

Natural Enemies of *Tuta absoluta* (Lepidoptera: Gelechiidae) in Oued Righ Region, An Arid Area of Algeria

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Abstract: In this study, information about natural enemies of the invasive tomato leafminer moth *Tuta absoluta* is presented from the southern part of Algeria. First, the most important natural enemies associated with this pest were listed. Furthermore, the life-history traits of the most representative larval parasitoid *Necremnus artynes* (Walker) at laboratory conditions was presented. Among predators of *T. absoluta*, three species were recorded: *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) belonging to Miridae family and *Orius* (Heteroptera: Antocoridae). The generalist bug *N. tenuis* is the most abundant because of its resistance to high temperatures. Parasitoids of *T. absoluta* are mainly: *Hemiptarsenus zilahisebessi*, *Bracon hebetor*, *Neochrysocharis formosa* and *Stenomesus* sp. This last one was not announced in the North of Algeria. The species identified as *N. artynes* was the most often found in the samples but its performances do not make it possible to use as biological agent of control under the conditions of the arid areas. At temperature conditions of 17°C as minimum and 23°C as maximum, 16 L: 08 D photoperiod and 60±10 % RH, the parasitoid *N. artynes* showed a parasitism rate of 20,38 ± 01,19 %, a female fecundity of 03,33 ± 01,87 eggs/female, a duration of life cycle (egg-adult) of 17,96 ± 02,53 days, an adult longevity of 05,45 ± 01,79 days and 04,27 ± 01,08 days for females and males respectively.

Key words: *Necremnus artynes* • Biological Parameters • Southeastern Arid Region of Algeria

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important crop in Algeria with a production of 1,47 million tons [1]. Tomato leaf-miner (*Tuta absoluta*) is a new pest that attacks tomato plant since its detection in Mediterranean area in 2006 [2]. It is originating from South American countries where it is a key pest of tomato [3, 4]. In Algeria, *T. absoluta* was detected for the first time in spring 2008 in the vicinity of Mostaganem [5]. One year later, it invaded several locations throughout the most important tomato growing regions of the country such as Biskra, an important area of tomato greenhouses. Many plants in different families were recorded as host plants of *T. absoluta* [6-9]. However in our region mines were only observed on *Chenopodium album* L. (Chenopodiaceae), in addition to Solanaceae, when there is absence of tomato plants or when heavy infestations of this pest were recorded. On tomato,

T. absoluta larvae feed on all the growing stages and mines in leaves, buds, flowers and fruits. At high densities, it can severely reduce yields or kill the plants [10, 11]. The success of its spread was related to the favourable climatic conditions, wide distribution of its hosts and a low efficacy of native natural enemies because tomato growers applied large quantities of insecticides that affected parasitoid abundance in the vegetable agro-system [12]. Due to its behavior of feeding inside the tissue, of leaves and fruits, control measures through insecticides foliar spraying have not achieved sufficient control levels. Furthermore, several studies have shown that *T. absoluta* can develop resistance forms due to the overuse of pesticides [13, 14]. In the same time several indigenous predators and parasitoids have been reported as natural enemies of *T. absoluta* in the northwestern Algeria [12, 15, 16] while in the South part, little is known both on the bioecology of *T. absoluta* and its antagonists.

The aim of this study was to record the complex enemies of the pest in a desert area that shows particular climatic conditions with a very high summer temperatures and to study biotic parameters of *Necremnus artynes*, an indigenous ectoparasitoid of *T. absoluta* larvae which is considered as the most efficient in many Mediterranean countries.

MATERIALS AND METHODS

Study Area: This study was conducted in the region of Oued Righ. This area is located in the Southeastern part of Algeria (Fig. 1). It is a saharian region with a temperate winter (Fig. 2) and a hot summer. The annual pluviometry is about 2 mm, the maximum of temperature is recorded in July and August with month mean of about 42°C and the minimum in December and January with about 5.5° C. This very low region is located at an altitude of only 69 m at 06°4' E and 33°7'N.

There is a large date palm tree grove in this area (Fig. 3). Farmers of this region cultivate vegetables and fruit trees under date palm trees. They still use ancestral methods to do that. In this region, tomato is cultivated mainly associated with palm trees (Fig. 4). Producers use only tomato cherry variety. The crop seeds are usually obtained by selection in the farm and they do never use chemicals to control tomato pests and diseases. The harvest is used to auto-consumption or sold at the local market. This agricultural technics allow producing tomato cycles per year: early season (from September to January) in green-houses and late season (from March to November) associated with date palm trees.

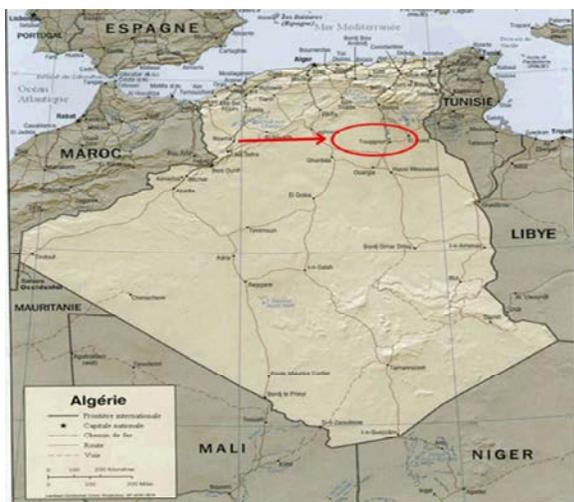


Fig. 1: Study site (Google earth, 19/03/2015)

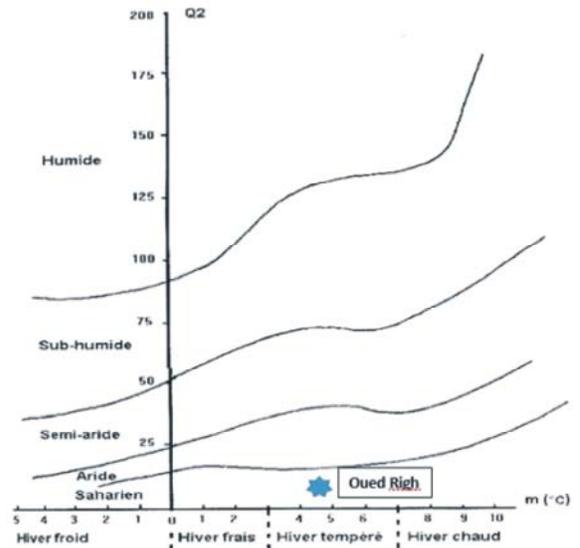


Fig. 2: Climatic gramme of EMBERGER (2005-2014)

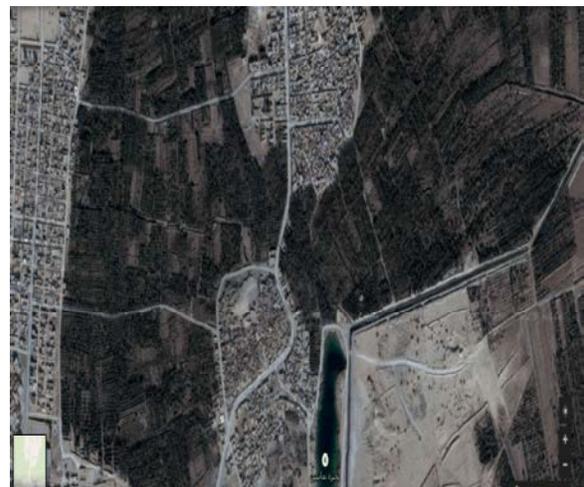


Fig. 3: Date palm tree groves (In black) in the region of Oued Righ (Google earth, 19/03/2015)



Fig. 4: Tomato crop associated with date palm trees

Source of Tuta Absoluta and Maintenance of Culture

Stock: Infested leaves with *T. absoluta* larvae were collected from tomato crops in the region of Ouargla. Samples were placed in ventilated plastic boxes until emergence of adult moths. Mated females were placed with tomato plants high about 20 cm in ventilated cages. The rearing experiment was carried out in laboratory environment conditions.

Source of Parasitoids: Observations were carried out during the period 2009-2014 from the plantation to the harvest of tomato. Leaves were collected from open fields and greenhouses infested by *T. absoluta* and brought to the laboratory in ventilated plastic boxes. Leaves were examined under a stereo-microscope to look for parasitoids. Each parasitized larva was maintained in laboratory environmental conditions (temperature: $18.45 \pm 1.56^{\circ}\text{C}$, photoperiod: 16 L: 08 D and relative humidity: $60 \pm 10 \%$) until emergence of adults. Obtained hymenopterans were used in biological experiments. They were maintained with *T. absoluta* larvae in ventilated plastic boxes (50 cm X 30 cm X 20 cm) and served as stock culture. Specimens were conserved in vials with 70% alcohol for identification.

Parasitoids Rearing: *T. absoluta* larvae were reared on cut leaves of tomato which were put in vial with water to keep the tissue turgescient. Each mated *N. artynes* female was introduced in a glass vial with ten L2/L3 tomato leafminer larvae. The parasitoids were fed with a solution of honey (10 %) and water. Every day the parasitoids were removed to another vial with ten new host-larvae. This experiment was repeated until the death of females. The larvae exposed to the parasitoids were daily examined under stereomicroscope (X20 and X40) to get the number of eggs laid per female. The development time of the progeny, sex-ratio and longevity of male and female were calculated.

RESULTS AND DISCUSSION

Parasitoid Inventory: In the Southeastern Algeria, five parasitoids belonging to the Eulophidae and Braconidae families had been recorded only in spring every year because it is the most favorable period in open field. However, some of them had also been found in summer on tomato crop conducted under date palm trees that constitute a micro-climatic area.

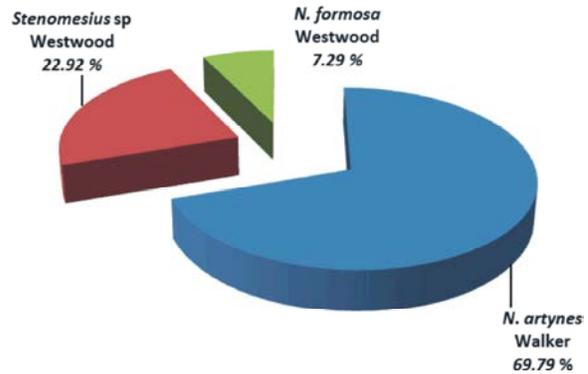


Fig. 5: Abundance of native parasitoids found on *Tuta absoluta* in the South of Algeria

These five species are the ecto-parasitoids *Necremnus artynes* (Walker), *Stenomesius* sp. (Westwood), *Hemiptarsenus zilahisebessi* (Erdos), *Bracon hebetor* (Say) and the endoparasitoid *Neochrysocharis formosa* (Westwood). *N. artynes* represented the most important number among the other species with a rate of 67 % (Fig. 5). Species of the genera *Necremnus* are the most economically important [17] and can attack micro-lepidoptera [18], Coleoptera [19], Hymenoptera or Diptera [20]. *N. artynes* have been recorded on *T. absoluta* in several locations of Algeria [12, 15, 16]. In Mediterranean countries, more than 50 hymenopteran species were recorded developing on tomato leafminer in the newly infested regions [21]. Our samples were sent to Pr. Verdu (a specialist of hymenoptera in Spain) for identification. The most part of samples were identified as being the species *N. artynes*. This first identification was based only on morphology but a recent study by Gebiola *et al.* [22] shows that the species identified through all the Mediterranean basin regions is misidentified. In their work, the authors discovered a new species that they call *Necremnus tutae* sp. Nov. RIBES and BERNARDO regarding to the molecular analysis of specimens recorded in Mostaganem area.

Life Traits of Necremnus Artynes: *N. artynes* is an idiobiont species that halt host development after attacking it by the injection of venom. It feeds on his host, prefers the third instar larval host to oviposit and grows protected inside the leaf mines. Eggs are usually observed one day after the confinement of the parasitoids with the hosts. The female deposits one to three eggs inside the mine near or on the larvae (Fig. 6) but just one egg

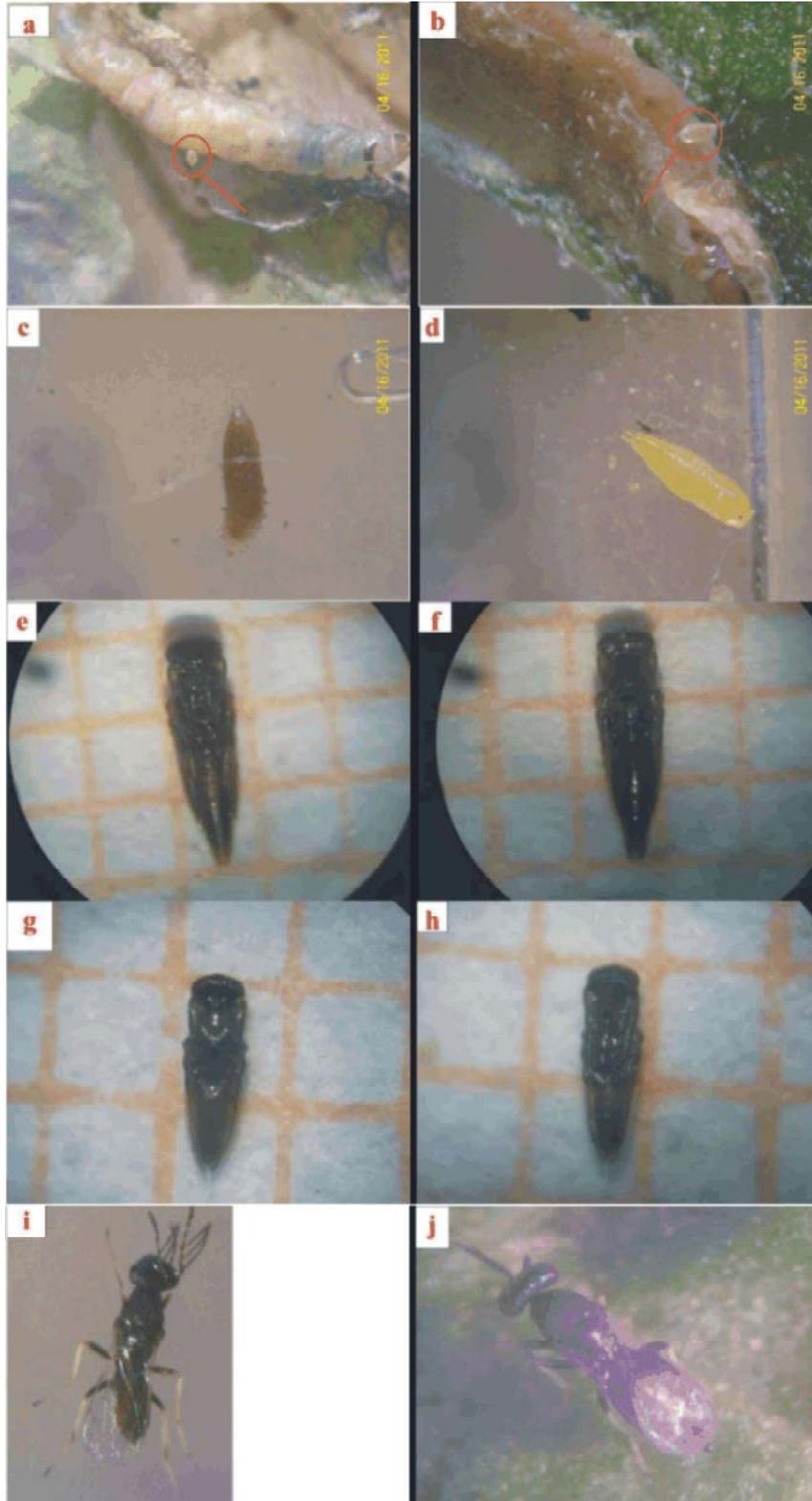


Fig. 6: *Necremnus artynes*: a- Egg, b- Young larva, c- Old Larva, d- Pre-pupa, e- Pupa ♀ (ventral view), f- Pupa ♀ (dorsal view), g- Pupa ♂ (dorsal view), h- Pupa ♂ (ventral view), i- Adult ♂, j- Adult ♀ (original photos X 40)

Table 1: Life history development of *Necremnus artynes* on L2/L3 instars of *Tuta absoluta* at 18.45 ± 1.56° C, 16 L: 08 D photoperiod and 60±10 % RH.

Trait	Value			N
	Mini	Max	Mean ± SE	
Fecundity of females (Eggs/female)	01	06	03,33 ± 1,87	09
Egg incubation period (Days)	?	?	02,30 ± 0,60	30
Larval period (Days)	?	?	05,85 ± 1,38	27
Pre-pupal period (Days)	?	?	01,00 ± 0,00	35
Pupal period (Days)	?	?	09,00 ± 1,14	35
Total developmental time (Days)	?	?	17,96 ± 2,53	27
Female longevity (Days)	02	08	05,45 ± 1,79	22
Male longevity (Days)	02	06	04,27 ± 1,08	22
Parasitism rate (%)	10	40	20,38 ± 1,19	13
Sex ratio (No. males/no. males + no. females)			0,54	27

Table 2: Monthly minimum and maximum temperatures recorded in 2014 in the region of Oued Righ.

	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
M (°C.)	18.4	21.8	22.7	30.3	33.8	37.8	42.3	42.6	38.8	32.4	25.1	18.4
m. (°C.)	6.1	7.7	9.7	14.9	20.1	23.4	27	27	25	16.9	11.8	4.9
(M+m)/2	12.2	14.7	16.2	22.6	26.9	30.6	34.6	34.8	31.9	24.6	18.4	11.6

M: Monthly mean of maximum temperatures. m: Monthly mean of minimum temperatures. (M+m) / 2: Monthly mean of temperatures.

hatches. Larva of the first instar has the same size of eggs but they may be recognized by their segmentations (Fig. 6). Parthenogenesis was observed but eggs laid by virgin females were not hatching. *N. artynes* parasitism occurs in the L2/L3 instars of *T. absoluta* [23, 24]. The mean parasitism rate obtained was 20.38 ± 1.19 % (Table 1). The same result was noted by PURE [25] in open field. The parasitoid fecundity was 3.33 ± 1.87 eggs per female with a maximum of 6 eggs per female. This mount is very low compared to those obtained by other authors at 30° C [26] probably due to the influence of low temperatures or the density of host-larvae [27]. However, Savino *et al.* [28] found the same result with *Dineulophus phtorimaeae* (Hymenoptera: Eulophidae) reared on *T. absoluta*. The mean duration of life cycle (egg-adult) was 17.96 ± 2.53 days (n = 35) with 2.30 ± 0.60 days for incubation, 5.85 ± 1.38 days for larval stage, 01 day for pre-pupal stage and 09 ± 1, 14 days for pupal stage. These results are similar to those obtained by Soriano *et al.* [29]. The sex-ratio was 0.54 (n = 27). [24] recorded the mount of 0.28. The mean longevity of adults was 5.45 ± 1.79 days and 4.27 ± 1.08 days for females and males respectively. This number is very low than which obtained by Arno, Berruezo and Gabarra [16] at 25° C (18 ± 3.1 days).

This study shows the important number of autochthonous parasitoids attacking the new pest *T. absoluta* in the Southern of Algeria. In this work, we investigate just the species *N. artynes* because it represented a large number in our samples. This parasitoid

can kill about 20 % of its host. It will be associated with other species such the indigenous eulophid *Stenomiesius* sp. which showed an important potential of control of *T. absoluta* (Unpublished data). In our study we counted just the parasitized host however, this parasitoid showed a host-feeding behavior because females killed 02 to 03 larva per day to feed without parasitizing which increase the control rate. This result is consistent with those obtained by Savino, Coviella and Luna [28] with *Dineulophus phtorimaeae* (Hymenoptera: Eulophidae) reared on *T. absoluta*. The lower mount of *N. artynes* fecundity obtained in our study may be due to low temperature because the experiment was conducted in the real conditions when the parasitoid was very active. Moreover, host density may have an influence on parasitism rate [4]. Savino, Coviella and Luna [28] recorded that increase in mean parasitized hosts was observed at higher *T. absoluta* densities.

These results were obtained in laboratory conditions and may be the parasitoid *N. artynes* will not be interest in field conditions because an intra-guild predation were noted when this parasitoid was tested on *T. absoluta* larvae with the autochthonous predatory bug *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae). In a study on the predation capacity, this bug showed no difference in consumption between parasitized and non-parasitized larvae (Unpublished data). Also, the fecundity of this species was very low in a laboratory conditions similar to those of the field when tomato is cropped (in March or November) (Table 2).

It may be real that *N. artynes* is regarded as the most frequent parasitoid of *T. absoluta* in the Mediterranean basin, but it does not have particular performances in the southern region of Algeria at the time of tomato culture. All these results show that this parasitoid may not be interest to realize to control *T. absoluta* populations in this area due to culture conditions and practices technics used by tomato producers.

Three predators had been found preying on eggs and larvae of the tomato leafminer in Southern Algeria. They mainly belong to Anthocoridae and Miridae families (Hemiptera). *Orius* sp., *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae) species were very abundant in both untreated tomato open fields and greenhouses. The most important of them was *N. tenuis* because it was more active and tolerant to high temperatures. It has been observed on tomato and pepper crops at more than 35° C, while *M. pygmaeus* was active at less than 28° C. A commercial strain of *N. tenuis* has been imported from Spain in 2010/2013 by the Algerian Ministry of Agriculture for releasing in tomato greenhouses throughout different sites [30]. These bugs are generalist predators that appeared very effective against *T. absoluta* [31] and they were regularly released to control whiteflies [32]. In the Western Palearctic countries, 15 arthropod species were recorded attacking *T. absoluta* [21]. In North of Algeria, *Dicyphus errans* Wolff, *Dicyphus tamaninii* Wagner, *Tapinoma nigerrimum* Nylander (Hymenoptera: Formicidae) were also recorded as predators of the tomato leafminer [12, 33].

CONCLUSION

In the current work, three predators and five parasitoids have been identified from South Algeria as autochthonous natural enemies of *T. absoluta*. The most abundant parasitoid in our samples of tomato leaves infested by *T. absoluta* was *N. artynes*. The life traits of this parasitoid were investigated. The study shows that this wasp may not be interest to use as control agent to control *T. absoluta* populations due to its low fecundity, the presence of an intra-guild predation and non-adapted conditions and technics used by farmers to produce tomato in this region. Nevertheless, further studies should be undertaken to understand the interaction between different indigenous natural enemies of *T. absoluta* in varied conditions of the southeastern of Algeria.

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