

## Predation of *Tuta absoluta* eggs during the nymphal stages of three Neotropical mirid predators on tomato

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### Abstract

*Tuta absoluta* (Meyrick), a key pest of tomato, is quickly spreading over the world. We are evaluating the biology and pest control capacity of three Neotropical mirid species, *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus basicornis* (Stal). Here we report about the predation of *T. absoluta* eggs by all nymphal stages of the three mirid species. A tomato leaflet with *ad libitum* prey was offered to a newly-emerged 1<sup>st</sup> instar nymph of a mirid predator and kept at  $24 \pm 1$  °C,  $70 \pm 10\%$  RH and 12-h photophase. Daily, the developmental stage of the nymph, as well as the number of eggs consumed was noted, and a new leaflet with eggs was added. Observations ended after nymphs had developed into adults and their sex had been determined. The average number of prey eaten by nymphs increased with nymphal age, and the 5<sup>th</sup> nymphal instar consumed higher numbers of prey than all earlier instars together. Total nymphal predation was 315, 393 and 331 *T. absoluta* eggs and total nymphal development took 16.9, 16.6 and 17.9 days for *C. infumatus*, *E. varians* and *M. basicornis*, respectively. Female nymphs of *M. basicornis* consumed significantly more prey than male nymphs. Nymphal survival of the three mirid species was 93%. The adult sex ratios of *E. varians* and *M. basicornis* did not deviate from a 1:1 ratio, whereas the sex ratio of *C. infumatus* was significantly female biased. Nymphal predation of these three Neotropical mirids was higher than values reported for any other mirid predator, which, together with their earlier published positive characteristics, make them interesting candidates for biological control of *T. absoluta*.

**Key words:** *Macrolophus basicornis*, *Engytatus varians*, *Campyloneuropsis infumatus*, biological control, Miridae, tomato borer.

### Introduction

The tomato borer *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae), native to South America, is quickly developing into a serious worldwide pest of tomato (EPPO, 2015). Due to the leaf-mining habit of this tomato borer, chemical control is difficult and frequent sprays are needed, resulting in a fast development of resistance to pesticides by the insect and reduction of natural enemies in the tomato agroecosystem (Guedes and Picanço, 2012). This, together with consumer concern for pesticide residues on tomato fruit necessitates a search for other ways of controlling this pest, such as biological control.

Both in Latin America and in Europe, many natural enemies of *T. absoluta* were found and some of these have been evaluated for biological control of this pest (e.g. Ferracini *et al.*, 2012; van Lenteren, 2012; Bueno *et al.*, 2013; Zappalá *et al.*, 2013; Gabarra *et al.*, 2014). Our research group collected native Brazilian generalist predators which might be used against several tomato pest species, and we reported the first promising results in Bueno *et al.* (2013). We are currently studying and comparing the biology and predation capacity of five species of heteropteran generalist predators present on tomato infested with *T. absoluta*. Our results indicated that three zoophytophagous mirid predators which we found in the field, *Campyloneuropsis infumatus* (Carvalho), *Engytatus varians* (Distant) and *Macrolophus*

*basicornis* (Stal), might be good candidates for the control of tomato borers, because they easily establish populations on tomato (Bueno *et al.*, 2013), show high daily predation rates in the laboratory (van Lenteren *et al.*, 2016) and appear to cause limited injury to tomato plants and fruit (Silva *et al.*, 2016a).

In order to select the most promising mirid species, we are determining various life-table characteristics. The data will be used to eventually calculate life-time predation by immature and adult mirids similarly to the study of Tommasini *et al.* (2004) on *Orius* species (Hemiptera Anthocoridae). Life-time predation data are not yet available for Neotropical Miridae, though predation results have been published for three Palearctic mirid species (Abbas *et al.*, 2014; Mollá *et al.*, 2014). Life-time predation can be expressed as total average number of prey killed by an individual, or by the kill rate ( $k_m$ ), which is the average number of prey killed per unit of time. We hypothesize that the kill rate will be a good predictor for selecting the most promising mirid species for control of pests on tomato, like it was for selecting the best *Orius* species for thrips control (Tommasini *et al.*, 2004).

In the current paper, we present data about predation of *T. absoluta* eggs by all nymphal stages of *C. infumatus*, *E. varians* and *M. basicornis*, which will later be complemented with data on predation by the adult stages similarly to the study of Tommasini *et al.* (2004). In addition to life-time predation data, other characteris-

tics, such as climate matching of predator and prey, searching efficiency under field conditions, potential of establishment prior to pest arrival, survival on alternative food and prey during periods of low pest numbers, are additional important criteria for comparison of predator performance. The three mirids show perfect climate matching with the prey (Montes, 2013), and two of the three species significantly reduce *T. absoluta* populations in experimental greenhouses (J. C. van Lenteren, V. H. P. Bueno and A. M. Calixto, personal observation). The three mirids were found preying in the field on aphids, other lepidopterans, whiteflies and psilids (Castineiras, 1995; Ohashi and Urdampilleta, 2003; Martinez *et al.*, 2014), and they develop and reproduce well on the factitious host *Ephestia kuehniella* (Zeller) (Lepidoptera Pyralidae) (Montes, 2013). These mirids further show plant-feeding behavior, but appear to cause limited injury to tomato (Silva *et al.*, 2016a) and might, due to their phytophagy, survive periods without prey.

In this paper, we report predation data by all nymphal stages of three mirid species, *C. infumatus*, *E. varians* and *M. basicornis*, when offered eggs of *T. absoluta ad libitum* on tomato. We also provide data about the development and survival of nymphs, and the sex ratio of the emerged adults.

## Materials and methods

### Collection and rearing of *Tuta absoluta*

Eggs, larvae and pupae of *T. absoluta* were collected from tomato plants at the experimental area of the campus of the Federal University of Lavras, Minas Gerais, Brazil in 2010. Newly emerged adults from the collections were released in a mesh cage (5 × 3 × 2.5 m) with unsprayed tomato plants (*Solanum lycopersicum* L. cv. Santa Clara) inside a greenhouse. New tomato plants were introduced regularly to the cage with the stock colony of *T. absoluta*.

### Collection and rearing of the mirid predators

A survey for mirid predators was done in the municipalities of Ribeirão Vermelho, Minas Gerais State, Brazil (21°08.596'S 045°03.466'W, 808 m a.s.l.) from 2010 to 2013. Collection, identification and maintenance of stock rearings of the three mirid predators on unsprayed tobacco plants (*Nicotiana tabacum* L. cv. TNN) and eggs of *E. kuehniella* as food are described in Bueno *et al.* (2012; 2013). For the experiments, adult predators from the rearing on tobacco were transferred to new cages containing an unsprayed tomato plant (cv. Santa Clara), and after nymphal emergence, *E. kuehniella* eggs were added twice per week. The stock rearings on unsprayed tomato were kept in a climate room at 24 ± 1 °C, RH 70 ± 10% and 12-h photophase. Individuals from these stock rearings, which were kept for at least 10 generations on tomato, were used in the experiments.

### Total egg predation by mirid nymphs

A pilot experiment was done to determine the maximal number of *T. absoluta* eggs that nymphs of each stage could consume per day. The daily numbers of prey

eggs offered to each of the nymphal instars of three mirid predators - 10 eggs/day for the 1<sup>st</sup>, 20 eggs/day for the 2<sup>nd</sup>, 30 eggs/day for the 3<sup>rd</sup>, 40 eggs/day for the 4<sup>th</sup> and 80 eggs/day for the 5<sup>th</sup> instar - were considerably higher than the maximal number consumed in the pilot experiment.

Single, unsprayed tomato leaflets with the required number of prey eggs were placed with their stem in Eppendorf tubes (1.5 ml) filled with a 1% agar-water suspension to prevent wilting. Prey eggs were put on the tomato leaflet after carefully removing them from plants of the stock rearing with a wet paint brush. The leaflet was then placed in a Petri dish (15 cm diameter) together with a newly emerged 1<sup>st</sup> instar nymph of the predator. Daily, after a 24 h-interval, the developmental stage of the nymph, as well as the number of eggs consumed (i.e. under a stereomicroscope at 40× magnification, non-damaged, collapsed, totally empty eggs were counted, whereby collapsed and empty eggs were considered to have been consumed by the nymphs) was noted, and a new leaflet with eggs substituted the previous one. Less than 0.001% of the *T. absoluta* eggs were missing on the leaflets after exposure to predators; we did not include missing eggs in further analyses. Observations ended after nymphs had moulted into adults and their sex had been determined according to the description given by Drake and Davis (1960). The number of replicates were 47 for *C. infumatus*, 45 for *E. varians*, and 43 replicates for *M. basicornis*. The experiment was carried out at 24 ± 1 °C, 70 ± 10% RH and 12-h photophase.

Data about 24 h mortality of *T. absoluta* eggs which were transferred from the stock rearing to a tomato leaflet, but in the absence of predators, were obtained from experiments with eggs from the same stock rearing, on the same tomato cultivar and at the same environmental conditions (van Lenteren *et al.*, 2016).

### Data analysis

Separate analyses were performed for the response variables “duration of developmental stage” and “number of eggs consumed”. The aim was to test for any differences between the species of predators, and the sexes, respectively. Separate univariate, fixed factor, two-way generalized linear models (GLMs) were performed for each developmental stage and the total across all developmental stages, assuming that the two response variables had equal variances and were normally distributed. The latter was confirmed to be the case for the number of eggs consumed per species separately, but not entirely for the duration of developmental stage (Kolmogorov-Smirnov test for normality). Post-hoc tests were performed where the model explained a significant proportion of the variation, and where the factor “species of predator” contributed significantly to the variation, where the Sidak method was applied to adjust the significance level. Binomial tests were applied to test for departure of a 1:1 sex ratio for each predatory species separately. After analyzing this general effect of species and sex on the development and consumption of the nymphs, we performed separate one-way univariate GLMs per species to detect whether within each spe-

cies, males and females differed in development time and egg-consumption per nymphal instar and during the whole immature period. All analyses were performed using IBM SPSS statistics version 22.

## Results

*T. absoluta* mortality after transferring eggs with a paint brush from the stock rearing to a tomato leaflet in absence of the predators was negligible during the 24 h test period: more than 10.000 eggs were checked and percentages of dead eggs due to handling or to natural mortality in the control treatments ranged from 0 to 1.12% (van Lenteren *et al.*, 2016). Because of this very low mortality in the control treatments, we have not corrected the predation data for “natural and handling mortality”.

During this nymphal predation experiment, we transferred more than 100.000 *T. absoluta* eggs from the stock rearing to the tomato seedlings, and 135 1<sup>st</sup> nymphal instar mirids, and determined egg predation and nymphal survival during circa 60 days. Nymphal survival was 93% for all three species (*C. infumatus*: 44 out of 47; *E. varians*: 42 out of 45; *M. basicornis*: 40 out of 43). The adult sex ratios of *E. varians* (21 females, 21 males) and *M. basicornis* (23 males, 17 females) did not

significantly deviate from a 1:1 ratio (Binomial test,  $P > 0.1$ ), whereas the sex ratio of *C. infumatus* significantly differed from a 1:1 ratio (Binomial test,  $P < 0.0001$ ) and was strongly female biased (41 females and 3 males). Table 1 shows the egg predation data for each nymphal stage of the three mirid species. As expected, the average number of prey eaten by nymphs increased with nymphal stage. The 5<sup>th</sup> nymphal instar consumed higher number of prey than all earlier instars together. In none of the GLM tests the factor “sex” contributed significantly to the observed variation, nor there was any significant interaction between sex and species (table 2). In the cases where the F-value in the GLM was significant, the factor “species” always explained a significant proportion of the variation (table 2). The post-hoc tests (table 1) indicate that overall, *E. varians* consumed the largest number of eggs, especially during the later nymphal stages.

Development times did not differ significantly between the sexes (table 2), and were similar across species. Overall, the developmental time of *M. basicornis* was significantly longer than the other two species, mostly influenced by the 5<sup>th</sup> instar (table 3). The univariate one-way GLM within each species between the sexes, for each nymphal instar and all instars combined, showed two marginally significant results out of the 36 tests that were performed: i) only for the 5<sup>th</sup> instar

**Table 1.** Predation of *T. absoluta* eggs by nymphal instars (means  $\pm$  SE) of the mirids *C. infumatus*, *E. varians* and *M. basicornis* on tomato at  $24 \pm 1$  °C,  $70 \pm 10\%$  RH and 12-h photophase.

Species	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	total	n
<i>C. infumatus</i>	10.7 $\pm$ 0.45 <sup>a</sup>	17.2 $\pm$ 0.75	33.9 $\pm$ 1.30 <sup>a</sup>	72.1 $\pm$ 2.44 <sup>a</sup>	181.3 $\pm$ 5.10 <sup>a</sup>	315.1 $\pm$ 4.42 <sup>a</sup>	44
<i>E. varians</i>	12.8 $\pm$ 0.47 <sup>b</sup>	20.4 $\pm$ 1.13	44.4 $\pm$ 2.23 <sup>b</sup>	82.1 $\pm$ 2.91 <sup>b</sup>	233.1 $\pm$ 6.40 <sup>b</sup>	392.8 $\pm$ 6.19 <sup>b</sup>	42
<i>M. basicornis</i>	13.4 $\pm$ 0.72 <sup>b</sup>	19.7 $\pm$ 0.81	40.4 $\pm$ 1.56 <sup>b</sup>	70.3 $\pm$ 1.92 <sup>a</sup>	187.0 $\pm$ 4.24 <sup>a</sup>	330.7 $\pm$ 5.26 <sup>a</sup>	40

Values with different letters within columns indicate significant differences in pairwise post-hoc tests with Sidak-correction of significance level; these post-hoc tests were only performed if the overall GLM (table 2) explained a significant proportion of the variation.

**Table 2.** Results of GLM analysis of variance of development time and predation for each of the nymphal instars and the sum across all instars of the mirids *C. infumatus*, *E. varians* and *M. basicornis*. Fixed factors were “species” (3 levels) and “sex” (2 levels), and also the interaction between these factors was tested. The table gives F values for the overall model, and only if this was significant, F values for each of the factors (sex and species) are also given, as well as the p values.

Development time	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	total
F value complete model	2.185	1.257	1.919	0.499	10.630	14.423
(Significance)	(NS)	(NS)	(NS)	(NS)	( $p < 0.0001$ )	( $p < 0.0001$ )
F value sex					0.987	0.095
(Significance)					(NS)	(NS)
F value species					16.468	34.547
(Significance)					( $p < 0.0001$ )	( $p < 0.0001$ )
Predation	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	total
F value complete model	3.451	1.723	4.353	2.950	12.024	25.513
(Significance)	( $p = 0.006$ )	(NS)	( $p = 0.001$ )	( $p = 0.015$ )	( $p < 0.0001$ )	( $p < 0.0001$ )
F value sex	0.460		0.160	0.838	0.333	0.006
(Significance)	(NS)		(NS)	(NS)	(NS)	(NS)
F value species	5.568		3.419	6.291	20.926	42.353
(Significance)	( $p = 0.005$ )		( $p = 0.036$ )	( $p = 0.003$ )	( $p < 0.0001$ )	( $p < 0.0001$ )

**Table 3.** Nymphal development time (days; means  $\pm$  SE) of the mirids *C. infumatus*, *E. varians* and *M. basicornis* on eggs of *T. absoluta* on tomato at  $24 \pm 1$  °C,  $70 \pm 10\%$  RH and 12-h photophase.

Species	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	5 <sup>th</sup> instar	Total development	n
<i>C. infumatus</i>	2.9 $\pm$ 0.10	2.6 $\pm$ 0.07	2.6 $\pm$ 0.07	3.3 $\pm$ 0.08	5.5 $\pm$ 0.10 <sup>a</sup>	16.9 $\pm$ 0.12 <sup>a</sup>	44
<i>E. varians</i>	2.6 $\pm$ 0.08	2.4 $\pm$ 0.08	2.7 $\pm$ 0.11	3.2 $\pm$ 0.09	5.8 $\pm$ 0.10 <sup>a</sup>	16.6 $\pm$ 0.14 <sup>a</sup>	42
<i>M. basicornis</i>	3.0 $\pm$ 0.08	2.5 $\pm$ 0.10	2.9 $\pm$ 0.08	3.2 $\pm$ 0.07	6.4 $\pm$ 0.08 <sup>b</sup>	17.9 $\pm$ 0.09 <sup>b</sup>	40

Values with different letters within columns indicate significant differences in pairwise post-hoc tests with Sidak-correction of significance level; these post-hoc tests were only performed if the overall GLM (table 2) explained a significant proportion of the variation.

of *E. varians*, the development between males and females was significantly different ( $F = 4.324$ ;  $p = 0.044$ ; males developed in  $5.95 \pm 0.498$  days, whereas females took  $5.57 \pm 0.676$  days); and ii) for *M. basicornis*, the total number of eggs consumed over all instars combined differed significantly between males and females ( $F = 4.122$ ;  $p = 0.049$ ; males consumed  $318.71 \pm 8.311$  eggs, and females  $339.48 \pm 6.301$ ).

## Discussion

Among the three studied mirid species, only *E. varians* was earlier found on tomato on Cuba (Castineiras, 1995) and in Mexico (Martinez *et al.*, 2014). Initially, we tried to collect predators of the tomato borer on tomato but hardly found any, supposedly due to the frequent sprays - 3 to 5 times per week during the whole tomato production cycle - with cocktails of pesticides (Bueno *et al.*, 2012). Next, we successfully collected predators including the three mirid species tested in this study on a tobacco crop which was rarely sprayed (Bueno *et al.*, 2013). Further, various biological characteristics of these three mirids were studied, showing that they successfully develop, reproduce and establish populations on tomato with *T. absoluta* eggs and larvae as food (Bueno *et al.*, 2013; Silva *et al.*, 2016b). All performance data of the three Neotropical predators are either similar or better than those of the Palearctic mirids *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) (Hemiptera Miridae), which are successfully used for control of *T. absoluta* in Europe (Calvo *et al.*, 2012; Mollá *et al.*, 2014).

To our knowledge, this is the first time that total predation of *T. absoluta* eggs by nymphs of Neotropical mirids was determined. Another paper reports nymphal predation of *T. absoluta* eggs by two European mirids (Mollá *et al.*, 2014) and two papers present data on nymphal predation of *E. kuehniella* eggs by three European mirids (Abbas *et al.*, 2014; Mollá *et al.*, 2014). Egg predation data for the three Neotropical mirid species are rather impressive. On average, they ate circa 20 *T. absoluta* eggs per day, starting at about 4 eggs per day during the 1<sup>st</sup> nymphal instar and culminating in about 35 eggs per day for the 5<sup>th</sup> instar. Total average egg predation by nymphs was 315 for *C. infumatus*, 393 for *E. varians* and 331 for *M. basicornis*. These numbers are twice as high as found for nymphal predation of

*T. absoluta* eggs by *N. tenuis* (148) and *M. pygmaeus* (175) (Mollá *et al.*, 2014) and also higher than nymphal predation found for *E. kuehniella* eggs by *Dicyphus maroccanus* Wagner (Hemiptera Miridae) (Abbas *et al.*, 2014). We found that *M. basicornis* female nymphs ate significantly more eggs than male nymphs, and a similar, though not significant, sex related difference in predation was reported by Abbas *et al.* (2014). Available data about daily predation of *T. absoluta* eggs by adults are all of limited value because of short-term measurements over one or a few days and, due to a period of starvation before the test, tend to overestimate adult predation (Urbaneja *et al.*, 2009; Bueno *et al.*, 2013; van Lenteren *et al.*, 2016). Life-time predation data by adults of the three Neotropical mirid species are not yet available. We have collected data for the first 14 days of adult life of *M. basicornis*: on average 563 eggs were consumed, resulting in a predation rate of 40 eggs/day/adult (J. Smit, personal observation). Based on life-span data for *M. basicornis* (Silva *et al.*, 2016b), we estimate that an adult predator might consume on average circa 800 eggs.

Total development times of nymphs of the three mirids (16-18 days) are similar to times found for the same mirid species with *T. absoluta* eggs as prey (16-19 days) (Silva *et al.*, 2016b). Nymphal developmental times with *T. absoluta* eggs as food are considerably shorter for *N. tenuis* (13 days), similar for *M. pygmaeus* (16 days) (Mollá *et al.*, 2014), considerably longer for *Dicyphus errans* (Wolff) (Hemiptera Miridae) (24 days) (Ingegno *et al.*, 2013), and slightly longer for *D. maroccanus* with *E. kuehniella* as prey (19.5 days) (Abbas *et al.*, 2014).

Nymphal survival of the three mirids was 93% in our experiments, which is clearly higher than the 70-75% survival found for the same three mirids by Silva *et al.* (2016b) with the same prey on the same host plant and with the same climate conditions. The lower survival found by Silva *et al.* (2016b) might have been caused by a difference in methodology: they put 40 newly emerged nymphs in a glass jar (1.7 L) on a tomato seedling with three leaves, while we put individual nymphs on a leaflet in a Petri dish. The high numbers of mirid nymphs on the three leaves in the experiment of Silva *et al.* (2016b) may have caused higher mortality. Survival percentages of the European mirids were 90% for *N. tenuis* and *M. pygmaeus* (Mollá *et al.*, 2014), 83% for *D. errans* with *T. absoluta* eggs as prey (Ingegno *et al.*,

2013), and 85% for *D. maroccanus* with *E. kuehniella* eggs as prey (Abbas *et al.*, 2014). Based on the high predation rates, high survival percentages and nymphal development times found, we can safely conclude that the methodology we used was appropriate and that regular manipulation of the predators did not negatively influence nymphal performance.

Little is known about the variation of sex ratios in mirid predators, and ratios reported for mirids are usually 1:1 (Wheeler, 2001), similar to what we found for *E. varians* and *M. basicornis*. We are currently investigating the cause of the strongly female biased sex ratios for *C. infumatus* which we consistently found in the experiments during the past 4 years.

Many new data about mirid predators attacking *T. absoluta* and other pests on vegetables have been made available during the past decade, which is a very positive development as it has become clear that these generalist predators can play an important role in biological control and IPM programmes (Urbaneja *et al.*, 2012). However, in order to evaluate their biological control potential, ranking based on a common aggregate parameter would be helpful. Currently papers, including some of our own papers, often conclude that based on one or another characteristic a certain predator might be a good candidate. In several papers, the aggregate parameter intrinsic rate of population increase ( $r_m$ ) is used for comparison of various *T. absoluta* predators (e.g. Mollá *et al.*, 2014). But the intrinsic rate of population increase only provides information how quickly a predator population can grow and does not provide an estimate on how many preys it can kill. Therefore, instead of using  $r_m$ , we propose to use the prey kill rate,  $k_m$ , as an aggregate parameter for comparison, though we realize that the kill rate might not be the only parameter which finally determines the efficacy of a natural enemy. For zoophytophagous and polyphagous mirid predators, such as the three species discussed in this paper, other criteria determining their success in pest control could be how well they establish on a plant in the absence of prey (Messelink *et al.*, 2015), and how they perform on alternative prey and food prior to arrival of the target pest(s) (Messelink *et al.*, 2014). If mirid predators could build up large populations by plant feeding, which we doubt for the three species we studied, and/or by feeding on alternative prey and food, a relatively low individual kill rate might be compensated by the presence of many predators.

For calculation of the kill rate, life-time predation data for all developmental stages are needed, as well as data on developmental times, survival rates, sex ratios and adult life-span (Tommasini *et al.*, 2004). This paper provides predation data for all nymphal stages of three Neotropical mirids, and we are currently determining life-time predation of adults of these mirids. With the resulting kill rates we will be able to estimate and compare the biological control potential of these predators. Another advantage of kill rate data for predators is that they can be compared with intrinsic capacity of population growth ( $r_m$ ) data and/or kill rate data of parasitoids in order to rank and select best candidates for biological control (van Lenteren, 2010).

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