Bionomics of the *Tamarix* leaf beetle, *Cryptocephalus sinaita moricei* Pic, 1908 (Chrysomelidae: Cryptocephalinae)

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ABSTRACT

Cryptocephalus sinaita moricei Pic, 1908 is a phytophagous leaf-beetle, whose adults and larvae feed on *Tamarix* shoots and leaves. After mating, the female oviposits and simultaneously covers each egg with dark anal secretion. Approximately half of the eggs are dropped beneath the tree, while the rest are adhered to the tree shoots. The larva constructs a case that apparently provides protection from natural enemies. Developmental duration from the egg to adult takes three months under laboratory conditions at $25 \pm 2\,°C$. In contrast to most congeners, *C. s. moricei* larvae can survive only on fresh shoots and leaves but not on decaying ones. Accordingly, the ground-hatching larvae quickly locate a tree and climb up to the canopy to feed. When fully fed, the majority of larvae drop off the tree and pupate on the ground, whereas a minority pupate on the tree, attaching themselves to twigs. Life history strategies, particularly regarding oviposition, feeding and pupation, are discussed.

KEYWORDS: Chrysomelidae, phytophage, larval behavior, mating behavior, oviposition, life history.

INTRODUCTION

The leaf beetle *Cryptocephalus sinaita moricei* Pic, 1908 (Chrysomelidae) is endemic to the Sinai Peninsula, Dead Sea area, and the Arava Valley of Israel (Lopatin *et al.* 2003), feeding solely on the shoots and leaves of the saltcedar (*Tamarix* sp.) (Lopatin and Chikatunov 1997). The genus *Cryptocephalus* includes ca. 660 Palearctic species (Lopatin *et al.* 2010), but data on its biology are scarce and fragmentary. The adults are stenophagous feeding on fresh shoots and leaves, and females of several biologically studied species drop their eggs from the host plant onto the ground (Reid 1999). It is there where the larvae usually develop to pupation, feeding on decaying leaves of the host plant (Lawrence 1991; Owen 1999). Variations in feeding habits have been observed in several species. Larvae of the hazel pot beetle (*Cryptocephalus coryli* L., 1758) feed on decaying leaves below the Hazel tree (*Corylus avellana* L.), as well as on decaying leaves of other plant species (Owen 2000). However, fitness was greater when the larvae were fed on fresh leaves of the host trees (Owen 2002). Larvae of other Cryptocephalinae, such as *Cadmus auriantiacus* Chapuis, 1875 and *Lexiphanus saponatus* Fabricius, 1801 were infrequently observed climbing on their host trees, *Eucalyptus fraxinoides*

saplings and *Cassandra caliculata* (L.) Moench, respectively, and feeding on their leaves (Reid 1999; Le Sage 1986). Reineck (1913) showed the larva of *Cryptocephalus janthinus* Germar, 1824 subsist completely on fresh leaves.

The larvae of *Cryptocephalus* species are case-bearing grubs. The case is apparently constructed of feces (Owen 1999), with an aperture from which the head and thorax usually protrude during feeding and moving, while the abdomen always remains concealed (Lawrence 1991). The larva withdraws its head and thorax entirely into its case when disturbed and during molting. While growing, the *C. coryli* grub cuts a slit along the case wall and expands it by adding chewed plant material (Owen 2002). Most *Cryptocephalus* species have five larval instars (Le Sage 1986), whereas *C. coryli* has six (Owen 2002). Pupation usually takes place on the ground beneath the host tree. During emergence the adults use their mandibles to break through the case wall (Lawrence 1991).

The present study focuses on the *Tamarix*-feeding *C. s. moricei*, for which no biological data have been available. We investigated its life cycle, trophic relationships and reproductive mode. This study, besides adding new knowledge to the currently sparse data on the genus *Cryptocephalus*, also provides information that can be used in case of possible introduction of *C. s. moricei* as a potential agent for the control of invasive *Tamarix* species.

MATERIALS AND METHODS

Study site

The research project was undertaken at the 'Enot Ẕuqim Nature Reserve, along the north-western shore of the Dead Sea, Israel (31°43'N 35°27'E, alt. -410 m).

Mating and oviposition

We carried out direct observations on *C. s. moricei* in the field using head-fixed 10× magnifying lenses. We also collected adult beetles for laboratory study. These were kept at a temperature of $25 \pm 2\,°C$ in a wooden rearing cage (80 (height) × 35 × 35 cm), with a glass window, three screen-covered windows and a fabric sleeve. A potted *Tamarix nilotica* plant was introduced through a hole at the bottom of the cage. A total of 50 copulation and 100 oviposition events were documented, some with a video camera. Additionally, 10 females were individually confined, in a wooden rearing cage for detailed oviposition observations.

Larval development and pupae

Observations on larval behavior were mostly conducted in the lab, while pupation tactics were usually followed in the field. We collected pupae from the tree using a sweeping net, while ground-living pupae were collected by sieving leaf litter from under the trees. All pupae were kept in small, mesh-covered plastic vials ($25 \pm 2\,°C$) and examined weekly.

Determination of the number of larval instars

We collected 150 larvae of different sizes and 50 eggs at the 'Enot Ẕuqim Nature Reserve by net-sweeping shoots and leaves. The larvae were sorted to

instars according to the following criteria: (1) weight of a larva together with its case, (2) length and diameter of the larval case, and (3) head width of the larva. Additionally, larvae hatched from the collected eggs were reared in the laboratory and the number of their molts was recorded. Weighting was done using a digital balance (SCALTEC®, SBA-32, d=0.0001 g). The length and diameter of the larval case were measured using a standard caliber, and head capsule measurements were taken using a stereoscopic microscope (Wild-Heerbrugg M8) with an ocular micrometer.

Host range and duration of development

We examined the host range in respect to two *Tamarix* species native to the Dead Sea area, *T. tetragyna* and *T. nilotica*, and native to Central Asia *T. ramossisima*. One hundred eggs laid on each *Tamarix* species were hand collected together with shoots on which they were laid. They were then introduced into a new rearing cage with the same *Tamarix* species from which they had been collected. The development of the different larval stages and their mortality were observed and noted daily. The same procedures were followed for all three plant species.

Adult and larval feeding performance

Adults and 1st instar larvae were kept in rearing cages on four diet regimes: (1) potted *T. nilotica* trees, (2) freshly cut shoots of *T. nilotica* that were replaced every 48 h, (3) abscised shoots and leaves of *T. nilotica* that were collected under the trees in 'Enot Ẕuqim, (4) starvation. Each regime was tested in 10 rearing cages: five replications with 20 larvae each, and five replications with 10 adults each.

Statistical analysis

One way ANOVA was used to analyze larval and adult feeding performance and survival. Paired t-test was used to analyze female oviposition tactics; χ^2 tests were used to analyze host range and larval development duration. A Kendall rank correlation test was used to determine correlation coefficient significance in egg oviposition tactics and hatching rates. In the parametric tests the numbers were transformed using square root.

<div align="center">

RESULTS

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Mating and oviposition

Mating was observed all year round. It started one week after female emergence, with no indication for season or time of day, and lasted 21±10.1 min (N=20, range 10–30 min). The male mounts the female without apparent courtship and the female walks and halts occasionally on the *Tamarix* shoot throughout the mating process. Oviposition commences immediately after mating.

When the egg begins to extrude from the tiny ovipositor, the female smears it with a dark sticky secretion, covering the bright yellow chorion. Once the egg is fully exposed, the female keeps rolling and smearing it, using her hind legs. During egg rolling, the female remains nearly motionless for 14.1±4.3 min (N=20). The length of the coated egg is 1.58±0.06 mm (N=53). The egg, now attached to the

Table 1: Oviposition site location in 10 isolated *C. s. moricei* females.

Female	Longevity (in days)	Total number of laid eggs	Oviposition location			
			tree	ground	%, tree	%, ground
1	30	223	80	143	36	64
2	32	192	91	101	48	52
3	35	198	50	148	26	74
4	40	281	114	167	41	59
5	40	285	107	178	38	62
6	44	210	81	129	39	61
7	55	277	144	133	52	48
8	56	333	121	212	37	63
9	56	298	137	161	46	54
10	60	310	140	170	46	54
Average	**45**	**260**	**106.5**	**154.2**	**41**	**59**

ovipositor, either adheres to the tip of a shoot or is shaken off and dropped onto the ground beneath the tree. The combination of these two oviposition tactics is reported here for the first time in leaf-beetles. The number of eggs laid per female correlates well with female longevity (Table 1) (Kendall's Correlation coefficient, $R=0.796$, $p=0.013$).

Both oviposition tactics were observed at the 'Enot Ẕuqim field site and in the laboratory (Table 1). Significantly more eggs were dropped on the ground (59%; Paired t-test, $t=-4.324$, $df=9$, $p=0.0017$), and the average total rate of egg hatching was 57%, showing no significant difference between dropped and adhered eggs (Paired t-test, $t=1.548$, $df=9$, $p=0.1550$; Correlation coefficient, $R=0.796$, $p=0.013$). There was no clear pattern or sequence of eggs adhered vs. dropped during a female's lifetime.

Larval development and pupae

The newly hatched larva is a case-bearing yellow grub with a black head and dark brown legs. While the abdomen of the larva is always hidden in the case, its head and thorax protrude during feeding and walking. However, if disturbed, the larva retracts its head and thorax into the case, using its sclerotized frons as a plug (phragmosis). At the beginning of each molt the larva seals the case aperture with feces and re-opens it at the end of the process. Once the larvae complete their development, $90\% \pm 6.17$ ($N=237$) of them drop off the tree, seal the case aperture, and pupate on the ground. The remaining larvae adhere to the tree shoots and pupate there. The pupae on the ground were parasitized by parastioids (*Heterocoelia punctatus* (Argaman) (Hymenoptera: Bethylidae) different from those
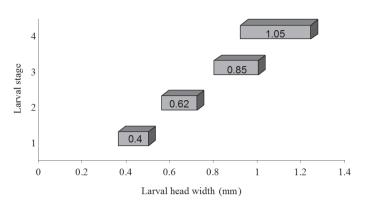
Fig. 1: Larval head width distribution within the different instars (198 larvae). The cube width re-
presents the larval head width range of each instar while the number on each cube stands for
the average value of the head width.

on the tree (*Hockeria* sp. (Hymenoptera: Chalcididae)), with parasitism rates of
60% (N=1195 pupae) and 54% (N=864 pupae), respectively.

Determination of the number of larval instars

In order to determine the number of larval instars we used the head width ob-
tained during daily measurements of larvae throughout their development. The
results indicated that *C. s. moricei* had four larval instars. However, although head
width measurements proved to be the best tool for estimating larval growth they
allowed a clear distinction only between three size groups, corresponding to the
first, second, and third + fourth instars (Fig. 1). Thus, some doubt exists as to the
existence of three or four instars. Larval weight including the case, or case size
alone, did not allow clear separation between all instars.

Host plant range

All *C. s. moricei* larvae feed exclusively on fresh shoots and leaves of *Tamarix*
trees. According to our observations, *C. s. moricei* develops equally well on the
two tested indigenous Israeli *Tamarix* species (Fig. 2). However, when cultured
on the introduced *T. ramossisima*, the larval stage takes significantly longer (i.e.,
two more weeks; $\chi^2=72.52$, df=29, p<0.001) than on the native saltcedar species
(Fig. 2). This difference is most apparent in the last larval instars. Mortality is
high (about 60%) on all three host species (Table 2). The highest partial mortality
occures during the egg and larval stages and the lowest is during the pupal phase.
No survival differences of the larvae were detected for different tree species (χ^2 for
heterogeneity, p>0.05).

Adult and larval feeding performance

Larva: The survival rate of larvae feeding on shoots and leaves of potted *Tamarix*
trees was significantly higher than that of larvae feeding on freshly cut *Tamarix*
shoots (ANOVA, F=33.34, df=1, p=0.0004) (Table 3). However, larvae of both
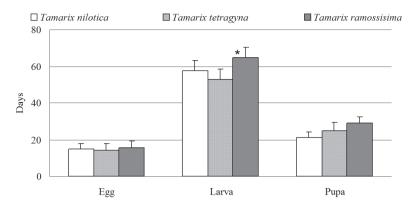
Fig. 2: Duration of development of *C. s. moricei* on three *Tamarix* species (300 eggs, 100 per tree; *=χ^2, P<0.05).

groups completed their life cycle and emerged as adults. Larvae that either starved or were fed abscised shoots and leaves survived less than 10 days, although the latter survived significantly longer (6±0.6 d vs. 4.2±0.4 d) (ANOVA, F=68.45, df=1, p< 0.001).

Adults: Feeding on potted trees vs. freshly cut shoots did not affect adult survival (ANOVA; F=0.51, df=1, p=0.49) (Table 3). Adults that either starved or were fed on abscised shoots and leaves survived less than 10 days (4.3 and 4.9 days, respectively), with the difference between the two groups being statistically insignificant (Table 3).

DISCUSSION

The beetle, *C. sinaita moricei*, lives in southern Israel and the Sinai. We studied its development and dietary demands as well as larval behavior in procurement of food, development and pupation. The relationship of its whereabouts to parasitism was also studied.

As determined through head-capsule measurements, development from the egg to adult probably includes four larval instars. However, this point must be further studied since the differentiation between the last two instars is unclear. Similar

Table 2: Survival/mortality of *C. s. moricei* immatures and adults on three *Tamarix* species under laboratory conditions.

Plant sp.	Eggs / mortality (%)		Larvae / mortality (%)		Pupae / mortality (%)		Adults	Total mortality / survival eggs– adults (%)	
nilotica	**100**	35	**65**	16.9	**54**	16.6	**45**	55	45
tetragyna	**100**	41	**59**	20.3	**47**	19.1	**38**	62	38
ramossisima	**100**	37	**63**	23.8	**48**	22.9	**37**	63	37

Table 3: Survival of *C. s. moricei* 1st instar larvae and adults kept on four diets
(100 larvae and 50 adults on each diet).

Diet	Potted trees		Fresh shoots		Abscised shoots		Starvation	
Stage	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults
Survival after 10 days, %	92	86	45	80	0	0	0	0
Average day of death	–	–	–	–	6±0.6	4.9±0.3	4.2±0.4	4.3±0.3

to other species of the genus, *C. sinaita moricei* adults are phytophagous with a narrow host plant range. However, unlike in its congeners, the mixed oviposition and pupation strategies (on the tree and on the ground) of *C. s. moricei* are unique and their larval dietary dependence upon live shoots and leaves is rare.

Larvae of *Cryptocephalus* never leave their case. According to Owen (1999), the case provides protection, commensurate with their soil and leaf-litter habitats. The larvae of *C. s. moricei* are atypical in not being ground-dwelling compared to other members of the genus. We suggest that their obligatory plant-feeding habit resulted from the need to obtain nutritious, relatively salt-free shoots and leaves, which abscised *Tamarix* shoots and leaves seemingly do not provide.

The beetle can survive and develop on at least two native *Tamarix* species (*T. tetragyna* and *T. nilotica*), as well as on the introduced *T. ramossisima*; all three species can support three or four beetle generations per year. However, development time on *T. ramossisima* takes longer (Table 3). It is possible that, due to its restricted distribution, *C. s. moricei* has adapted to the nutritive values, allelochemicals, and salt excretions typical to the native tree species. Thus, it exploits the introduced *T. ramossisima* with less efficiency, prolonging the generation time, and therefore reducing the net reproduction rate. This also results in longer exposure to adverse biotic and abiotic factors, which may lead to higher mortality (Price 1997). However, the true effect of such slower development upon the species fitness can only be ascertained through actual exposure in the field, where multiple factors affect the individual fitness.

Oviposition behavior includes both dropping some eggs to the ground and adhering others to young foliage. These two concurrent tactics point to a mixed strategy. About 41% of eggs are laid on foliage and 59% are dropped on the ground; in the latter case larvae need to find a tree to climb on to reach a suitable feeding site. This mixed strategy exposes eggs and the newly hatched larvae to very different ecological trade-offs. These include the physical environment, food availability, and exposure to natural enemies.

Many of the existing theories on fitness and survival concern trade-offs between survival and reproduction that often involve risk spreading (Stearns 1992). The

oviposition behavior of *C. s. moricei* may imply spreading the risk encountered by the same genotype within different environments. However, Hopper (1999) noted that no experiments with clear results have demonstrated that risk-spreading has been a major factor in the evolution of insect behavior or life histories, and that risk spreading would more typically develop in small populations (e.g. n < 100). Since *C. s. moricei* forms extensive populations (hundreds of individuals) in the study site and lays eggs in two environments only (on the tree and on the ground), it is unlikely that its oviposition strategy reflects a risk spreading mechanism.

The obligatory phytophagous habit of the *C. s. moricei* larvae is exceptional, as most other studied *Cryptocephalus* species feed primarily on detritus (Lawrence 1991) and only rarely on fresh plant material (Reid 1999; Le Sage 1986). The unsuitability of detritus or abscised *Tamarix* shoots and leaves to serve as food for the larvae of *C. s. moricei* is probably associated with the fact that leaf shedding in *Tamarix* constitutes part of the trees' salt removal mechanism. Abscised *Tamarix* shoots and leaves are salt-laden (Waisel 1991), forcing the larvae that hatch on the ground to find alternative, more suitable food in the form of fresh shoots and leaves. However, in our experiments, abscised *Tamarix* shoots and leaves prolonged larval survival. The ability of the 1st instar larvae to survive on detritus is probably commensurate with their need to travel across the ground and up the tree trunk prior to reaching shoots and leaves on which they can feed. The fact that all the heretofore studied congeners are detritus feeders that drop their eggs onto the ground, suggests that the present oviposition and feeding behaviors in *C. s. moricei* are secondary. We suggest that the unsuitability of *Tamarix* leaf litter to serve as larval food was an important driving force in the evolution of the present feeding traits of *C. s. moricei*. Once plant feeding had evolved, on-tree oviposition followed, with the consequent mixed oviposition strategy. From our findings it is unclear whether the mixed oviposition strategy is truly an evolutionary stable strategy, or a transitional state that may still change with time, possibly in favor of the on-tree oviposition. As far as we could determine, the cost of both strategies, in the form of mortality occurring prior to initiation of larval feeding, is approximately equal. Therefore, both oviposition habits have prevailed, each with its unique risks and benefits.

The search for enemy-free space by larvae that are about to pupate is one of the defensive strategies of a species. Two cases that support our hypothesis that pupation sites are selected to lower the probability of predation have been reported, one in Tenebrionidae and one in Coccinellidae. Tschinkel (1981) observed that tenebrionid larvae tend to disperse just before pupation presumably to reduce cannibalism, while Lucas *et al.* (2000) concluded that the selection of a pupation site away from an aphid colony by *Coleomegilla maculata* (De Geer) (Coccinellidae) may be an adaptive response to the intra-guild predation.

The dual pupation sites of *C. s. moricei*, on the tree and on the ground, suggest a strategy developed to minimize parasitism and predation risks, since both locations, tree and ground, exhibit high parasitism rates together with high emergence failure

rates (Malihi 2004). However, without additional data it is impossible to determine the conditions under which a particular strategy, pupation on the tree or on the ground, is more advantageous.

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