LIFE CYCLE OF THE CYST FORMING NEMATODE *CACTODERA TORREYANAE* CID DEL PRADO AND SUBBOTIN, 2014 AND ITS RELATIONSHIP WITH ITS HOST *SUAEDA EDULIS* FLORES OLV. & NOGUEZ

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ABSTRACT

Evans, G., I. Cid del Prado Vera, P. Yáñez-Jiménez, and R. Noguez-Hernández. 2015. Life cycle of the cyst forming nematode *Cactodera torreyanae* Cid del Prado and Subbotin, 2014 and its relationship with its host *Suaeda edulis* Flores Olv. & Noguez. Nematropica 45:189-197.

The goal of this study was to examine the biology of *Cactodera torreyanae* and its interaction with *Suaeda edulis*. Both pathogen and host are recently described organisms, but little is known about either of them, although they appear to be well-adapted to soils with a very high salinity and pH. The anatomical changes that *C. torreyanae* incited in its host roots were studied. *Cactodera torreyanae* induced multinucleate syncytia to form in parenchymatous cells located in the vascular tissue of the primary phloem of the *Suaeda edulis* root. The syncytia were irregular in shape and size: perimeter 0.427 - 1.748 (0.784 ± 0.43) mm; area 0.012 - 0.105 (0.032 ± 0.03) mm^2. The nematode completed its life cycle in 25 days after 447-degree days, at a base temperature of 20ºC. None of the post-embryonic stages completely penetrated the host roots to complete its life cycle, so *C. torreyanae* can be considered to be a sedentary ecto-parasite.

Key words: *Cactodera torreyanae*, life cycle, *Suaeda edulism* syncytium.

RESUMEN

Evans, G., I. Cid del Prado Vera, P. Yáñez-Jiménez, y R. Noguez-Hernández. 2015. Ciclo de vida del nematodo formador de quistes *Cactodera torreyanae* Cid del Prado & Subbotin, 2014 y su interacción con su hospedante *Suaeda edulis* Flores Olv. & Noguez. Nematropica 45:189-197.

El presente estudio fue llevado a cabo para conocer el ciclo de vida de *Cactodera torreyanae* y su interacción con su hospedante *Suaeda edulis*. Ambos nematodo y hospedante son recientemente descritos y poco se conoce de ellos. Ambos organismos están bien adaptados a suelos con alta salinidad: Na 690 mg.kg^{-1}, con un pH de 9.1. La primera parte de ésta investigación fue estudiar los cambios anatómicos que *C. torreyanae* induce en las raíces del hospedante; *C. torreyanae* forma un sincitio multinucleado en las células parenquimatosas localizadas en el cilindro vascular del floema primario. El sincitio es irregular en forma y tamaño; el perímetro va de 0.427 - 1.748 (0.784 ± 0.43) mm; área en mm^2 0.012 - 0.105 (0.032 ± 0.03). El segundo objetivo fue describir el ciclo de vida y así aceptar o rechazar la hipótesis de Cid del Prado y Subbotin (2014), quienes observaron que este nematodo, a diferencia de otros géneros de la misma familia Heteroderidae, no requiere penetrar completamente a la raíz de su hospedante para completar su ciclo de vida y su desarrollo es fuera de la raíz. El nematodo completo su ciclo de vida en 25 días a una temperatura de 20°C requiriendo 447 días grado, y ningún estadio postembrionario requirió penetrar completamente la raíz para su desarrollo y se concluye que esta especie vive como un ectoparásito sésil.

Palabras clave: *Cactodera torreyanae*, ciclo de vida, *Suaeda edulis* sincitio.

INTRODUCTION

Nematodes are major plant pathogens in temperate and tropical regions and cause global economic losses in different crops each year (Sasser and Freckman, 1987; Evans and Rowe, 1998; Luc *et al.*, 2005). About 80% of these losses are due to two main groups of nematodes: the root-knot nematodes (*Meloidogyne* spp.) and certain genera of cyst nematodes. During the spring of 2012, second-stage juveniles and males of a cyst nematode were detected from soil around *Suaeda torreyana* (Chenopodiaceae) plants, growing naturally in a saline soil. *Suaeda edulis* (syn. *Suaeda torreyana*) was described from

Mexico as a new plant species (Noguez-Hernandez *et al*., 2013). This plant, known by the Mexican people as romerito, is a traditional food plant at Christmas. During observations of nematodes from soil samples, the second-stage juvenile showed a conspicuous increase in the width of the body and increase in size of the nucleus of the genital primordium cells. This finding was the first indication that the nematode may have an unusual postembryonic development. Morphological and molecular studies identified the nematode as *Cactodera torreyanae* (Cid del Prado and Subbotin, 2014). In the process leading up to the description of this new species, it had been observed that the advanced juvenile stage was abundant in the soil and the third and fourth stages were attached to the outside of the roots. This observation led to the hypothesis that these nematodes may not enter the root of the host to complete their life cycle. In this paper, the results from a study of the post-embryonic development of *C. torreyanae*, in relation to accumulated degree days, and the histological changes that this species induces in *Suaeda edulis*, are presented.

MATERIALS AND METHODS

The study was conducted in a field in which *Suaeda edulis* and *C. torreyanae* occur naturally in the Colegio de Postgraduados in Montecillo, Texcoco, Estado de Mexico (latitude 19.46339° and longitude -98.90476°; altitude of 2286 m above sea level). The soil type was a sandy clay loam (46.8% sand, 22% silt, 31.2% clay) with a pH ranging from 8.6 to 10.1 and electrical conductivity from 1.25 to 11.22 at 0- to 30-cm depth, with 690, 90, and 125 Mg.kg^{-1} of Na, K, and Ca, respectively, and apparent density of 1.46 g.cm^3. The study was conducted during the 5-mon period from January to May, 2014. *Suaeda edulis* plants grown from seeds were planted in a 5 m^2 section of a field. Ten soil samples and 10 plants with root were collected every 5 and 7 d beginning immediately after planting, during the 5-mon period, and nematodes attached to the roots were collected.

A standard sieving-flotation-centrifugation method (Jenkins, 1964) was used to extract the vermiform nematodes from 200-g samples of soil. To extract cysts, the Fenwick can flotation device was used (Shepherd, 1986). For histological studies, roots were washed gently and samples with attached nematodes were cut into segments of 5-mm length. Root segments were fixed in FAA (formalin, acetic acid, and ethanol), dehydrated in an ethanol series, and embedded in paraffin wax. Samples were then sectioned at 10 μm, stained in safranin (1.0%) and fast green (0.03%) (Johansen, 1940), mounted in synthetic resin, and examined using a compound microscope. The nematodes were fixed in formalin (4%) for 8 d and then processed to glycerine using a modification of Seinhorst (1959) method, as described by Cid del Prado and Subbotin (2012), and mounted on slides.

The experiment was repeated three times. When the plants reached a height of 5 cm 15 d after germination, they were transplanted at 5-cm depth into the 5 m^2 naturally moist area, where both *C. torreyanae* and *S. edulis* occurred naturally. A single Lascar EL-USB-1 data-logger (Lascar Electronics Ltd., Salisbury, UK) was buried in the ground at 10 cm in the center of the plot to record the soil temperature.

RESULTS

Recently hatched, second-stage juveniles, and some in the process of developing to the third-stage, were found in the soil during all 5 mon of the experiment, and these stages always became attached to the roots in the process of developing to the third stage (Fig.1). Adult males and cysts were also found in soil. Occasionally, some third-stage nematodes were present in the soil and we believe that these became detached from the roots when the plants were collected. Cysts were collected from the soil at all sampling times, likely because the experimental site was naturally infested with *C. torreyanae* and was the natural habitat of *S. edulis*. The number of specimens of the different stages that was collected was variable. Some plants had more than 10 specimens attached to the roots, while
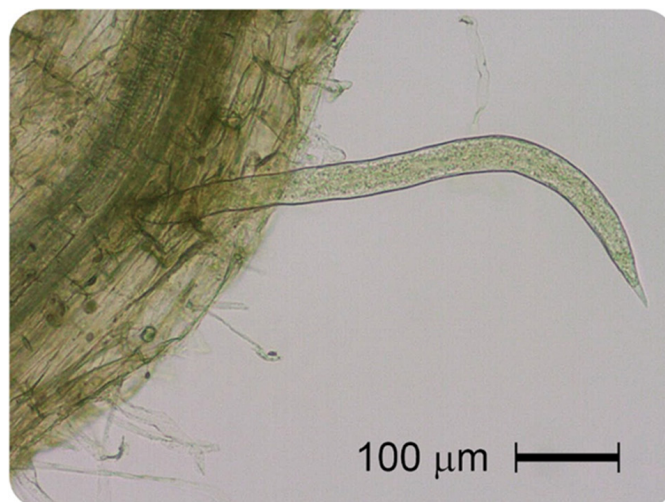

Fig. 1. Second-stage juvenile of *Cactodera torreyane* attached to a root of *Suaeda edulis*.
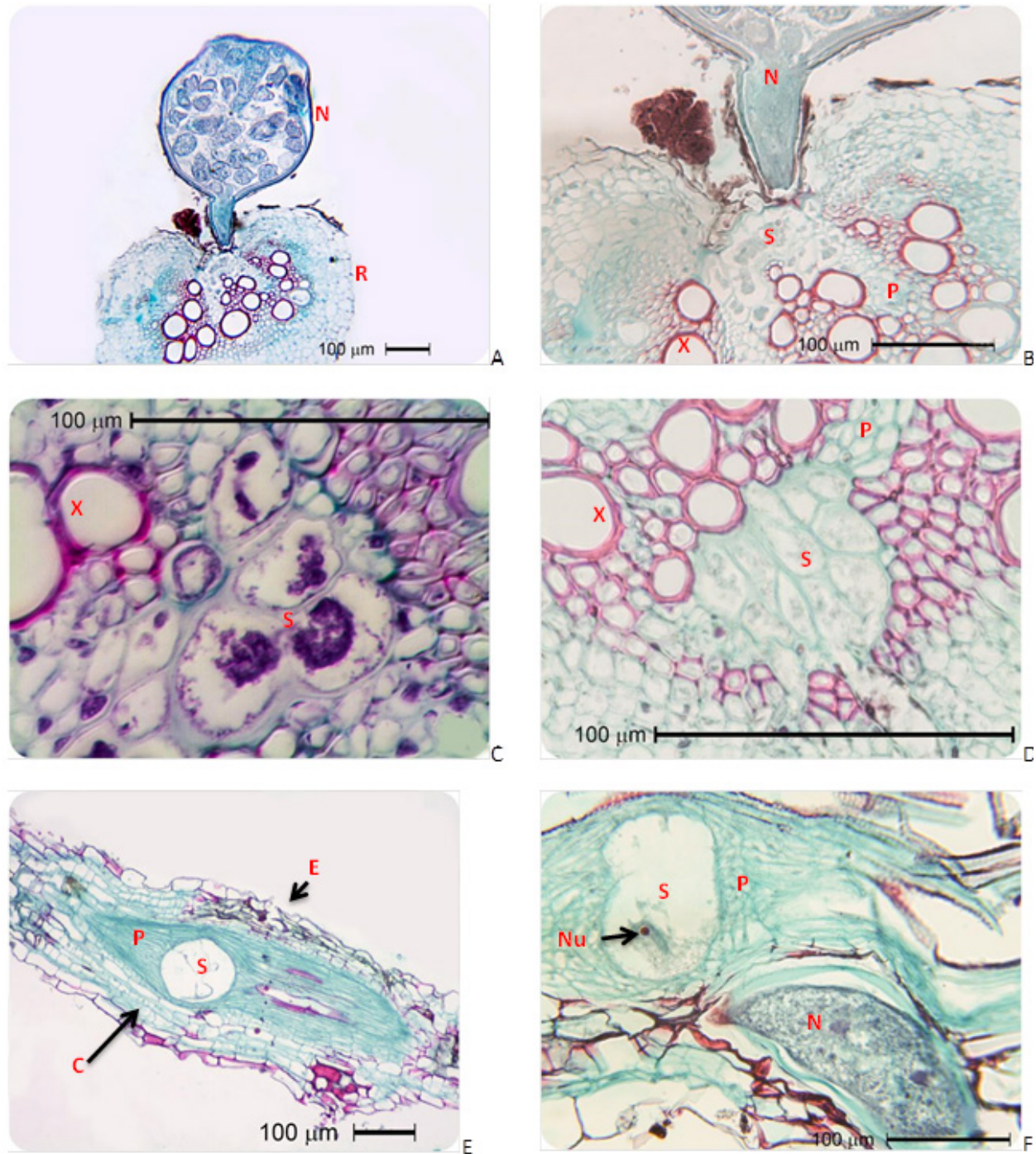
Fig. 2. A) Nematode attached to root of *Suaeda edulis*; B) Nematode attached to root of *Suaeda edulis* next to syncytium; C) Dissolution of cell walls in the initial phase of symcytium formation; D) Dissolution of cell walls in the initial phase of symcytium formation; E) Longitudinal section showing syncytium obstructing phloem tissue; F) Nematode close to syncytium.

N = Nematode; X = Xylem; P = Phloem; R = Root; C = Cortex; E = Epidermis; Nu = Nucleolus; S = Syncytium.
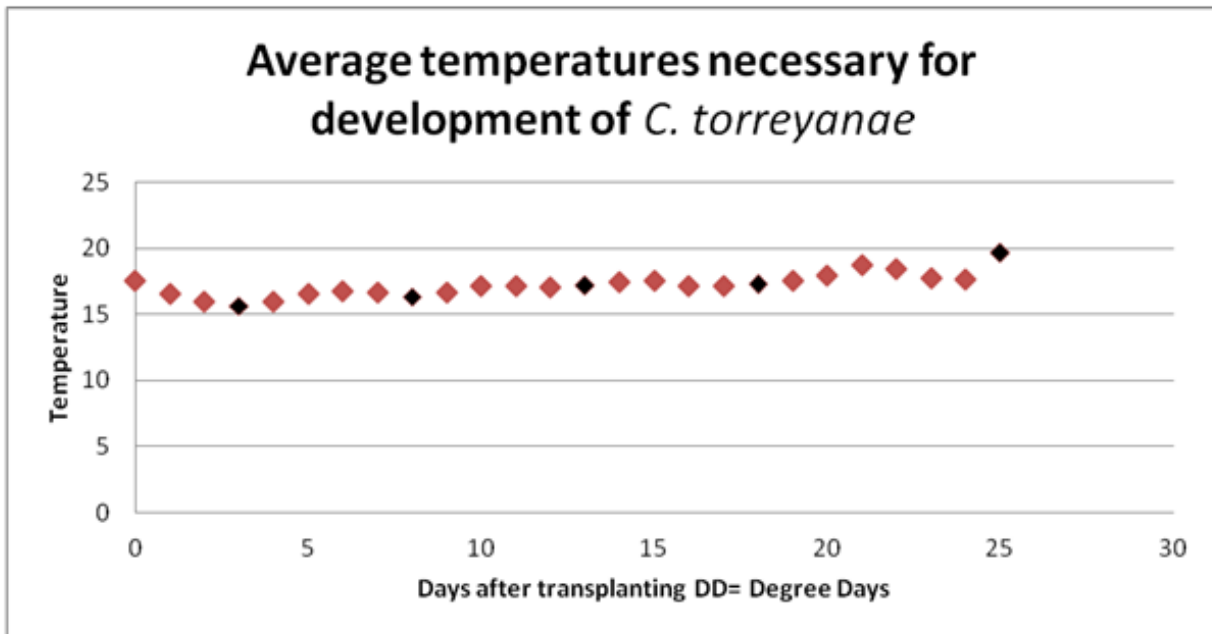
Fig. 3. Degree-days and base temperature necessary for the development of *Cactodera torreyanae*.

Table 1.   Dimensions of the various stages of *Cactodera torreyanae* in μm.

| Factor	J2	J2A	J3	J4♀	J4♂	Mature ♀
n	10	9	12	14	10	17
L	908 - 1093 (997 ± 18)	814 - 1089 (1017 ± 29)	613 - 1080 (922 ± 35)	871 - 1126 (998 ± 21)	864 - 923 (898 ± 17)	1121 - 1942 (1351 ± 54)
Body width	42 - 58 (50 ± 1.9)	49 - 76 (63 ± 3.1)	97 - 265 (164 ± 12.9)	277 - 486 (371 ± 17.0)	156 - 165 (160 ± 2.7)	486 - 971 (663 ± 34.5)
Oesophagus	194 - 372 (273 ± 15.9)	219 - 400 (283 ± 21.7)	222 - 281 (251 ± 7.7)	209 - 307 (270 ± 9.7)	-	300 - 345 (320 ± 9.5)
a	17 - 24 (20 ± 0.7)	13 - 21 (16 ± 0.9)	3 - 10 (6 ± 0.5)	2 - 3 (2.7 ± 0.1)	5.2 - 5.8 (5.6 ± 0.2)	1.5 - 2.6 (2.07 ± 0.1)
b	2.9 - 4.9 (3.75 ± 0.2)	2.6 - 4.7 (3.83 ± 0.3)	3.0 - 4.4 (3.87 ± 0.2)	3.0 - 4.1 (3.62 ± 0.1)	-	3.2 - 4.1 (3.71 ± 0.2)
Hyaline portion of tail	45 - 91 (64 ± 5.2)	49 -90 (64 ± 4.6)	-	-	-	-
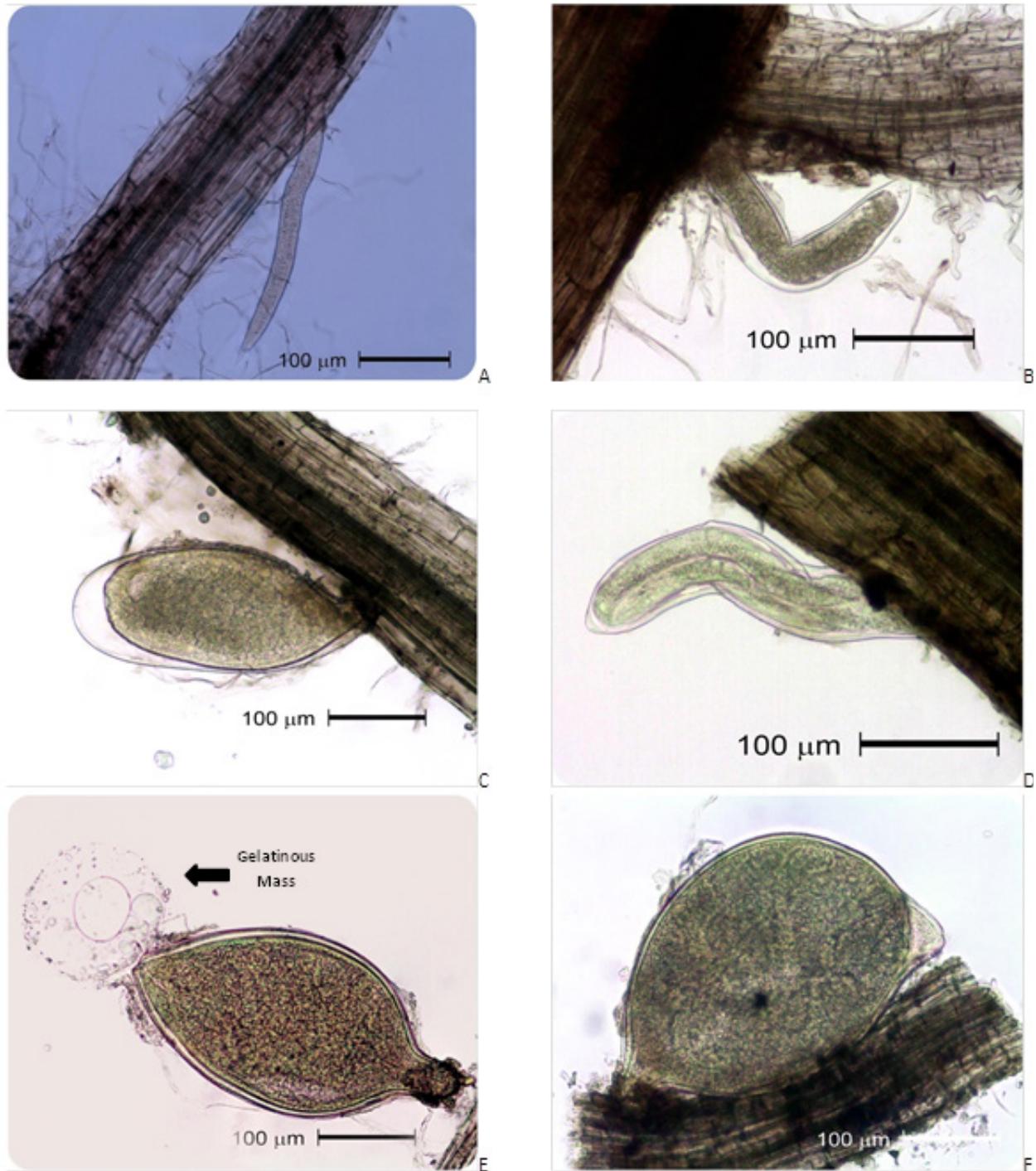
Fig. 4. Different developmental stages of *Cactoderra torreyanae* attached to root of *Suaeda. edulis*. A) Second-stage juvenile; B) Third-stage juvenile; C) Fourth-stage female; D) Fourth-stage male; E) Young female; F) Adult female.
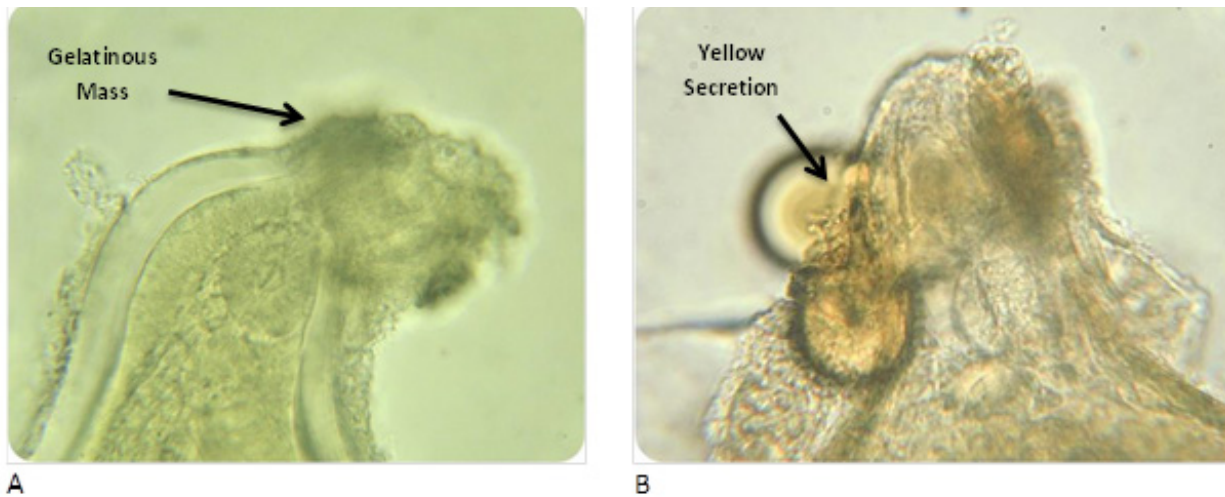
Fig. 5. A) Young female nematode with secretion near head/neck area; B) Yellow secretion on anterior end of young females.
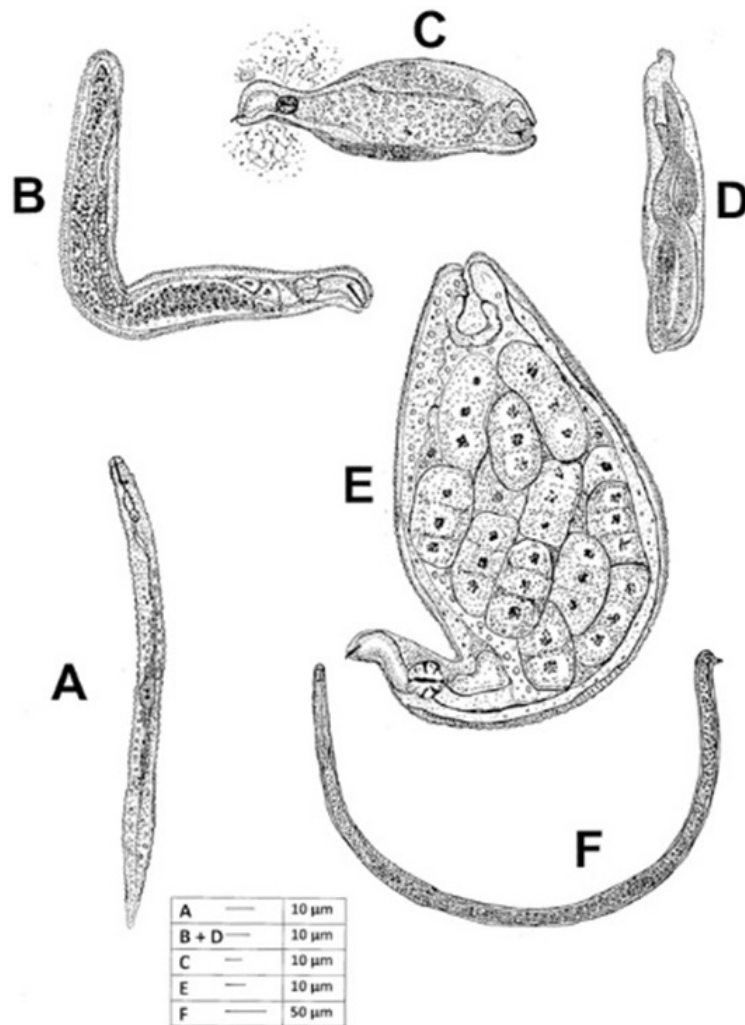

Fig. 6. Morphological changes observed in *Cactodera torreyanae* on *Suaeda edulis*. A) Advanced second-stage juvenile; B) Third-stage juvenile; C) Fourth-stage female; D) Fourth- stage male; E) Adult female; F) Adult male.
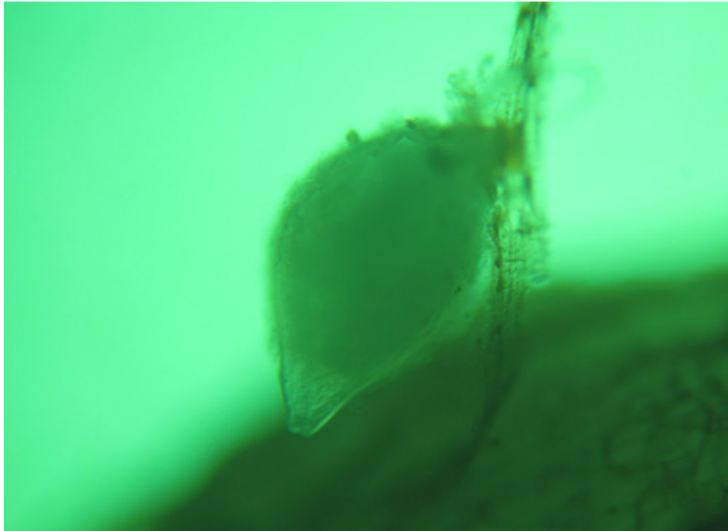
Fig. 7. Adult female attached to tiny root of *Suaeda edulis*.

others had fewer, and this was likely dependent on the population density of the nematode in the place where each plant was growing. Nematodes were always observed to be outside the roots. At the same time that experimental plants were collected, some wild *S. edulis* plants growing nearby were collected, to compare the development of the nematode. The same stages were found on both types of plant and were always living as sessile ectoparasites.

The second-stage juveniles partially penetrated the root with the anterior part of the body. Migration to the vascular tissue, primarily the phloem, resulted in damage to the parenchymal cells of the epidermis and the cortex. The damaged cells displayed severe necrosis and collapse (Fig. 2A-B). Syncytia formed from multiple cells after dissolution of adjacent cell walls in response to the nematode (Fig. 2C-D). The syncytia were much larger than surrounding cells (perimeter (0.427 - 1.748 (0.784 ± 0.430) mm; area (0.012 - 0.105 (0.032 ± 0.026) mm^2), and in some instances, the point at which two or more of the cells joined did not appear to have a cell wall (Fig. 2C). The cells of the phloem showed hyperplasia caused by the nematode. Longitudinal cross sections of root samples showed the cytoplasm of the cells to be dense and granular (Fig. 2E-F). No large vacuoles were observed in this cytoplasm and the xylem tissue was not damaged. Cells surrounding the syncytia were smaller than normal cells of the vascular tissue, and the cells were isodiametric in shape.

The life cycle was completed in 447 degree days below 20°C (Fig. 3). The infective second-stage juvenile does not penetrate the roots but attaches itself to a young root, inserts its head and neck (Fig. 4A), and feeds on parenchymal cells of the primary phloem. This stage was detected at 66 degree days, the third juvenile stage was detected at 148 degree

days (Fig. 4B), and the fourth stage (Fig. 4C) was found after 234 degree days. The young females started producing a gelatine-like material near the vulva (Fig. 4E) with white females detected at 320 degree days and the first cyst at 447 degree days (Fig. 4F). The nematode remained sedentary and completed its entire life cycle attached to the root in this manner at a temperature of 16º to 20ºC (mean of 17ºC). It was possible to differentiate between males and females when they reached the fourth-stage juvenile as the vermiform shape of the male could be seen through the cuticle, whereas the females remained swollen (Fig. 4C-D). In the fourth stage, the primordial cells increased in number to more than 100 cells, and a small cluster of primordial cells near the posterior end of the nematode that would likely give rise to the vagina and vulva were bigger than the others. A yellow secretion was observed next to the head and neck region (Fig. 5A-B). Fully mature females with eggs were white in colour and, at about 25 d after planting, turn brown in colour, forming the first cyst. All the eggs were retained inside the female bodies.

All stages of the developing nematodes were measured and catalogued (Table 1 and Fig. 6A-F). The nematode width increased with each moult [second stage: 42 - 58 (50 ± 1.9) μm; late second stage: 49 - 76 (63 ± 31) μm; third stage: 97 - 265 (164 ± 12.9) μm; fourth stage: 277 - 486 (371 ± 17) μm; mature females 486 - 971 (663 ± 34.5) μm]. The cuticle and the hypodermis thickened and the oesophageal glands were more distorted after each moult. The nuclei of the internal primordial cells were more prominent in the late second stage (Fig. 6A) and by the third stage, the number of primordial cells had increased to a cluster of more than 20 cells (Fig. 6B). The stylet remained robust until the end of the second stage. All the nematodes were swollen at the third stage, and it was not possible to differentiate the sex. At this stage the robust stylet was replaced by a slender and flexible one. Mature females and cysts were seen on roots on the 25th day or after 447 degree days (Fig. 7).

DISCUSSION

Cactodera torreyanae was described in 2014 (Cid del Prado and Subbotin, 2014) and its effects on the host are still, for the most part, unknown. The nematode can survive in soils with an extremely high salt content as can its only known host, *S. edulis*. The absence of aerial symptoms in infected plants

was in keeping with comments by Baldwin and Mundo-Ocampo (1991), who stated that damage to host plants by nematodes of this genus is not of economic or commercial importance. The changes that this nematode species causes in the cells of its hosts are similar to those described by Hernández-López *et al.* (2006) for another *Cactodera* species (*C. galinsogae*). In accordance with observations made by other researchers in other genera of nematodes, syncytia are irregular in shape and size (Baldwin and Bell, 1985; Suárez *et al.*, 1985; Hernández-López *et al.*, 2006) and only are found in cells of the primary root phloem tissue. An important observation was that a very large number of small cells surround the syncytium, suggesting that secretions from the nematode result in both hypertrophic growth of cells and their nuclei and cellular hyperplasia. These small cells may lead to an increased metabolic rate. It is likely that this could lead to the creation an area of high osmotic pressure (a nutrient sink), increased biosynthesis, and increased metabolic activity, which translates to a high energy demand (Itaya *et al.*, 1988; Puthoff *et al.*, 2003; Hammes *et al.*, 2005; Szakasits *et al.*, 2009).

Only late second-stage juveniles and mature male nematodes were recovered from soil samples. The presence of the juveniles in the soil is a clear indication that this nematode does not have to enter the root of its host to complete its life cycle, a detail also observed by Cid del Prado and Subbotin (2014). Observations made in the present study confirmed that all the stages of this nematode develop outside the root of its host. Upon hatching, the second-stage juveniles remain in the soil until they find a suitable root and then penetrate an area close to the root tip. It inserts the anterior part of the body into the young root until it reaches the undifferentiated parenchyma cells of the primary phloem tissue. As to why it does not fully penetrate the roots, we can hypothesize that the roots the nematode prefers to infect are thin and their bodies are too large to fully enter the roots that they parasitize (Fig. 7). Once the nematode has partially penetrated the plant roots, it begins to feed, and in the process, modifies host cells. Three moults occur, ending in the adult stage. The time taken for *C. torreyanae* to complete its life cycle is 25 d after planting or 447 degree days at 20°C, which is less than the 30 to 35 d observed by Koenning and Sipes (1998) for other *Cactodera* species. In a more recent study of *Cactodera galinsogae* by Tovar-Soto *et al.* (2008), the life cycle required 56 d.

The gelatinous matrix produced near the vulva by the young females, unlike in other nematodes of the Heteroderidae, does not contain eggs. *Cactodera torreyanae* retains all the eggs within the female body. We noted, however, that males may become 'trapped' in this gelatinous mass. We hypothesize that this substance may contain pheromones that attract mature males to females. The purpose and chemical composition of the yellow secretions near the head of the female nematodes remains unknown.

Cactodera torreyanae has a sessile ectoparasitic feeding habit. In contrast to most members of Heteroderidae that are sessile endoparasites, postembryonic development occurs outside the root, and the *C. torreyanae* second-stage juveniles penetrate roots only with the anterior portion of the body with the posterior part of the body protruding from the root surface.

Juveniles (third and fourth stages) develop into swollen adult females, which also are attached to the roots by the anterior portion of their bodies. Gelatinous material protrudes from the posterior end of the young female body but no eggs were observed in it, which is in contrast to other members of the Heteroderidae, such as *Heterodera schachtii*, where females commonly deposit eggs in the gelatine (Raski, 1950). It is unknown if other hosts for *C. torreyanae* exist that may be agriculturally important, but an extensive evaluation of potential hosts for this nematode would be of value.

ACKNOWLEDGMENTS

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