Susceptibility of *Tetranychus cinnabarinus* and *Tetranychus urticae* (Trombidiformes: Tetranychidae) to *Neozygites floridana* (Entomophthorales: Neozygitaceae)[1]

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Abstract *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae) is a cosmopolitan spider mite species that infests more than 900 species of plants, including commercial species. *Tetranychus cinnabarinus* (Boudreaux) is characterized by a red coloration, in contrast to the green-colored *T. urticae*. Both are occasionally found coexisting in warm environments, and, for that reason, they are considered by some to be synonyms. In this study, we identified specimens of the two morphospecies using optical and electron microscopy and amplification of a segment of the mitochondrial cytochrome oxidase I (COI) gene. Bioassays determined the level of susceptibility and survival of both morphospecies when they were infected with the entomopathogenic fungus *Neozygites floridana* (Weiser and Muma). For the evaluation of the susceptibility of these morphospecies to *N. floridana*, bioassays were conducted under conditions of 25 ± 2°C, 60 ± 5% relative humidity, and a 12:12-h photoperiod regime. From these bioassays, we found that *T. cinnabarinus* was less susceptible to *N. floridana* and that there is a higher level of fertility, survival, and development in the *T. cinnabarinus* than in the *T. urticae* mites. Within a mass rearing system, *T. cinnabarinus* appears be a more efficaceous functional food for predatory mites, such as *Phytoseiulus persimilis* (Athias-Henriot) (Mesostigmata: Phytoseiidae), than *T. urticae* to support rearing and release of biological agents for integrated pest management.

Key Words biological control, Tetranychidae, entomopathogen, mortality, bioassay

Pesticide misuse has caused an ecological imbalance between predator populations and their prey in agricultural crops (Badii and Abreu 2006). As a result, some species of mites have become highly destructive pests in agriculture and thus increased their pest status and importance (Badii et al. 2010). Mites of the Tetranychidae family feed on a wide variety of vascular plants throughout the world (Sun et al. 2012). These mites represent a risk of introduction and establishment as

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pests for any country that imports plant material. Many species of this family are polyphagous and have high development and fertility rates (Arguelles 2006, Sun et al. 2012).

Tetranychus cinnabarinus (Boisduval) and *T. urticae* (Koch) (Trobidiformes: Tetranychidae) are spider mits pests with a wide range of plant hosts (Peralta and Tello 2011, Xien et al. 2008). The morphologic and genetic distinction between the two species has been the subject of many studies and discussions about whether they are distinct species. Both forms have a worldwide distribution, although *T. urticae* is found predominantly in temperate climates, whereas *T. cinnabarinus* lives mainly in warm and tropical climates. They can infest crops in greenhouses and in natural environments (Sun et al. 2012). Some characteristics used by researchers to differentiate between *T. cinnabarinus* and *T. urticae* are the shape of the dorsal integumentary lobes, the shape of the aedeagus, chaetotaxy, the ability to enter diapause, adaptation to host plants, various physiologic traits, and population genetics; however, Auger et al. (2013) failed to confirm the taxonomic position of *T. cinnabarinus* as a different species from *T. urticae*. Bolland et al. (1998) do not recognize *T. cinnabarinus* as a different species from *T. urticae*. Similarly, investigations with molecular tools have not found genetically significant distinctions between the two tetranychids (Mendoza et al. 2011). Therefore, *T. cinnabarinus* is considered a phenotype of *T. urticae*, and both forms can coexist in the same crop, causing significant economic losses (Sun et al. 2012).

To combat tetranychid mite infestations in Mexico, a wide variety of acaricides with different chemical structures are used (Márquez-Chávez et al. 2019), including neurotoxins, insecticides such as organophosphates, carbamates, pyrethroids, and specific acaricides such as transport inhibitors, avermectins, and tetronic acids (Van Leeuwen et al. 2010). In mites of the Tetranychidae family, there is a rapid selection of resistance genes towards acaricides and insecticides, which is based on their arrhenotokous parthenogenesis, high fecundity, short life cycle, and inbreeding (Van Leeuwen et al. 2010). The abuse of abamectin to combat tetranychids has caused numerous cases of resistance around the world (Stumpf and Nauen 2001).

Biological control is one of the techniques used in the integrated pest management (IPM), where the pest-natural enemy interaction is essential for the knowledge of the other interactions that occur within a tritrophic system where the plant, the pest, and the enemy intervene in the search to optimize control tactics (Rodríguez et al. 2013). The deployment and use of *Phytoseiulus persimilis* (Athias-Henriot) (Phytoseiidae) to manage tetranychid mites is an excellent example of this tritrophic system (Daza et al. 2010). In the commercial-scale mass rearing system of phytoseiids, the predatory mites are fed with tetranychid mites, which, in turn, are fed with plants, mainly *Phaseolus vulgaris* (L.), thus creating a tritrophic system requiring synchrony among the different components of the process (Morales et al. 2014, Rodríguez et al. 2013). However, achieving this synchrony is not easy in that it also requires an economically and technically viable mass reproduction system (Rodríguez et al. 2013). The use of predatory phytoseiids in the agricultural field is limited by the scarce availability of them in the market. Despite continuous improvements in mass rearing methods, two important problems are the high demand of tetranychids within the rearing system and their vulnerability to

entomopathogens such as *Neozygites floridana* (Weiser and Muma) (Entomophthorales: Neozygitaceae) (Rodríguez et al. 2013).

The entomopathogenic fungus *N. floridana* is a natural enemy of species of the genus *Tetranychu*s and is the main factor causing natural decline of populations of these mites (Klingen et al. 2009). This fungal species can form resistant spores that survive under adverse conditions. These resistant spores have been found in the soil, throughout all the seasons of the year (Da Silveira et al. 2013).

Delalibera et al. (2004) reported *N. floridana* as an obligate pathogenic fungus of mites. The first observation of this species was in 1966 by Weiser and Muma in Lake Alfred, Florida, USA, where it was found infecting *Eutetranychus banksi* (McGregor). Since then, this fungus has been reported infecting at least 18 species of mites, all belonging to the Tetranychidae family (Ribeiro et al. 2009). Roggia et al. (2009) indicate that even with this list, the host range of *N. floridana* is not yet fully defined. Epizootics of *N. floridana* in tetranychid populations routinely occur in rainy seasons resulting in high percentages of mortality in active populations of *Tetranychus* (Barta and Cagan 2006).

Neozygites floridana infections have completely eliminated *Tetranychus* populations in an average of 3.38 d at 25°C and in 11.02 d at 15°C (Castro et al. 2013, Smitley et al. 1986). It is a specific entomopathogen of tetranychid species, so phytoseiids are not directly affected by it; however, the decrease in the tetranychid population is a key factor in phytoseiid production (Ribeiro et al. 2009). The difficulty of maintaining a continuous production of *P. persimilis* encourages producers to prefer to control pests with chemical products; then, to provide continuity to biological control with natural enemies, it is necessary to have the immediate availability of beneficial organisms (Daza et al. 2010, De la Peña et al. 2011).

In preliminary observations, the senior author (V.L.G.-P.) found that the red form, identified as *T. cinnabarinus*, appeared to be less susceptible to infection by *N. floridana* than the green form, identified as *T. urticae*. The study reported here was conducted to determine whether there was indeed a difference in the susceptibility of the red and green forms to *N. floridana*. Our ultimate objective is selecting a population that could be offered as a diet in the mass rearing of *P. persimilis* with reduced impact on the colony by the pathogenic fungus.

Materials and Methods

Collection and identification of biological material. The mites were collected on raspberry plants (*Rubus idaeus* L.) grown in organic and conventional macrotunnels in the municipality of San Isidro Mazatepec, in Tala, Jalisco, Mexico, during February, March, and April 2020. The plant samples infested with the mites were transported to the laboratory in an ice chest with cooling gels wrapped in newspaper to avoid excess humidity. Likewise, cadavers of tetranychid mites showing signs of infection by a pathogenic fungus, preliminarily identified as *Neozygites* sp., were collected in greenhouses of the Biobest México, S.A de C.V. company located in the same town, during the months of January and February 2020. To observe the characteristic structures of *Neozygites,* described in Westrum et al. (2014), samples were mounted on temporary slides with lactophenol. For the morphologic

identification of the mites of both morphospecies, between 80 and 100 specimens of the mites were fixed in 70% ethyl alcohol (molecular biology grade). Approximately 60 adult specimens of the two morphospecies, including males, were mounted on slides with Hoyer's mounting medium following the method of Krantz and Walter (2009). Additionally, 20 specimens were processed for observation with electron microscopy according to the procedure developed by Bozzola and Russel (1999). Finally, 40 specimens were kept in molecular biology grade alcohol to be processed for DNA extraction and polymerase chain reaction (PCR).

The specimens mounted on slides were observed with a Carl Zeiss Primostar phase contrast microscope (Oberkochen, Germany), whereas the specimens mounted on brass specimen stubbs were observed with a Jeol JSM 6390 electron microscope (Jeol USA, Inc., Peabody, MA). The keys of Bolland et al. (1998) and the redescriptions of *T. cinnabarinus* and *T. urticae* by Tuttle et al. (1976) were used to identify the mites.

Molecular identification. To extract total DNA from the mites, the DNeasy Blood & Tissue Kit (QIAGEN, Germantown, MD) was used following the manufacturer's instructions. The PCR test ran with the COIF primers: 5′-AAGAGGAGGAGGAGACCCAA-3′ and XR: 5′-AAACCTCTAAAAATAGCGAATA-CAGC-3′, which amplify a fragment of the COI subunit I gene (Folmer et al. 1994, Márquez-Chávez et al. 2019). The PCR reaction volume was 30 μl with 3 μl 10× buffer, 2 mM dNTPs, each primer 20 pM, TaqDNA 0.5 U, and 5 μl DNA template. The thermal program was run in a Thermo Scientific Type 5020 thermal cycler (model ITCA0096) with one cycle of 94°C for 3 min, 35 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final extension cycle at 72°C for 10 min. The PCR products were visualized on a 1.5% agarose gel with ethidium bromide (10 mg/ml) in 1× TAE buffer (Tris-acetate at pH 8.3 + ethylenediaminetetraacetic acid [EDTA]) and were sent to Macrogen Inc. (Seoul, South Korea) for Sanger sequencing. The sequences obtained were analyzed with the MEGA program (version 10.0.5) to obtain the consensus sequences. These were aligned (BLAST) with sequences deposited in GenBank for the identification of species by similarity in the respective nucleotide sequences.

The group separation of the morphospecies was conducted with the maximum likelihood method and the Tamura-Nei model using a bootstrap of 1,000 repetitions. The tree with the highest logarithmic probability (−1,135.54) was obtained. The initial trees were obtained by applying the BioNJ method to a matrix of distances by pairs estimated using the Tamura-Nei model. A discrete gamma distribution was applied to model the evolution rate differences between the sites [five categories (+ G, parameter = 0.2106)].

For the specific identification of the fungus, groups of approximately 10 cadavers of *Tetranychus* sp. mites infected by a fungus, tentatively identified as *Neozygites* sp., were assayed. The mites were macerated in 350 μl lysis buffer (2% triton, 1% SDS, 100 mM NaCl, 10 mM Tris-Cl, pH 8, 1 mM EDTA) and 500 μl of phenol-chloroform-isoamyl alcohol (25:24:1), vortexed for 30 min, and centrifuged to separate the aqueous from the oily phase (1,200 rpm, 4°C for 5 min). Then, 500 μl of the aqueous phase of each sample was collected and transferred to a new tube, and 50 μl sodium acetate, pH 5.2, and 1 ml absolute ethanol were added at −20°C, incubated on ice for 30 min and centrifuged (1,200 rpm, 4°C, for 5 min), and the

supernatant was discarded by decantation. One milliliter of 70% ethanol was added to each tube at −20°C, and the tubes were shaken by rotation, incubated on ice for 15 min, and then centrifuged for 15 min (12,000 rpm, 4°C). The supernatant was discarded by decantation and allowed to air dry, and the DNA was resuspended in 100 μl TE buffer (0.1 mM EDTA).

PCR of the total DNA extract was run using the Dynazyme EXT DNA Polymerase kit (Thermo Fisher Scientific, Madrid, Spain) and the 5′-CTGGTTGATTCTGCCAGT-3′-5′-AATGATCCTTCCGCAGGT-3′ primers, which amplify a ribosomal DNA segment, with the following mixture reaction mixture: 10 μl Dynazyme PCR master mix (2×), 0.5 μl sense primer, 0.5 μl antisense primer, 4 μl ultrapure water, and 5 μl DNA template to a final volume of 25 μl. The PCR program was 94°C for 3 min, 35 cycles of 94°C for 30 s, 52°C for 45 s, and 72°C for 60 s, with a final extension cycle at 72°C for 5 min, plus 4°C indefinitely. The PCR products were visualized on a 1.5% agarose gel with ethidium bromide (10 mg/ml) in 1× TAE buffer. The results of the PCR were sent to Macrogen Inc. for Sanger sequencing. The sequences were processed for purification using the BioEdit (v.7.2.5; 1997–2013) program and aligned using the Mega X v.10.1.8 (1995–2018) program with sequences deposited in GenBank.

Once the mites were identified, colonies of each morphospecies were founded, maintained on *P. vulgaris* var. "Peruvian" or "Normal." Cadavers were stored at 5°C in Eppendorf tubes in which small silica capsules were placed before sealing with Parafilm and placed in plastic containers with more silica capsules.

Development, mortality, and fertility of mite morphospecies. Experimental arenas were constructed with acrylic pots (2.5 cm diameter × 3 cm high), the lids of which were perforated in the shape of a square (approximately 0.5 × 0.5 cm). The opening was covered with screen mesh to prevent the escape of the mites. Each pot contained cotton previously moistened with 10–12 drops of water. A 2.5-cm-diameter leaf disk of *P. vulgaris* was placed on the cotton with the abaxial side facing upward (just the diameter of the bottle to prevent the mites from falling onto the cotton). These were maintained at 25 ± 2°C and 60 ± 5% relative humidity (RH) on a 12:12-h photoperiod regime in an environmentally controlled room. These conditions were the same for all the experimental arenas.

To evaluate the development and mortality of the morphospecies, a female in the quiescent deutonymph stage (i.e., nearing adulthood) and two adult males were placed in each experimental arena to ensure mating once the female reached adulthood. After 24 h, the adults were removed, leaving only the eggs, which were counted and monitored every 24 h to document their passage through the stages of development, as well as mortality over time. The monitoring continued until the death of the mites that emerged from the eggs. The leaf discs, food for the mites, were replaced every 2–3 d, as well as the cotton to prevent contamination.

As in the previous test, 60 experimental arenas per morphospecies were used to monitor the development and mortality of *T. urticae* and *T. cinnabarinus* specimens exposed to infection by *N. floridana*. The mites were handled as previously described, except that a mite cadaver sporulating with *N. floridana* was placed in close proximity to healthy mites. That cadaver had previously been placed for 3 h in a humid chamber at 25 ± 2°C, 95 ± 5% RH, and 12:12-h photoperiod to promote its sporulation (Da Silveira 2013). After 24 h in the arena, the female, the males, and the cadaver were removed, and the eggs present were monitored until they reached

the adult stage and death. All the dead mites were mounted on slides with lactophenol to observe if they were infected by *N. floridana*, which was interpreted as the cause of death. If the cadavers did not have spores of the fungus, death was considered from a natural or unknown cause.

The stages analyzed for each morphospecies were egg incubation time, larva-adult stage duration, and adult longevity. In the case of mites exposed to infection by *N. floridana*, the longevity of adults was compared between those that were infected by this fungus, which was determined by the presence of spores attached to their body, and those that died by other causes, not determined.

To compare the fecundity of females between morphospecies, 60 experimental arenas were prepared for each morphospecies. The arenas were the same as those used in the previously described experiments. Incubation conditions were 25 $\pm$ 2°C and 60 $\pm$ 5% RH on a photoperiod of 12:12 h. In this experiment, quiescent females in the deutonymph stage were used (to approximate the females to a known age, i.e., 1 d old) with two males per female to ensure mating. Throughout the life of each female, the eggs that she laid every 24 h were counted and then these were removed with a fine brush. The leaf that served as food for the females and males was replaced every third day.

To estimate the fecundity of the morphospecies in the presence of *N. floridana*, the procedure was the same as described in the previous paragraph with 60 experimental arenas per morphospecies. On this occasion, a cadaver infected by *N. floridana* was placed next to the female and the males (the corpse had previously been moistened in a humid chamber to ensure sporulation). The cadaver was removed after 24 h, and the eggs that the female laid were counted until she died.

Analysis of data. To compare the development time between *T. cinnabarinus* and *T. urticae*, with and without exposure to infection by *N. floridana*, an analysis of variance was performed, with which the significance of both factors and their interaction were estimated. The data were grouped by stage of the two morphospecies, in the presence and absence of fungus, and an analysis of variance was conducted for a 2×2 factorial experiment once the assumptions of the model were corroborated. The response variable was the time elapsed by each stage of development. To evaluate the differences between groups, the Tukey test ($\alpha = 0.05$) was used. In the same way, the longevity of the mother females used in each experimental arena was analyzed.

Mite mortality curves were constructed and compared between morphospecies with and without exposure to *N. floridana*, using the nonparametric log-rank test. The percentage of mites killed by *N. floridana* was calculated using Abbot's (1925) formula.

To evaluate the fecundity of the morphospecies, both in the presence and in the absence of *N. floridana*, an analysis of variance was conducted per day, and the assumptions of the model were properly validated. The statistical analysis was conducted using the programming language R (v 4.0.5).

Results and Discussion

Identity of the mite morphospecies. According to Tuttle et al. (1976), one of the main morphologic differences to distinguish *T. cinnabarinus* from *T. urticae* is

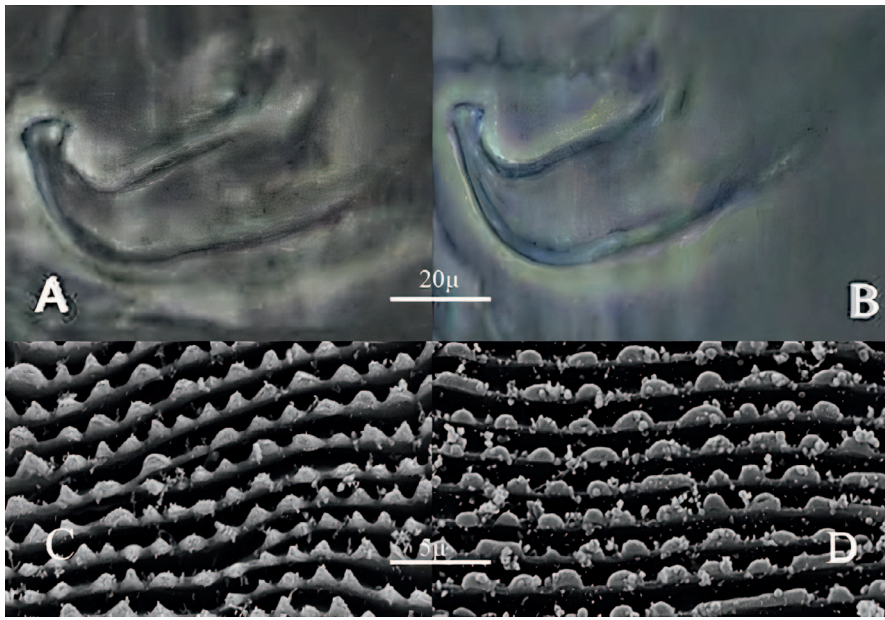

Fig. 1. Phase contrast micrographs of the aedeagus of the male *T. cinnabarinus* (A) and male *T. urticae* (B) and electron micrographs of the dorsal lobes of the female *T. cinnabarinus* (C) and female *T. urticae* (D).

the shape of the aedeagus, where in *T. cinnabarinus* the head of the aedeagus is slightly rounded, whereas in *T. urticae*, it is slightly more pointed (Fig. 1A, B). Yet, we found no evident and consistent differences in the shape of the aedeagus of these morphospecies (Fig. 1A, B), which may be attributed to the high degree of polymorphism that exists with these two forms (Auger et al. 2013).

The shape of the lobes or microtubercles of the striae on the back of females also has reportedly been used as a distinguishing feature separating *T. cinnabarinus* and *T. urticae* (Tuttle et al. 1976). On *T. cinnabarinus*, the lobes are taller than wide and have a pointed shape (Fig. 1A), whereas on *T. urticae* the lobes are rounded (Fig. 1B). We also observed that this characteristic clearly differed between the two morphospecies, coinciding with the redescriptions of Tuttle et al. (1976). On this basis, the red morphospecies was confirmed as *T. cinnabarinus*, whereas the green morphospecies was confirmed as *T. urticae*, although we do not define them as separate species. Furthermore, Auger et al. (2013) thoroughly reviewed the morphologic characteristics used to separate *T. cinnabarinus* from *T. urticae* and concluded that the lobe and aedeagus morphology should not be used for identification and separation of the two forms due to the variation in the sizes of the lobes found on the same morphospecies.

Although the morphologic features examined did not reliably separate the morphospecies, the two were grouped as two distinct species based on genetic differences found in the DNA regions encoded by the COI molecular marker. The

two morphospecies exhibited high percentages of similarity with nucleotide sequences deposited in GenBank and identified as *T. urticae*. We found three haplotypes of the *T. urticae* morphospecies and six haplotypes of *T. cinnabarinus*. These results agree with the genetic differences found by Li et al. (2009) who, using microsatellites as molecular markers, found molecular differences between *T. cinnabarinus* and *T. urticae*. However, Auger et al. (2013) did not support the monophyletic distinction of the two morphospecies.

The phylogenetic tree, with the lengths of the branches measured in number of substitutions per site, was constructed from results obtained from the alignment sequence with bootstrap values greater than 50% based on 1,000 replicates (Fig. 2). This analysis involved 15 nucleotide sequences, with a total of 926 positions. The tree clearly shows genetic separation of the two forms (Fig. 2). To make comparisons by groups, the COI sequences AB736076 (*T. urticae*, green form) and AB736079 (*T. cinnabarinus*, red form) used by Matsuda et al. (2014) were included. The sequence of the green morphospecies, designated here as *T. urticae*, was grouped with the sequence cited above, AB736076, of green mites, whereas the sequence of the red morphospecies, identified here as *T. cinnabarinus*, was grouped with the sequence of the species cited as red, AB736079.

Identity of the fungus. Keller (1997) listed the primary structures for the identification of *N. floridana* as rounded hyphal bodies (or rounded at some end), unbranched conidiophores, oblong or spherical primary conidia with three to eight nuclei, capillary tube capilliconidia, and zygospores ellipsoid or spherical, binucleate, and dark brown in color reaching black. Azygospores are rarely found, and cystidia and rhizoids are absent. These characteristics coincide with those listed by Robert et al. (2005) in the description of *N. floridana*. Based on these morphological criteria, we identified our collected material as *N. floridana*.

We detected only one haplotype of *N. floridana* in our molecular analyses, reflecting the single collection of the fungus. This single *Neozygites* haplotype had 99.84% similarity with the sequence identified as extracted from *Neozygites* sp. isolate ARSEF 662 (access code gi | 22535856 | AF296759.1), as well as 99.84% similarity with the sequence identified as extracted from *N. floridana* from isolate ARSEF 5376 (AY233984.1). Therefore, the identification of the fungus we collected and analyzed was corroborated as *N. floridana*.

Response of morphospecies to infection *N. floridana*. In terms of mortality, a greater percentage of *T. urticae* (81%) died from infection by *N. floridana* than *T. cinnabarinus* (55.34%). An explanation for these different levels of mortality between two closely related mite morphospecies may be carotenoid concentration in the tetranychid mites. Altincicek et al. (2012) found that *T. cinnabarinus* (red form) had a significantly higher concentration of carotenoids than *T. urticae* (green form). Perhaps the cartenoids interact with the physiology or immune activity of the mite to cause the observed differences in mortality. Similarly, changes in the genome of *T. cinnabarinus* and *T. urticae* could lead to a differentiation process.

In comparing the two uninfected morphospecies, we found no significant differences in the duration of the egg stage ($F = 0.0407$; df = 1, 224; $P = 0.8403$); however, the duration of the *T. urticae* larva-adult stage was significantly longer than that of *T. cinnabarinus* ($F = 3.2252$; df = 1, 224; $P = 0.0738$), but adult longevity was significantly shorter ($F = 52.186$; df = 1, 224; $P < 0.0001$) (Table 1). For both morphospecies, infection with *N. floridana* significantly reduced the duration of the

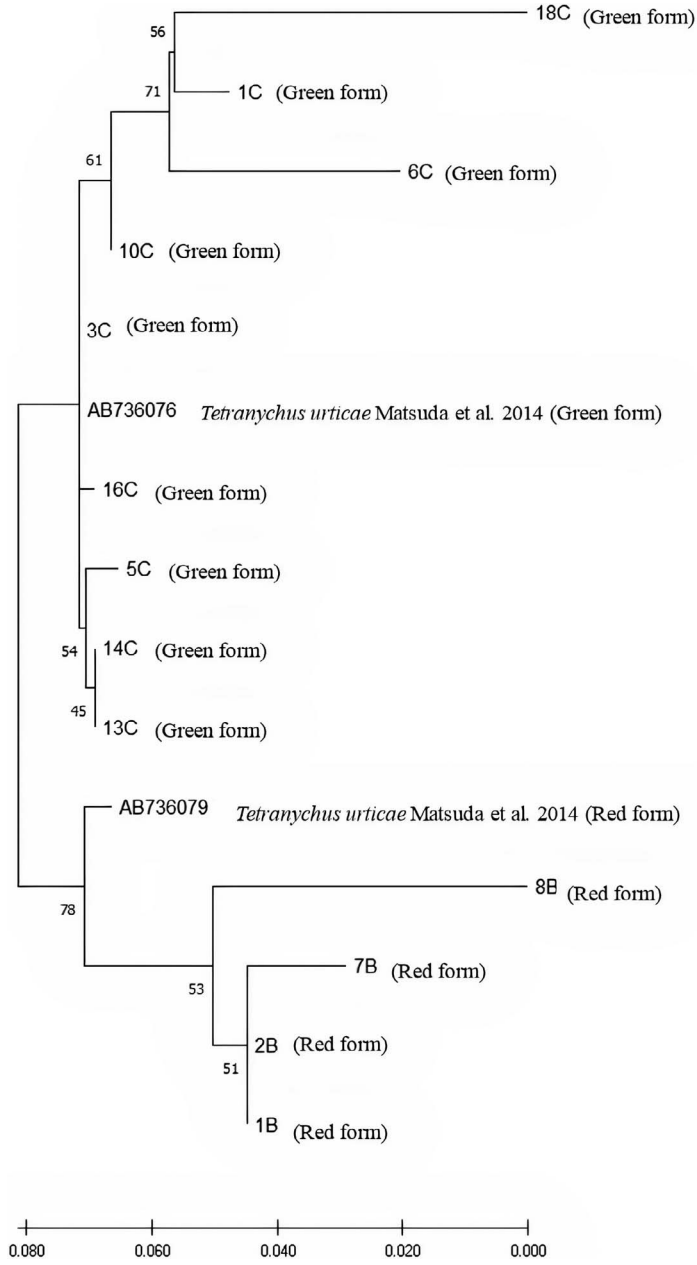

Fig. 2. Phylogenetic tree of mite segregation, with the maximum likelihood method, *T. cinnabarinus* (red form) and *T. urticae* (green form), based on the COI mitochondrial gene, using the Tamura-Nei model with a Gamma distribution. Bootstrap values based on 1,000 replicates.

Table 1. Mean ± SE duration (days) of developmental stages of *T. urticae* and *T. cinnabarinus* infected and not infected with *N. floridana*.*

Treatment	Egg	Larva-Adult	Adult Longevity
T. urticae, not infected	4.56 ± 0.196a	6.80 ± 0.252a	17.04 ± 1.413b
T. urticae, infected	3.69 ± 0.067b	4.01 ± 0.083c	0.81 ± 0.234d
T. cinnabarinus, not infected	4.49 ± 0.093a	5.42 ± 0.137b	20.77 ± 0.433a
T. cinnabarinus, infected	3.82 ± 0.165b	4.57 ± 0.349c	2.26 ± 0.318c

* Means within columns followed by the same lowercase letter are not statistically different (Tukey, $\alpha = 0.05$).

egg stage ($F = 29.88$; df = 1, 224; $P < 0.0001$), and the larva-adult stage ($F = 62.7271$; df = 1, 224; $P < 0.0001$). There are no previous data on this reaction to *N. floridana* infection; it appears to be a SOS reaction that speeds the development of egg and larva-adult stage ($F = 2346.102$; df = 1, 224; $P < 0.0001$; Table 1). In comparing the duration of these life stages in the two morphospecies infected with *N. floridana*, we found no significant differences in the duration of the egg or larva-adult stages, but adult longevity of *T. cinnabarinus* was longer than that of *T. urticae* ($F = 10.052$; df = 1, 224; $P < 0.001$).

The results of the survival curves showed significant differences between the morphospecies in the presence versus absence of *N. floridana*. The comparison of the survival curves between morphospecies without the presence of *N. floridana* is expressed by the equation Tc − Tu ($\chi^2_1 = 38.70$, $P = 0.0002$). On the other hand, the curves analyzed where one morphospecies was exposed to the entomopathogen and another morphospecies was not, are expressed by the equations Tc − TcN ($\chi^2_1 = 86.82$, $P = 0.0002$), Tu − TuN ($\chi^2_1 = 68.95$, $P = 0.0008$), Tc − TuN ($\chi^2_1 = 85.70$, $P = 0.0002$), and Tu − TcN ($\chi^2_1 = 871.06$, $P = 0.0008$). Finally, the mortality curves of both morphospecies exposed to *N. floridana* were compared and are expressed as TcN − TuN ($\chi^2_1 = 18.36$, $P = 0.006$; Fig. 3). The highest percentage values of individual survival were observed in *T. cinnabarinus* when the treatments with or without exposure to *N. floridana* were compared.

The average fecundity of the females of both morphospecies, infected or not infected with *N. floridana*, showed significant differences between the mean number of eggs laid per day. In general, the average fecundity of females infected and not infected with *N. floridana* was higher for the red form (*T. cinnabarinus*) than the green form (*T. urticae*) (Fig. 4). We postulate that the infection of the adults negatively impacted the average fecundity, likely through impacts on normal physiologic functions. Similarly, the significant reduction in developmental time since egg stage, larva to adulthood and in the longevity of adults infected with *N. floridana* is likely also attributed to the negative impacts of the ongoing fungal infection and the eventual mortality caused by the infection.

The developmental times, sex ratio (*T. cinnabarinus*, 3:1; *T. urticae*, 4:1), and levels of fecundity we observed agree with those reported by Badii et al. (2010) and Peralta and Tello (2011). None of those results showed marked differences in development and sex ratios between the two morphospecies; yet, we observed significant differences in adult longevity of *T. cinnabarinus* and *T. urticae,* where *T.*

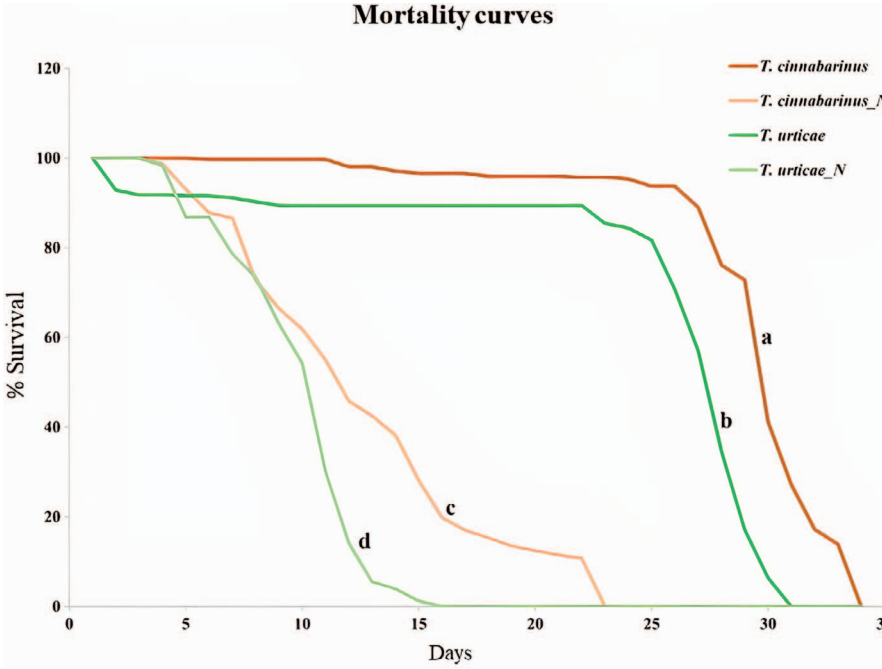

Fig. 3. Mortality curve from egg to death of *T. urticae* and *T. cinnabarinus*, infected and not infected with *N. floridana*.

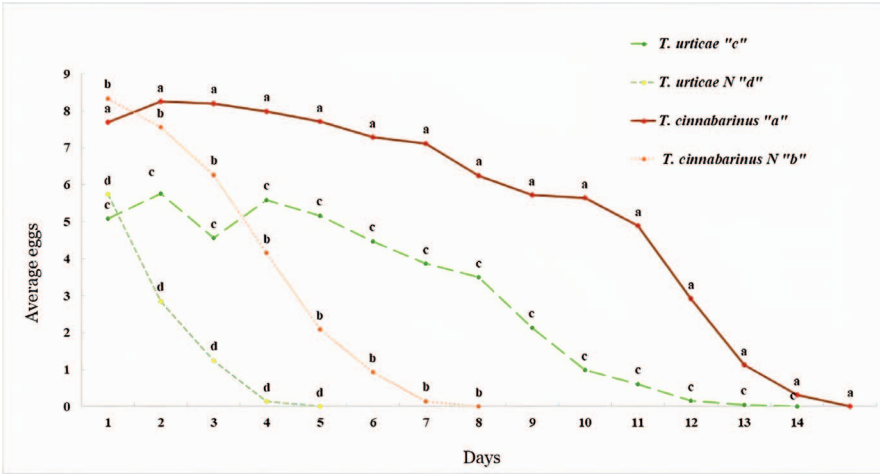

Fig. 4. Average fecundity per day between treatments (letters correspond to comparisons between days).

cinnabarinus females lived longer (20.77 d) than did *T. urticae* females (17.04 d; Table 1). We further demonstrated these differences in our derived mortality curves derived (see below; Fig. 3). We also found differences in the sex ratios of the offspring produced by females infected with *N. floridana* (*T. cinnabarinus*, 8: 1; *T. urticae*, 3: 1), which may at least partially explain the high female fecundity rate of *T. cinnabarinus*.

Mortality curves show significant differences (log rank, $P = 0.05$) between mites infected with *N. floridana* and uninfected mites (Fig. 3). The frequency of infection by *N. floridana* observed in the two morphospecies indicates that *T. urticae* is more susceptible to death due to infection by *N. floridana*.

From the factorial variance analysis, the morphospecies did not show significant difference in the development of the eggs; however, infected mites differ in terms of egg and larva-adult development and adult longevity; it is remarkable that longevity of *T. urticae* females is shorter than that of *T. cinnabarinus*. Trandem et al. (2015) hypothesized that males are attracted to females infected with *N. floridana,* thus dispersing the spores to healthy females in mating activities and increasing the potential for infection of other adult females. According to these results, *T. cinnabarinus* has a greater reproductive potential than *T. urticae* due to its longer longevity and and higher fecundity rate when infected with *N. floridana*. This translates into a greater number of offspring, greater adaptability, and greater persistence in crops.

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References Cited

Abbot, W. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Altincicek, B., J.L. Kovacs and K.N. Gerardo. 2012. Horizontally transferred fungal carotenoid genes in the two-spotted spider mite *Tetranychus urticae*. Biol. Lett. 8: 253–257.

Arguelles, A., N. Plazas, L. Táutiva, F. Cantor, A. Bustos and D. Rodríguez. 2006. Evaluación de un método a campo abierto para la producción de *Tetranychus urticae*: Koch (Acari: Tetranychidae), como suministro de presas para *Phytoseiulus persimilis*–Athias. Rev. Fac. Ciencias Básicas 1: 117–122.

Auger, P., A. Migeon, E. Ueckermann, L. Tiedt and M. Navajas. 2013. Evidence for synonymy between *Tetranychus urticae* and *Tetranychus cinnabarinus* (Acari, Prostigmata, Tetranychidae): Review and new data. Acarol. 53: 383–415.

Badii, M. and J. Abreu. 2006. Control biológico una forma sustentable de control de plagas. Daena Intern. J. Good Consc. 1: 82–89.

Badii, M., J. Flores and E. Cerna. 2010. Regulación poblacional de ácaros plaga de impacto agrícola. Intern. J. Good Consc. 5: 270–302.

Barta, M. and L. Cagán. 2006. Aphid-pathogenic Entomophthorales (their taxonomy, biology and ecology). Biología 61: 543–616.

Bolland, H., J. Gutiérrez and C. Fletchmann. 1998. World catalogue of the spider mite family (Acari: Tetranychidae). Brill Academic Publ., Leiden, The Netherlands.

Bozzola, J. and L. Russell. 1999. Electron microscopy: Principles and techniques for biologist. Jones and Bartlett, Boston, MA.

Castro, T., W. Wekesa, A. Moral, G. Demetrio, I. Delalibera and I. Klingen. 2013. The effects of photoperiod and light intensity on the sporulation of Brazilian and Norwegian isolates of *Neozygites floridana*. J. Invertebr. Pathol. 114: 230–233.

Da Silveira Duarte, V., K. Westrum, A. Lopes Ribeiro, M. Guedes, I. Klingen and I. Delalibera. 2013. Abiotic and biotic factors affecting resting spore formation in the mite pathogen *Neozygites floridana*. Intern. J. Microbiol. 2013:276168.

Daza, V., R. Cantor, C. Rodríguez, A. Bustos and J. Cure. 2010. Criterios para la producción de *Phytoseiulus persimilis* (Parasitiformes: Phytoseiidae) bajo condiciones de invernadero. Acta Biol. Colombiana 15: 37–46.

De La Peña, A., P. Niño, A. Bustos, F. Cantor and D. Rodríguez. 2011. Efecto de diferentes tiempos de almacenamiento en frío sobre algunos parametros de *Phytoseiulus persimilis* (Athias Henriot) Y *Neoseiulus californicus* (Parasitiformes: Phytoseiidae). Acta Biol. Colombiana 16: 63–74.

Delalibera, I., A. Hajek and R.A. Humber. 2004. *Neozygites tanajoae* sp. a pathogen of the cassava green mite. Mycologia 96: 1002–1009.

Keller, S. 1997. The genus *Neozygite*s (Zygomycetes, Entomophthorales) with special reference to species found in tropical regions. Sydowia 49: 118–146.

Klingen, I., G. Waersted and K. Westrum. 2009. Overwintering and prevalence of *Neozygites floridana* (Zygomycetes: Neozygitaceae) in hibernating females of *Tetranychus urticae* (Acari: Tetranychidae) under cold climatic conditions in strawberries. Exp. Appl. Acarol. 46: 231–245.

Krantz, G. and D. Walter. 2009. A manual of acarology. Texas Tech Univ., Lubbock, TX.

Li, T., X. Chen and Y. Hong. 2009. Population genetic structure of *Tetranychus urticae* and its sibling species *Tetranychus cinnabaribus* (Acari: Tetranychidae) in China as inferred from microsatellite data. Ann. Entomol. Soc. Am. 102: 674–683.

Márquez-Chávez, A., A. Guzmán-Franco, M. Santillán-Galicia, F. Tamayo-Mejía and J. Rodríguez-Maciel. 2019. Effect of host plant on the genetic diversity of *Tetranychus urticae* Koch populations and their susceptibility to fungal infection, Biol. Contr. 137: 104015.

Matsuda, T., M. Morishita, N. Hinomoto and T. Gotoh. 2014. Phylogenetic analysis of the spider mite sub-family Tetranychinae (Acari: Tetranychidae) based on the mitochondrial COI gene and the 18s and the 59 end of the 28s rna genes indicates that several genera are polyphyletic. PLoS One 9: e108672.

Mendoza, R., D. Navia, I. Diniz, P. Auger and M. Navajas. 2011. A critical review on some closely related species of *Tetranychus* sensu stricto (Acari: Tetranychidae) in the public DNA sequences databases. Exp. Appl. Acarol. 55: 1–23.

Morales, J. and M. Rojas. 2014. A modular cage system desing for continuous medium to large scale in vivo rearing predatory mites (Acari; Phytoseiidae). Psyche 2014: 1–8.

Peralta, O. and V. Tello. 2011. Tabla de vida de *Tetranychus cinnabarinus* (Acari: Tetranychidae) sobre tres variedades de melón, *Cucumis melo*. Rev. Colombiana Entomol. 37: 21–26.

Ribeiro, A., M. Guedes, E. Calderan and I. Delalibera. 2009. Host range of *Neozygites floridana* isolates (Zygomycetes: Entomophthorales) to spider mites. J. Invertebr. Pathol. 102: 196–202.

Robert, V., G. Stegehuis and J. Stalpers. 2005. The MycoBank engine and related databases. 10 May 2021. (https://www.mycobank.org/).

Rodríguez, H., A. Montoya, Y. Pérez-Madruga and Y. Ramos Mayra. 2013. Reproducción masiva de ácaros depredadores Phytoseiidae: retos y perspectivas para Cuba. Rev. Prot. Vegetal. 28: 12–22.

Roggia, S., J. Vanderei, R. Roppa, G. Nascimiento, D. Navia and I. Delalibera. 2009. Predatory mites and the fungus *Neozygites floridana* associated with the spider mites on soybean, in Rio Grande du Sul, Brazil. Pesquisa Agropecuária Brasileira 44: 107–110.

Smitley, D., G. Kennedy and W. Brooks. 1986. Role of the entomogenous fungus, *Neozygites floridana*, in population declines of the twospotted spider mite, *Tetranychus urticae*, on field corn. Entomol. Exp. Appl. 41: 255–264.

Stumpf, N. and R. Nauen. 2001. Cross-resistance, inheritance, and biochemistry of mitochondrial electron transport inhibitor-acaricide resistance in *Tetranychus urticae* (Acari: tetranychidae). J. Econ. Entomol. 94: 577–583.

Sun, J., C. Lian, M. Navajas and X. Hong. 2012. Microsatellites reveal a strong subdivision of genetic structure in Chinese populations of the mite *Tetranychus urticae* Koch (Acari: Tetranychidae). BMC Genet. 13: 8.

Trandem, N., UR. Bhattarai, K. Westtrum, GK. Knudsen, I. Klingen. 2015. Male spider mites prefer females killed by the mite-phatogenic fungus *Neozygites floridana*. J. Invertebr Pathol. 128: 6–13.

Tuttle, D., E. Baker and M. Abbatiello. 1976. Spider mites of Mexico (Acari: Tetranychidae). Intern. J. Acarol. 2: 1–108.

Van Leeuwen, T., J. Vontas, A. Tsagkarakou, W. Dermauw and L. Tirry. 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. Insect Biochem. Mol. Biol. 40: 563–572.

Westrum, K., V. Duarte, R. Humber, I. Delalibera and I. Klingen. 2014. Confirmation of *Neozygites floridana* azygospore formation in two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions. J. Invertebr. Pathol. 122: 1–5.

Xien, L., R, Xie, K. Zhang and X. Hong. 2008. Genetic relationship between the carmine spider mite *Tetranychus cinnabarinus* (Boisduval) and the two-spotted mite *T. urticae* Koch in China based on the mtDNA COI and rDNA ITS2 sequences. Zootaxa 1726: 18–32.