Foreign Exploration for the *Nezara viridula* (L.) egg parasitoid, *Trissolcus basalis* (Wollaston), in Southern Spain.

Field Trip Report
July 20-29, 2009

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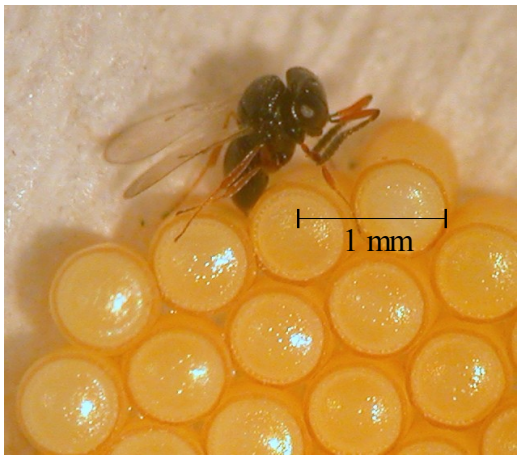
Contents

1 – Background

The southern green stink bug, *Nezara viridula (*L.), 1758 (Heteroptera: Pentatomidae) (photo 1), was accidentally introduced into many countries since 1798. It was first discovered in Florida in 1885 and in Hawaii in 1961. It feeds on a very wide range of host plants including many crops.

The egg parasitoid, *Trissolcus basalis (*Wollaston), 1858 (Hymenoptera: Scelionidae) (photo 2), is generally considered to be effective in controlling its host, *N. viridula*.

1 – *Nezara viridula* adult (15mm)



2 – *Trissolcus basalis* adult (1mm) laying eggs on *N. viridula* fresh egg mass

Trissolcus basalis has been present in the southern U.S. for a long time. But surveys indicated that it has not been as effective in limiting *N. viridula* populations as previously thought.

In 1986, the southern green stink bug was discovered in California, destroying tomato plants and the European Parasite Laboratory near Paris, France, was asked by the University of California, Davis, to help control the new invader.

Following climate matching using Klammadeagrams, Dr. Walker Jones decided to make *T. basalis* collections in matching sub-climate types areas in Spain, France and Italy.

The collections were made and samples were shipped, evaluated and further released in California in 1987 and 1988. The parasitoid became established and since has effectively controlled *N. viridula*.

2 – Purpose

EBCL has proposed to conduct a retrospective study to try and determine the geographic origin of the populations of *T. basalis* that successfully established in the U.S.

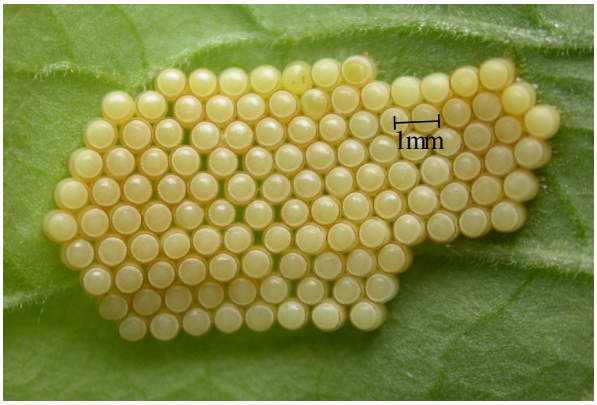
This will be done using molecular neutral markers such microsatellites. These markers are being developed by an Italian cooperator and will be transfered to EBCL. Dr. Marie-Claude Bon will be in charge of the population genetic analysis.
This information would contribute to the long-running discussion about the comparative importance of climate matching versus genetic matching in biocontrol and possibly lead to the identification of unique qualities of the successfully established populations in California.

Original specimens of the three *T. basalis* populations were kept in alcohol since they were collected twenty-two years ago.

To enhance the precision of the genetic comparisons, new collections from California, from the same site in Italy and some sites in southern France have already been made. The Spanish population remained to be sampled.

An exploration was conducted in Spain in July 2009 to collect *Trissolcus basalis* from the Granada area, the original location.

During the survey, we will first look for the presence of *Nezara viridula* adults and nymphs on appropriate plants. This will give us an idea as for the chances to discover egg masses as it is easier to find mobile stages than eggs.
Then, if the host is present, we will look for its egg masses. A fresh egg mass, which is of a pale yellow color, without a parasitoid ovipositioning must not be neglected as it could have been already parasitized (photo 3). Parasitized eggs begin to turn darker until they turn completely black prior to emergence in about ten days (photo 4).
And, as a last resort, if *N. viridula* has been observed or even not observed at all and no egg masses found, we will expose fresh sentinel *N. viridula* egg masses from a rearing culture (front cover photo).



3 – *Nezara viridula* fresh egg mass

4 – *Nezara viridula* parasitized egg mass by *Trissolcus basalis*.
T. basalis progeny is hatching

3 – Summary

The route was planned to survey two areas to increase the chances of finding the target parasitoid: north of Valencia, there is an Institute, the IVIA (Instituto Valenciano de Investigationes Agrarias), where some researchers used to work in biocontrol of *Nezara viridula* and Granada is the original sampled location.

1 – Valencia area

We had a good contact in Valencia area at the IVIA with Dr. María Verdú and Dr. Francisco Beitia.

The search of *Trissolcus basalis* was successful. Parasitized *N. viridula* egg masses were discovered in the spot by Dr. Francisco Beitia on old tomato plants.

A few sentinel *N. viridula* egg masses from our rearing were placed in another tomato plot where we didn't observe any sign of *N. viridula* presence. No *T. basalis* emerged.

2 – Granada area

We had no previous contact in the Granada area before leaving EBCL and hence the survey for *Trissolcus basalis* was not successful. No *Nezara viridula* were found. No *T. basalis* emerged from the sentinel egg masses placed in the area.

However, I made contact with a farmer from southern Granada who agreed to later ship us parasitized *N. viridula* eggs if found.

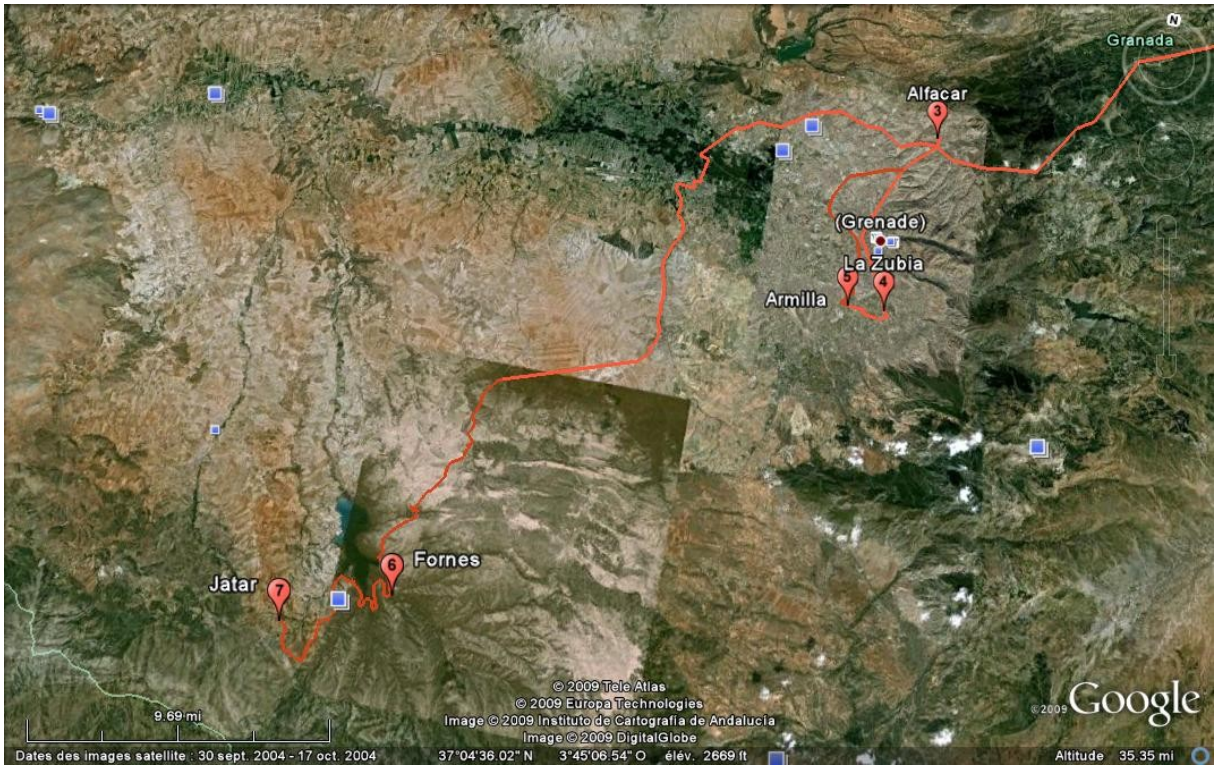
4 – Itinerary

Date	Route	Map #
July 20	EBCL Montferrier sur Lez-Montpellier to Moncada-Valencia	1
July 21	Moncada downtown to IVIA. Moncada & back	2, 3
July 22	Moncada downtown	2
July 23	Moncada-Valencia to Alfacar-Granada	1, 2, 4
July 24	Alfacar to Armilla & back	2, 4
July 25	Alfacar to Fornes & back	2, 4
July 26	Alfacar to Játar & back	2, 4
July 27	Alfacar to Fornes & back	2, 4
July 28	Alfacar-Granada to IVIA Moncada-Valencia	1, 2, 3
July 29	Moncada-Valencia to EBCL Montferrier sur Lez-Montpellier	1

Map 1- Field trip general map (red line)

Map 2- The two areas searched

Map 3- The Valencia area site detail



Map 4- The Granada area sites

5 – Daily schedule

Day 1, Monday, July 20th:
Travel from EBCL-Montferrier to Moncada-Valencia.
Brought 80 French *Nezara viridula* adults ♂ and ♀ along in rearing boxes in order to obtain fresh egg masses to be used as sentinels to attract ♀ *Trissolcus basalis*.

Day 2, Tuesday, July 21st:
Met with Dr. María Verdú and Dr. Francisco Beitia from the IVIA (Instituto Valenciano de Investigationes Agrarias) at Moncada-Valencia as planned.

Surveyed for *Nezara viridula* on tomato plants from one of their experimental plots on the IVIA site (photo 5). No *N. viridula* were observed but 5 *N. viridula* sentinel egg masses were attached to tomato plants (photo 6, map 3). Francisco Beitia and María Verdú confirmed the presence of both *N. viridula* and *T. basalis* on this site. Francisco Beitia agreed to check the sentinel egg masses until my return, a week later.

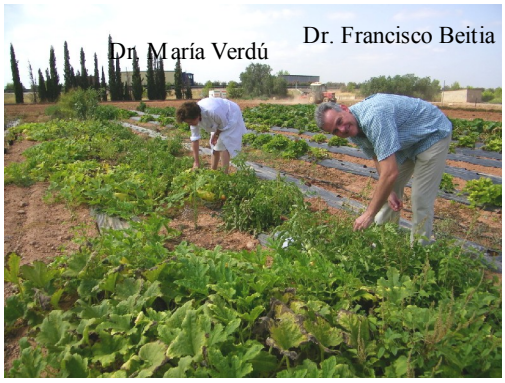
5 – Tomato experimental crop used for *Tuta absoluta* research

6 – Sentinel egg mass placed under a tomato leaf in the control (photo 5)

A survey for the presence of *N. viridula* were conducted on older tomato plants that were growing in the IVIA experimental plot (photo 7, map 3).

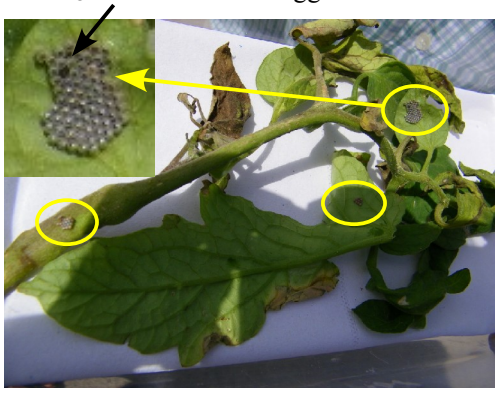
Nezara viridula nymphs and adults were found. We kept large nymphs and adults to add to our "mobile rearing". Three *N. viridula* parasitized egg masses were found by Francisco Beitia. Only one male *T. basalis* emerged from one egg mass (photo 8).

GPS coordinates of the spot are as follows: 39° 35' 13"" N, 0° 23' 52" W, altitude: 189 ft (57,6m).



7 – Search for *Nv* and its parasitoids

♂ *T. basalis* on the egg mass



8 – *Nv* egg masses parasitized by *T. basalis*

We visited Francisco Beitia's laboratory: he is working on *Ceratitis capitata* on citrus (photo 9 & 10), on *Tuta absoluta* (Lepidoptera: Gelechiidae) on tomato, a new invader from South America (photo 5 & see appendices p.15).

9 – Francisco's technician adding water to the *Ceratitis capitata* egg container in a rearing chamber

10 – Francisco Beitia and María Verdú standing next to the citrus experimental crop used for *C. capitata* research

Citrus orchards are planted everywhere in the area (photo 11).
Valencia: Latitude: 39.46, Longitude : -0.37, mean altitude: 10m.

11 – Citrus orchards

Day 3, Wednesday, July 22nd:
The *N. viridula* rearing boxes brought along were cleaned (photo 12), temperature inside the room averaged 26-29°C.

Provided honey for the emerging *Trissolcus basalis* (photo 13).
Organized the second part of the trip.

12 – The rearing boxes

13 – *Trissolcus basalis* from IVIA

Day 4, Thursday, July 23rd:
Travel from Moncada-Valencia to Alfacar-Granada.

Day 5, Friday, July 24th:
Bought a map of Granada, enquired from the salesman where one can find a horticultural zone in the east and southeast of the city.

Located the area taking back roads and finally discovered a vegetable garden in La Zubia.

Made contact with the owner who told us that he has no “chinche verde” (southern green stink bug). We looked at the plants with him and noticed the presence of *Tuta absoluta* on tomato leaves, but no *Nezara viridula* (photo 14, map 4).

14 – First vegetable garden explored and the owner

15 – Another vegetable garden

Learned that there are many vegetable gardens in this area.
Kept following back roads, found other gardens with tomato plants (photo 15).

Stopped at a second garden in Armilla (map 4) that was larger and fenced, with many different crop species, called “Villa María”. The retired gardeners are selling fruits and vegetables on the site (photos 16 & 17).

16 – Overview of the vegetable garden

17– The retail fruit and vegetable store and the owners

Together, we looked at the plants and no “chinche verde” was found.
Learned from them that there is an area where farmers are growing tomato plants infested by *Nezara viridula*. It is at about 50 km southeast of Granada (map 4).

Crops seen in the area: olive & some almond orchards, many irrigated cherry & walnut orchards, vegetable gardens, some Japanese medlar & fig trees, artichoke, grape vine, poplar.

It’s a “vega”, a fertile valley with a lot of water coming from the surrounding high mountains: Sierra Nevada (3482 m) & other mountains (photo 23). The temperatures can reach –5°C and there is snow in winter.
Armilla: latitude: 37-08N, longitude: 003-38-08W, altitude: 700 m.

Day 6, Saturday, July 25th:
Travel from Alfacar-Granada to Fornes (photo 18, map 4).
Discovered a tomato field in Fornes and stop nearby where I met the owner (photo 19, map 4).

18 – On the way to Fornes

19 – Cherry tomato plants and the owner

The “chinche verde” was present there. The owner cultivates 4.5 ha of cherry tomato and 2 ha of “pepinos” in contract with the Dutch and olive trees.

Searched the tomato plants and did not notice the presence of *N. viridula*, but a few *Tuta absoluta* damaged the leaves. The farmer is using insecticides against the new moth.

Placed 3 *N. viridula* sentinel egg masses on these tomato plants.

The following day, planned to go and see organic vegetable (“ecológica”, not “biológica”…?) crops in a village a little further away from Fornes, where the farmer has all his other crops.

Day 7, Sunday, July 26th:

Drove until Játar, saw many tomato and other vegetable crops (photo 20, map 4). Some of them were protected by nets (photo 21), farmers call them “invernaderos” (greenhouses).

Met the farmer again who showed me an organic runner bean crop (photo 22). He introduced me to the owner of this organic crop who confirmed the presence of “chinche verde” in his culture and allowed me to survey the plants.

20 – Other tomato crops

21 – Invernaderos

22 – Organic runner bean crop

23 – Crop irrigation system

Examined the organic plants extensively and used a sweep net, observed a few other organic crops nearby (cabbage, etc) and another non-organic tomato culture but did not find *Nezara viridula.*

Collected a few other Pentatomids: several *Graphosoma lineatum italicum* and one *Eurydema ornata* on *Daucus carota,* one *Aelia rostrata* on beans and one *Eurydema ventralis* collected earlier in the day, in Alfacar (J-C Streito det.).

Checked the sentinel egg masses previously placed on tomato plants in Fornes the day before. They are still yellow with no evidence of parasitism.

Day 8, Monday, July 27th:
Returned to Fornes, retrieved the egg masses placed 2 days ago. One of them has turned orange (not parasitized).

Spoke with the farmer who agreed to send us *N. viridula* egg masses if he could find some later. He will also place 3 newly-collected *N.viridula* egg masses from our colony on organic plants in Játar to be send to us if they don't become juvenile stink bugs.

Main crops in the area: olive, almond (not profitable) and vegetable crops, mainly cherry tomato plants and zuchini.

Day 9, Tuesday, July 28 July:
Travel from Alfacar-Granada to IVIA Moncada-Valencia.

Met Dr. Francisco Beitia again at the IVIA, Moncada.
Retrieved the sentinel egg masses exposed on July 21st at the Institute. *N. viridula* emerged from 3 masses, nothing emerged from the other 2.

Day 10, Wednesday, July 29th:
Travel from Moncada-Valencia to EBCL Montferrier sur Lez-Montpellier.

6 – Results / Conclusion

1 – Valencia area

54 females and 5 males of *Trissolcus basalis* emerged from the 3 *Nezara viridula* egg masses found in Moncada, Valencia area.

We obtained an F1 to ensure we had sufficient females for the genetics study.

The samples were placed in absolute ethanol in small plastic vials, separating the females from the males and frozen at -20°C until their eventual use.
A few specimens were also kept frozen for dry mounting.

Egg masses of *N. viridula* were found very quickly thanks to our contacts on the spot. María Verdú and Francisco Beitia were very helpful in this regard.

2 – Granada area

Without any contact in the Granada area, exploration took a longer time than expected.

However, tomato fields were identified. Although growers said that “las chinches verdes” were present in their plots, we did not observe any *N. viridula* in any fields searched.

One reason was due to the fact that most of the tomato crops were treated with insecticides against *Tuta absoluta*. Even in the few organic crops surveyed, we did not observe any *N. viridula*.

A good contact was made with a fruit and vegetable grower from southern Granada. He was willing to send us sentinel egg masses from our rearing once they turned black. The egg masses were placed in organic tomatoes.

He eventually did send us one egg mass. Unfortunately, it looked dead although two parasitoids other than *Trissolcus basalis* emerged: *Ooencyrtus* sp., W. Jones det. (see appendices p.15).

3 – General conclusion

The field trip to Spain was successful considering the lack of information and contact prior to departure.

Considering the recent reinforcement of the Spanish regulations regarding the collection of living materials, I would like to stress the need to have contacts with governmental institutions like IVIA.

7 – Acknowledgements

► To Dr. Walker Jones, my supervisor, who gave me the possibility to organize and conduct such trip in a foreign country. This was my very first exploration. He also established the contact in Valencia for me and helped me correcting this report.

► To Dr. María Verdú and Dr. Francisco Beitia from the IVIA Moncada-Valencia who have been very helpful in my search for parasitoid. They are excellent contact for EBCL.

► To all the Spanish people who have helped me during my exploration and more particularly the farmers met in Granada area.

► And to Dr. Marie-Claude Bon for having helped me in correcting this report.

8 – Contact addresses

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► **Farmer's address from south Granada:**

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CP – 18127
GRANADA – ESPAŇA

9 – Appendices

► Page 16: photo of the two parasitoids of the *Ooencyrtus* genus (p. 14) from a mail sent to the farmer during fall.

► Page 17: photos of *Tuta absoluta* damage on tomato fruit and leaf (p. 9) from Internet.

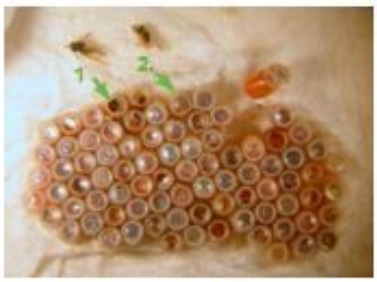
Parásitos de huevos de la chinche verde

Los huevos en el circulo verde son los que me has enviados. Muchas gracias otra vez. Parecen de color oscuro pero, vistos de cerca, no son todos del mismo color.

En comparación, éste es una foto, en el marco rojo, de huevos que se han vueltos muy negro. Son los que buscamos.

Pero, dentro de los huevos que has enviados, dos han dado dos insectos. ¡Míra!

Son las dos micro avispas que han salido de los dos huevos indicados por las flechas verdes. Una ha salido cavando un agujero encima del huevo (1) y el otro ha salido en el lado del huevo (2).
Todavía, no fue possible idintificar la especie de avispa.



Additionnal information on *Tuta absoluta* from www.tutaabsoluta.com (P.2 from 9)

Nature of damage

The larvae of *Tuta absoluta* mine the leaves producing large galleries and burrow into the fruit, causing a substantial loss of tomato production in protected and open filed cultivations. The larvae feed on mesophyll tissues and make irregular mine on leaf surface. Damage can reach up to 100%. This pest damage occurs throughout the entire growing cycle of tomatoes. *Tuta absoluta* has a vey high reproduction capability. There are up to 10-12 generations in year in favourable conditions. The larvae are very unlikely to enter diapause as long as food source is available. *Tuta absoluta* can overwinter as eggs, pupae and adults. Adult female could lay hundreds of eggs during her life time. Tomato plants can be attacked from seedlings to mature plants. In tomato infestation found on apical buds, leaves, and stems, flowers and fruits, on which the black frass is visible. On potato, mainly aerial parts are attacked. However damage on tuber also recently reported.

Tuta absoluta reduced yield and fruit quality of Tomato grown in green house and open field. Severely attacked tomato fruits lose their commercial value. 50–100% losses have been reported on tomato (EPPO, 2005). On potato, CIP (1996) considers that is one of the major pests of foliage, occurring in warm zones of low altitudes (below 1000 m). As larvae are internal feeders it is difficult to achieve an effective control through application of chemical insecticides. Moreover, *Tuta absoluta* can rapidly evolve strains with reduced susceptibility to insecticides that have been previously effective. Failure by synthetic insecticides has also been reported in many countries.