Exp Appl Acarol (2015) 65:219–226
DOI 10.1007/s10493-014-9870-3

Life cycle of *Cosmolaelaps jaboticabalensis* (Acari: Mesostigmata: Laelapidae) on *Frankliniella occidentalis* (Thysanoptera: Thripidae) and two factitious food sources

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Received: 23 January 2014 / Accepted: 2 December 2014 / Published online: 10 December 2014

Abstract The aim of this work was to study the life cycle of *Cosmolaelaps jaboticabalensis* Moreira, Klompen and Moraes preying on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), a serious cosmopolitan pest of different crops, as well as on *Protorhabditis* sp. (Nematoda: Rhabditidae) and *Tyrophagus putrescentiae* (Astigmatina: Acaridae), prospective factitious foods for the mass rearing of the predator. Experiments were conducted in a chamber at 25 ± 1 °C, 70 ± 10 % RH and in the dark. Total immature development (egg-adult) was completed in 12.3 ± 5, 6.6 ± 0.6 and 7.1 ± 0.6 on *F. occidentalis*, *Protorhabditis* sp. and *T. putrescentiae*, respectively. Fecundity and intrinsic rate of increase were higher on *Protorhabditis* sp. (71.6 ± 9.1 eggs/female; 0.28 female/female/day) than on *F. occidentalis* (63.8 ± 14.8; 0.23) and *T. putrescentiae* (43.1 ± 8.9; 0.23). *Cosmolaelaps jaboticabalensis* reproduces by thelytokous parthenogenesis and its larval stages can be completed without feeding. Protonymphs and deutonymphs can survive in the absence of food for about a month, and adults for almost 2 months. It was concluded that *C. jaboticabalensis* is a promising biological control agent of *F. occidentalis* and that it may be mass reared with the use of *Protorhabditis* sp. or *T. putrescentiae*.

Keywords Predatory mites · Biological control · Hypoaspidinae · Life table

Introduction

About 108 species are presently known to belong to *Cosmolaelaps*, a genus of laelapid mites of the subfamily Hypoaspidinae composed of edaphic and free-living species

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(Moreira et al. 2014). Nothing is known about the biology and potential as control agents of the vast majority of these species, but information about some species of this group indicates that they may be useful as biological control agents of organisms which spend all or part of their lives in the soil (Afifi and Van der Geest 1984; Al Rehiayani and Fouly 2005).

Cosmolaelaps jaboticabalensis Moreira, Klompen and Moraes was recently described from specimens collected in São Paulo state, southeastern Brazil (Moreira et al. 2014). The potential of this species as a biological control agent of the edaphic stages of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) was shown. The latter is a cosmopolitan and important pest of many crops that can also act as vector of plant pathogens (Reitz 2009).

The successful use of a biological control agent requires, among other things, the availability of an efficient process for its mass production (van Lenteren 2000). Soil mites have been produced in large scale using the mite *Tyrophagus putrescentiae* (Schrank) (Astigmatina: Acaridae) as factitious food (Steiner et al. 1999; Freire and Moraes 2007b). Discovery of alternative factitious food sources for mass rearing can reduce production cost of predatory mites, improving the chances of adoption of biological control. Free-living nematodes have been frequently mentioned in the literature as suitable prey for edaphic mites (Walter 1988; Berndt et al. 2004; Castilho et al. 2009), but have not been reported as food sources in predatory mite mass rearing processes. In our preliminary trials, the free-living nematode *Protorhabditis* sp. (Rhabditina: Rhabditidae) was observed to promote the highest oviposition level of *C. jaboticabalensis* in comparison with other food source.

Knowledge of the life cycle of *C. jaboticabalensis* should provide further insight on its potential for use in the control of *F. occidentalis*, especially in Brazil, where that predator is naturally found. The aim of this study was to evaluate the life cycle of *C. jaboticabalensis* on *F. occidentalis* and on *Protorhabditis* sp. and *T. putrescentiae* that could be used in its mass rearing.

Materials and methods

Organisms sampling and rearing

The population of *C. jaboticabalensis* used in this study was collected in litter under a loquat tree [*Eriobothrya japonica* (Thunb) (Rosaceae)], at Jaboticabal, São Paulo state, Brazil, in December 2011. It was maintained in the laboratory in units similar to those described by Freire and Moraes (2007b), kept in a Biochemical Oxygen Demand incubator at 25 ± 2 °C, 70 ± 10 % RH, in the dark, and fed a mixture of all developmental stages of *T. putrescentiae*, offered on pieces of a commercial dog food (Purina®).

Pre-pupae or pupae of *F. occidentalis* were obtained from a colony initiated with specimens collected from sweet pepper [*Capsicum annuum* L. (Solanaceae)] and roses [*Rosa* sp. (Rosaceae)], respectively at Iacanga and Atibaia, São Paulo, Brazil in 2011, and maintained on *Canavalia ensiformis* (L.) (Fabaceae) plants. All developmental stages of the free-living nematode *Protorhabditis* sp. were obtained from a colony started with specimens collected from manure, at Piracicaba, in 2010 and maintained on pods of green-beans [*Phaseolus vulgaris* L. (Fabaceae)]. All developmental stages of *T. putrescentiae* were obtained from a colony of the Acarology Laboratory of Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), maintained on the same previously mentioned commercial dog food for more than 5 years.

Biological parameters of *Cosmolaelaps jaboticabalensis*

The study was initiated with predator eggs laid in an experimental unit within a period of 12 h by females isolated from the stock colony. Each experimental units consisted of a Petri dish (3 cm diameter and 2 cm in height) whose base was covered with a layer of a solidified paste made of a mixture of gypsum and activated charcoal (9v:1v).

A single egg was transferred to an experimental unit, which was kept moist by daily addition of distilled water. For each treatment, a total of 74 eggs were used, each corresponding to a replicate. The predators were fed daily with surplus amounts of either designated prey. The units were examined at 12 h intervals until the mites reached adulthood and at 24 h intervals thereafter, until the death of the predator. When prey was *F. occidentalis*, daily predation rate was also evaluated.

Survival of *Cosmolaelaps jaboticabalensis* in the absence of food

A complementary test was conducted to evaluate the ability of each predator mobile stage to survive in the absence of food. The test was initiated with ten 0–6 h old mites of each stage, obtained by isolating mites of the corresponding previous stages in experimental units similar to those previously described, where they were fed with a surplus amount of *Protorhabditis* sp. Immediately before molting (when mites became more shiny and lethargic), mites were transferred to new experimental units without food, but whose solidified base was kept moist by daily addition of distilled water. After molting, the units were examined every 24 h to determine longevity.

Given that in the trial about prey consumption larvae were observed to develop to protonymphs without feeding, a complementary test was conducted to determine whether the larvae could feed when offered *Protorhabditis* sp. as prey. For this purpose, ten 2 h old larvae were isolated in experimental units similar to those previously described. They were kept without food for 6 h and then offered a surplus amount of all developmental stages of *Protorhabditis* sp. on pods of green-bean. Possible consumption of nematodes was checked for a period of 3 h after the nematodes were introduced into the units, under a stereomicroscope.

Statistical analysis

The mean duration of each stage, as well as of the total immature phase (egg-adult), and duration of the pre-oviposition, oviposition, post-oviposition, longevity and fecundity on different prey were submitted to analyses of variance, using non parametric Kruskal–Wallis test for comparisons of three means and Mann–Whitney *U* test for comparisons of two means. Statistical analyses of duration of egg and larval stages were not compared statistically because they are non-feeding stages and because the respective durations were too close to the precision of the observations (only one observation every 0.5 day). The survivorship rates of the immature stages were compared with Chi Square test. A fertility life table (Birch 1948) was constructed for predators reared on each prey. Net reproductive rate (R_o), intrinsic rate of population increase (r_m), finite rate of population increase (λ), mean generation time (T) and the standard errors were calculated using "jackknife" procedure and compared by Student's *t* test using "LifeTable.SAS" in the software "SAS System" (Maia et al. 2000).

Results

Development, reproduction and life table parameters

The durations of the egg or the larval stages were similar on the different prey species (Table 1). Durations of the protonymphal and deutonymphal stages as well as of the entire immature phase (egg-adult) were significantly longer on *F. occidentalis*, followed by *T. putrescentiae* and *Protorhabditis* sp., the latter also significantly different from each other ($\chi^2 \geq 62.5746$; *df* 2; $p < 0.0001$).

Survivorship of each stage was high on all prey species (76.9–98.0 %); thus, survivorship for the entire immature phase (egg-adult) ranged between 61.5 and 63.9 % on the three prey species. For each stage, no significant difference was observed between survivorship rates on the different prey.

The pre-oviposition period was significantly longer on *F. occidentalis* ($\chi^2 \geq 90.9672$; *df* 2; $p < 0.0001$) than on the other prey (Table 1), but no significant differences were observed between predators feeding on the other two prey. Oviposition period was statistically the same on *F. occidentalis* and *Protorhabditis* sp. but longer on *T. putrescentiae* ($\chi^2 \geq 36.0986$; *df* 2; $p < 0.0001$), whereas post-oviposition period was statistically the same on *F. occidentalis* and *T. putrescentiae*, but longer on *Protorhabditis* sp. ($\chi^2 \geq 46.4421$; *df* 2; $p < 0.0001$). The combination of these different periods led to a significantly shorter longevity on *F. occidentalis*, intermediate on *T. putrescentiae* and longer on *Protorhabditis* sp. ($\chi^2 \geq 35.8169$; *df* 2; $p < 0.0001$). Fecundity was intermediate when food was *F. occidentalis*, significantly lower on *T. putrescentiae* and significantly higher on *Protorhabditis* sp. ($\chi^2 \geq 54.4188$; *df* 2; $p < 0.0001$).

For all prey species, oviposition reached the highest rates at the beginning of the oviposition period, reducing slowly afterward, reaching very low levels at the end of the first month (Fig. 1). About 80 % of all eggs were laid in the first 20, 20 and 23 days of

Table 1 Duration of different developmental stages (days ± SE); survivorship (%, in parentheses) and mean duration (days ± SE) of pre-oviposition, oviposition and post-oviposition periods of *Cosmolaelaps jaboticabalensis* fed with *Frankliniella occidentalis*, *Protorhabditis* sp. or *Tyrophagus putrescentiae* at 25 ± 2 °C, 70 ± 10 % RH and in the dark

Stages	Prey		
	F. occidentalis	*Protorhabditis* sp.	*T. putrescentiae*
Egg	1.6 ± 0.3 (76.9a)	1.5 ± 0.4 (83.6a)	1.5 ± 0.4 (76.9a)
Larva	0.8 ± 0.3 (98.0a)	0.7 ± 0.2 (86.2a)	0.7 ± 0.2 (92.0a)
Protonymph	9.4 ± 5.9 c (91.8a)	2.2 ± 0.4 a (90.9a)	2.5 ± 0.4 b (91.3a)
Deutonymph	4.6 ± 3.1 c (88.8a)	2.1 ± 0.2 a (97.5a)	2.3 ± 0.3 b (95.2a)
Egg-adult	12.3 ± 5.0 c (61.5a)	6.6 ± 0.6 a (63.9a)	7.1 ± 0.6 b (61.5a)
Pre-oviposition	3.9 ± 0.9 b	2.3 ± 0.7 a	2.4 ± 0.6 a
Oviposition	32.4 ± 13.7 a	31.0 ± 14.5 a	50.2 ± 13.2 b
Post-oviposition	24.5 ± 17.4 a	54.8 ± 17.3 b	16.9 ± 11.7 a
Adult longevity	57.7 ± 16.3 a	86.9 ± 13.4 c	68.1 ± 17.4 b
Fecundity	63.8 ± 14.8 b	71.6 ± 9.1 c	43.1 ± 8.9 a

Means within a row followed by the same letter are not significantly different (Kruskal–Wallis test for three means and Mann–Whitney *U* test for two means, $p > 0.05$). Mean percentages (in parentheses) within a row followed by the same letter are not significantly different (χ^2 test, $p > 0.05$)

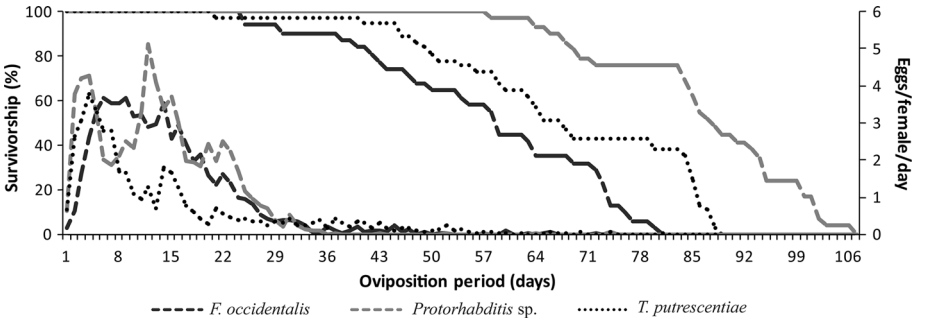

Fig. 1 Mean daily oviposition (eggs/female/day) and survivorship of *Cosmolaelaps jaboticabalensis* fed with *Frankliniella occidentalis*, *Protorhabditis* sp. and *Tyrophagus putrescentiae* at 25 ± 2 °C, 70 ± 10 % relative humidity and in the dark

Table 2 Life table parameters of *Cosmolaelaps jaboticabalensis* fed with *Frankliniella occidentalis*, *Protorhabditis* sp. or *Tyrophagus putrescentiae* at 25 ± 2 °C, 70 ± 10 % RH and in the dark

Prey	N	R_o	r_m	λ	T
F. occidentalis	31	38.98 ± 1.624 b	0.23 ± 0.005 a	1.26 ± 0.007 a	15.79 ± 0.312 b
Protorhabditis sp.	29	45.85 ± 1.084 c	0.28 ± 0.005 b	1.32 ± 0.007 b	13.55 ± 0.280 a
T. putrescentiae	37	26.04 ± 0.885 a	0.23 ± 0.005 a	1.26 ± 0.006 a	13.86 ± 0.293 a

Means within a column followed by the same letter are not significantly different (Student's *t* test; $p > 0.05$)
R_o net reproductive rate, r_m intrinsic rate of increase, λ finite rate of increase, *T* mean generation time in days

oviposition on *F. occidentalis*, *Protorhabditis* sp. and *T. putrescentiae*, respectively. Female survivorship reached 50 % after 58, 65 and 67 days of the emergence on *F. occidentalis*, *T. putrescentiae* and *Protothabditis* sp., respectively; all females died after 81, 89 and 107 days on *F. occidentalis*, *T. putrescentiae* and *Protorhabditis* sp., respectively (Fig. 1).

During periodic observations of the stock colony, no males were found and only females were generated from eggs isolated to initiate the life table studies. The eggs obtained from those populations also produced only females. These results indicate that the reproduction of *C. jaboticabalensis* occurs by thelytokous parthenogenesis.

On *F. occidentalis*, the population of *C. jaboticabalensis* increased approximately 39 times at each generation (R_o: 38.98), a rate slightly but significantly lower than on *Protorhabditis* sp. (about 46 times), which in turn was significantly higher than on *T. putrescentiae* (approximately 26 times) (Table 2). *Cosmolaelaps jaboticabalensis* produced about 0.23 female/female/day (r_m: 0.23) on *F. occidentalis* and *T. putrescentiae*, a value slightly but significantly lower than on *Protorhabditis* sp. (0.28). The daily increase in population size was about 26 % (λ: 1.26) on *F. occidentalis* and *T. putrescentiae*, a value that was also significantly lower than on *Protorhabditis* sp. (about 32 %). The mean duration of a generation of the predator was significantly higher on *F. occidentalis* (T = 15.79 days) than on *Protorhabditis* sp. (T = 13.55) or *T. putrescentiae* (T = 13.86), what was related to the longer duration of the immature phase on *F. occidentalis*.

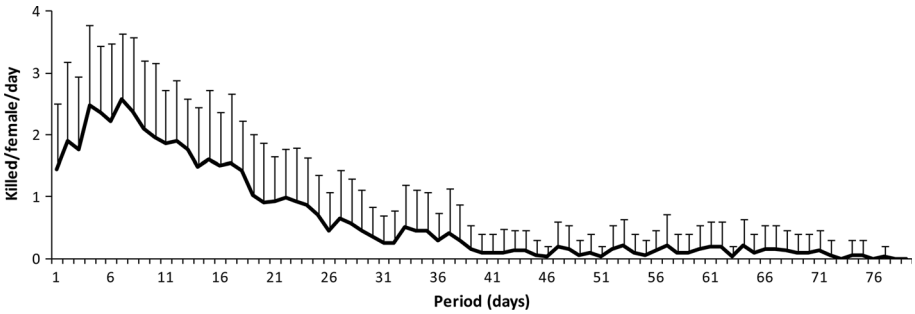

Fig. 2 Mean number of pre-pupae/ pupae of *Frankliniella occidentalis* killed by adult female of *Cosmolaelaps jaboticabalensis* at 25 ± 2 °C, 70 ± 10 % RH and in the dark

Predation rate

No pre-pupae/ pupae of *F. occidentalis* were killed by larvae of the predator. On average, 1.6 ± 0.8 and 1.7 ± 1.0 pre-pupae/ pupae were killed daily per protonymph and deutonymph of the predator, respectively, resulting in a mean total predation of 2.9 ± 1.3 prey per predator in the immature phase. Mean daily predation rate by adults increased until the end of the first week, reaching 2.6 ± 1.1 pre-pupae/ pupae per day, decreasing progressively thereafter (Fig. 2), reaching and maintaining a level below 1.0 prey per day after the first month of the adult stage. Mean total predation rate was 54.1 ± 9.9 pre-pupae/ pupae per adult predator (n = 31).

Survival in the absence of food

All larvae survived and developed to the protonymphal stage within 24 h. No significant differences were found between the time of survival of starving protonymphs and the deutonymph (31.7 ± 13.9 and 36.4 ± 7.6 days, respectively). However, none of the starving protonymph or deutonymph developed to the subsequent stage. Adults survived significantly longer without food (59.8 ± 7.5 days) than protonymphs and deutonymphs ($\chi^2 \geq 17.0199$; *df* 2; $p < 0.0001$). When *Protorhabditis* sp. was added to the experimental units, larvae that were starved for 6 h were not observed to feed on them.

Discussion

The results of this study demonstrated that *C. jaboticabalensis* was able to complete its life cycle on all organisms offered as prey. The similar durations of the egg as well as of the larval stages in the presence of the different prey was expected, because those are non-feeding stages. Al Rehiayani and Fouly (2005) and Cabrera et al. (2005) observed that larvae of *Cosmolaelaps simplex* Berlese as well as of the hypoaspidine *Stratiolaelaps scimitus* (Womersley), respectively, did not feed. Thus, non-feeding larvae seem to be common among the Hypoaspidinae.

Daily predation rates of immature fed *F. occidentalis* were lower than that of the adults, even at the final phase of the oviposition, when adult predation rate is greatly reduced. The maximum predation rate obtained in the first week was lower than determined by Berndt

et al. (2004) for *Gaeolaelaps aculeifer* (Canestrini) [mentioned as *Hypoaspis* (*Geolaelaps*) *aculeifer*] on pre-pupae/pupae of *F. occidentalis* (3.5 prey/day) but higher than determined by the same authors for *Stratiolaelaps miles* (Berlese) (1.6 prey/day), on the same prey. The latter two species have been commercialized for the control of *F. occidentalis*.

The values of intrinsic rate of population increase obtained in this and previous studies are comparable to what has been mentioned in the literature for the Phytoseiidae, as summarized by Sabelis (1985). Phytoseiids are the mite group most often used for biological control of phytophagous mites. As examples, rates close to 0.3 have been reported for *Phytoseiulus macropilis* (Banks) and *Phytoseiulus persimis* Athias-Henriot, phytoseiid species widely used commercially for the control of pest species of spider mites (Sabelis 1985).

The ability of biological control agents to survive in the absence of the pest they are released to control is of major importance. Wright and Chambers (1994) observed that protonymphs and deutonymphs of *S. miles* can survive between 12 and 24 days, respectively, in the absence of food, at 20 ± 1 °C. These values are much lower than determined for *C. jaboticabalensis* (31.7 ± 13.9 and 36.4 ± 7.6, respectively). Ability to survive under prey scarcity is a desirable attribute to consider in the selection of prospective candidates for practical use.

The results of the present study showed adequate biological performance of *C. jaboticabalensis* on *Protorhabditis* sp. Thus, this nematode and possibly others sharing the same habit may be important in maintaining this predator under natural conditions. These nematodes could also favor the persistence of that predator when the latter is released as part of a biological control program. In addition, *Protorhabditis* sp. seem promising for use as a factitious food source for the mass rearing of *C. jaboticabalensis*, provided adequate procedures are developed for mass production of the nematode and for its appropriate delivery to the predators in mass rearing units.

On *T. putrescentiae*, the oviposition period of *C. jaboticabalensis* was about twice as long, its fertility was about half and its intrinsic rate of increase was about the same determined by Freire and Moraes (2007a) for *Cosmolaelaps paulista* Freire and Moraes. In turn, Enkegaard et al. (1997) determined a much lower intrinsic rate of population increase (0.054) for *S. miles* on that prey. Thus, the results of the present work suggest that *T. putrescentiae* is also suitable for the mass production of *C. jaboticabalensis*. In a mass production program, the use of *T. putrescentiae* as prey may be more convenient than the use of other food sources, given the ease with which this prey can be produced.

In conclusion, the results of this study suggest that *C. jaboticabalensis* is a promising biological control agent of *F. occidentalis*, and that it could be mass reared with the use of *Protorhabditis* sp. or *T. putrecentiae* as factitious food sources.

Acknowledgments To State of São Paulo Research Foundation (FAPESP), for the financial support for the conduction of this research. To Élison Fabrício Bezerra Lima for the identification of *F. occidentalis*. To Hans Klompen for his valuable suggestions to a former version of this manuscript. To Maria Aparecida Reis and Jessica Oliveira for helping in the experiments. To Nazer F. Sourassou, for his help with the statistical analyses.

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