal and adult weights of individuals reared on the 5 and 20% cortex diets did not differ significantly. The average weights of the adults reared on the various diets ranged from 557.0 ± 104.2 to 571.9 ± 114.2 mg (Table 3), whereas the average weights of female and male adults collected in orchards during this study were 838.3 ± 277.5 and 611.0 ± 140.5 mg, respectively. None of the diets used for rearing became contaminated.

Development time of individual larvae of *C. tenebrionis* differed markedly on all the diets. Most of the larvae (about 80%) reared at 28°C pupated within 3 months. However, a few larvae had a prolonged larval development of 6–7 months and one individual took a year. Rearing the larvae at 24°C prolonged their development, with more than 80% of them pupating after 10–12 months. This extended development is associated with an increase in average adult weight to 859.4 ± 184.8 mg.

Adults reared on artificial diet emerged from October to January and actively fed on peach branches, but did not lay the eggs until February–March. The number of eggs laid by females reared at 28°C on artificial diet was relatively low, about 20 eggs per female. However, females which were reared at 24°C laid about 45–50 eggs over a period of 3 months. Close to 100% of the eggs laid by females reared on artificial diets and collected in orchards hatched.

The percentage larval mortality, percentage of larvae that pupated, mean time to pupation, and weights of pupae and adults of *C. tenebrionis* that hatched from eggs laid by beetles reared on an artificial diet (i.e., the second laboratory generation) did not differ significantly from those of beetles collected in the orchards (Table 3). The survival of the neonates of the laboratory generation was close to 100%. Although the average time to pupation of offspring of laboratory-reared beetles and wild beetles did not differ significantly, mainly because the results were very variable, the first pupae developed in the laboratory population 10–20 days earlier than in the population derived from wild beetles collected in the orchards. The average weight of 1-month-old larvae of adults reared in the laboratory was significantly greater (T-test: $t = 2.9$, $df = 33$, $P = 0.003$) than that of those born to adults collected in the field: 116.1 ± 53.6 and 71.4 ± 38.7 mg, respectively.

Rearing of *C. carbonaria* was limited by the small number of adults collected in the orchards. Therefore, *C. carbonaria* neonates were reared only on an artificial diet containing 5% cortex tissue. The feeding behaviour of the larvae was similar to that of *C. tenebrionis* and the average time to pupation was less than 2 months (Table 3).

DISCUSSION

The development of *C. tenebrionis* larvae in orchards takes between 6 and 18 months, depending on ambient temperature and rootstock (Martin, 1951; Garrido, 1984; García et al., 1996; Hmimina et al., 1998). Rivnay (1945) found that larvae reared in sections of stem in the laboratory, at a constant 27°C, took over 17 weeks before showing any signs of pupating. On the diet developed in this study the larval development of *C. tenebrionis* and *C. carbonaria* was shorter, lasting on average of 9–11 weeks at 28°C.

The best diets, measured in terms of larval survival and percentage pupation (about 80%), contained casein and dry brewer's yeasts, Wesson's salt mix, Vanderzant vitamin mix, sucrose, bactoagar, sorbic acid, methyl paraben and cellulose (Table 2). The results also suggest that for complete larval development and successful pupation root or stem cortex tissue (fresh or dry) need to be added to the diet. *Capnodis tenebrionis* completed its development on diets containing from 5 to 20% of cortex tissue. Increasing the percentage of cortex tissue in the diet significantly reduced the larval development time, but did not affect the weights of pupae or adults. Cortex tissue can be collected beforehand, dried, milled and kept at –20°C for 6 months, and probably longer (unpublished data). Diet no 10, with 5% cortex tissue, is recommended as preparing the cortex tissue is rather tedious and higher percentages of cortex tissue did not markedly improve the performance of the larvae.

The structure of the diet was a crucial factor determining its success as a medium for rearing both *Capnodis* spp. Our experiments indicate that the neonates and more advanced larvae require diets with different structures. This is possibly because these larvae differ in morphology and have different functions (Rivnay, 1945; Marannino & De Lillo, 2007b). The neonate larvae of *C. tenebrionis* have several complex structures, such as fine clavate hairs, sensory cones and various sensillae, on the head, thorax and abdomen, which facilitate locomotion while burrowing (Rivnay, 1945). These structures are present on larvae up to the third instar but not on older larvae (Rivnay, 1945).

Neonates successfully burrowed into the diet, making long tunnels in a viscous matrix consisting of 60–62% water. This starter diet was a key element in the successful development of young *Capnodis* larvae. The older (3rd instar and older) larvae survived only on drier crumbly diets, which do not obstruct their movement within the diet matrix. Compared with neonates and first or second instar larvae, older individuals form a wider tunnel when burrowing in the cortex of roots (Rivnay, 1945). Based on observations on larval feeding behaviour and records of mortality rates, it is clear that older larvae were unable to form wide tunnels in the viscous diet and died due the pressure of the diet matrix when they enlarged their thorax during moulting. In addition, our

observations revealed that *Capnodis* is cannibalistic and the larvae should be kept singly. Cannibalism reduces competition for space and provides the cannibal with high quality food. This behaviour is well documented for other tree borers (e.g., Hanks et al., 1993; Ware & Stephen, 2006); in the case of the red oak borer *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae) this behaviour results in significant weight gain (Ware & Stephen, 2006). The rearing of *Capnodis* on artificial diets enabled us to document larval cannibalism among buprestids for the first time.

Two successive generations of *C. tenebrionis* were successfully reared on an artificial diet. The second generation adults, which emerged from October to January, did not lay eggs until February–March (study in progress), which is similar to what happens in orchards during the overwintering period (Rivnay, 1946; Garrido, 1984).

Our main reason for developing an artificial diet for *Capnodis* larvae was to produce a rearing procedure that would enable us to examine the efficacy of bacterial toxins in improving the resistance of the rootstock against *Capnodis* spp. So far the diet has enabled us to demonstrate the efficacy of several Bt products (mainly *Bacillus thuringiensis* spp. *tenebrionis*), and toxins produced by the symbiotic bacteria of several nematode, and a dose-dependent response to systemic insecticides. Furthermore, using the artificial diet it is possible to determine the effect of temperature on the development of the larvae and the conditions under which their growth is arrested and pupation induced (Gindin et al., studies in progress). Observations of larvae reared on the artificial diets revealed the effect of cannibalism on larval survival and that early and later instar larvae feed in very different ways. Successful rearing of these *Capnodis* spp. on artificial diets will facilitate the development of artificial diets for other *Capnodis* pests, such as the pistachio borer, *Capnodis cariosa* Pallas, and poplar borer, *Capnodis milliaris* Klug., as well as other buprestid pests that develop cryptically within plant tissues. The present findings will facilitate the study of the effects of actual and potential control compounds on the larvae of these borers, and provide information on the phenology of the larvae from different climatic regions.

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