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North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas
MEXICO - USA - CANADA

SP 1
Surveillance Protocol for the Tomato Leaf Miner, *Tuta absoluta*, for NAPPO Member Countries

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Review

NAPPO Surveillance Protocols are subject to periodic review and amendment. The next review date for this NAPPO Surveillance Protocol is 2018. A review of any NAPPO Surveillance Protocol may be initiated at any time upon the request of a NAPPO member country.

Endorsement

This Surveillance Protocol was approved by the North American Plant Protection Organization (NAPPO) Executive Committee on March 19, 2013 and is effective immediately.

Approved by:

Greg Wolff
Executive Committee Member
Canada

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Executive Committee Member
United States

Javier Trujillo Arriaga
Executive Committee Member
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Implementation

Not applicable.

Amendment Record

Amendments to this Surveillance Protocol will be dated and filed with the NAPPO Secretariat.

Distribution

This Surveillance Protocol is distributed by the NAPPO Secretariat, to the Industry Advisory Group (IAG) and Sustaining Associate Members (SAM), the International Plant Protection Convention (IPCC) Secretariat, and to other Regional Plant Protection Organizations (RPPOs).

Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick), originated in South America and is a significant pest of tomato (*Solanum lycopersicum* L.), as well as other solanaceous crops. This pest may be responsible for losses of up to 80-100% in tomato plantations in both protected cultivation and open fields in native and introduced ranges if left uncontrolled (Desneux et al. 2010). It attacks all aerial parts of the host (leaves, stems and fruits). Once introduced, *T. absoluta* can be spread by seedlings, infested vines with tomato fruit, tomato fruit and used containers. Outdoor markets, vegetable repacking and distribution centres are potential introduction points in the spread of this pest (CFIA, 2010).

The impact that *T. absoluta* could represent for North America underscores the need for surveillance for this pest. To this end, the North American Plant Protection Organization (NAPPO) provides the following guidelines for surveillance for the tomato leaf miner in North America (Canada, Mexico and United States).

1. Purpose of the Surveillance Protocol

The purpose of this protocol is to establish a surveillance methodology that will allow for the early detection and delimitation of *Tuta absoluta* in the North American region (Mexico, USA, Canada), should it become introduced. Practical applications of detection and delimiting surveys (as defined in ISPM 5 and in ISPM 26: 2006) are provided. Suggestions for preparation, packaging and shipping of samples for identification are provided in Appendix 1.

Because tomato is the main crop known to be attacked by *T. absoluta*, the survey methodologies included here will focus on this crop. They may be adapted for application in other crops as required.

2. Information on the Target Pest

2.1 Pest name

Scientific name: *Tuta absoluta* (Meyrick), Lepidoptera: Gelechiidae

Common names: Tomato leaf miner, tomato borer, South American tomato moth, South American tomato pinworm (English); palomilla del tomate (Mexico), polilla del tomate, polilla perforadora, cogollero del tomate, gusano minador del tomate, minador de hojas y tallos de la papa (Spanish); traça-do-tomateiro (Portuguese); mineuse de la tomate (French).

2.2 Life cycle

Tuta absoluta is a microlepidopteran moth with a high reproductive potential, and although its biological cycle depends on temperature, it is capable of over 10 generations per year under optimal conditions. Its life cycle comprises four development stages: egg, larva, pupa and adult, and is completed within 24 days at 27^{o} C (Table 1). Low temperatures are a limiting factor for its survival, but *T. absoluta* can overwinter as eggs, pupae or adults, depending on environmental conditions.

Eggs are small, cylindrical, creamy white to yellow-orange, and 0.35 mm long. Larvae are cream coloured with a characteristic dark head and a lateral spot that extends from the ocellus to the posterior margin. They lack a typical dorsal plate in the prothorax. Instead they have a dark oblique band that does not cover the dorsal midline. Pupae are cylindrical in shape and greenish when just formed, becoming darker in colour as they near adult emergence. The pupae are often coated with a white silky cocoon. Adults are 5-7 mm long with a wingspan of 8-10 mm. The most important identifying characters are the filiform antenna, grey coloured scales and black spots present on the anterior wings. Females can live for two weeks, whereas the males live only one week.

Females usually lay eggs on the aerial parts of host plants, on the underside of leaves or stems and to a lesser extent on fruits. A mature female can lay up to 260 eggs. Egg hatch occurs in 4-6 days.

After hatching, young larvae penetrate plant tissue (leaves, aerial fruits or stems), begin feeding and create mines.

Larvae develop through four larval stages. As they develop from the second to fourth instar, they become greenish to light pink (by feeding on leaves) and measure between 1 and 8 mm. The larval stage is the most damaging to plants and is completed within 12-15 days under optimal conditions. Larvae do not enter dormancy when a food source is available (Desneux et al. 2010).

Larvae of *T. absoluta* attack the foliage by penetrating into the leaf and feeding on the mesophyll tissues. The feeding behaviour results in irregular mines on the leaf surface.

Older (3^{rd} - 4^{th} instar) larvae can feed on all parts of tomato plants. They can leave their mines and travel to new locations to mine again. This behaviour may result in damage to all stages of plant growth. The larvae produce large galleries in the leaves, burrow into stalks, apical buds, and green and ripe fruits. Fully-grown larvae usually drop to the ground on a silk thread and pupate in the soil, although pupation may also occur on leaves or in the calyx. Pupae have been found in the mines, outside the mines and in the soil, as well as beneath pots and under greenhouse benches.

Table 1: Average length of the life cycle of *Tuta absoluta* at different temperatures

Life Stage	Duration (Days)		
	14 ^{o}C	20 ^{o}C	27 ^{o}C
Egg	14.1	7.8	5.13
Larva	38.1	19.8	12.2
Pupa	24.2	12.1	6.5
Total Egg-Adult	76.4	39.7	23.8

Source: Barrientos, Apablaza, Estay and Noreno, 1997, quoted by Estay, 2000.

2.3 Dispersal mode

Tuta absoluta is nocturnal in habit. Adults usually remain hidden during the day, showing greater morning-crepuscular activity. There are indications that these moths can spread several kilometres by flying or drifting with the wind. It has been found in cold climate as far north as Switzerland and The Netherlands, but prefers hotter temperatures (Mediterranean, South America, Middle East) (Desneux et al. 2010).

2.4 Target hosts

Tomato leaf miner feeds mainly on solanaceous hosts; however, other hosts may be attacked occasionally. All of its known hosts are reported here.

- Major host: *Solanum lycopersicum* (= *Lycopersicon esculentum*), tomato.
- Minor hosts: *Solanum tuberosum* L. (potato) *Solanum lyratum* Thunb., *Solanum muricatum* Ait. (Peruvian pepino), *Nicotiana glauca* L. (tree tobacco), *Solanum melongena* L. (eggplant), *Nicotiana tabacum* L. (tobacco) (Desneux et al. 2010). Other minor hosts may include *Solanum habrochaites* Knapp & Spooner (= *Lycopersicon hirsutum* Dunal),and *Capsicum annuum* L. (peppers).
- Wild hosts: *Solanum bonariense* L. (granadillo), *Solanum nigrum* L. (common nightshade), *Solanum elaeagnifolium* Cav. (silverleaf nightshade, bull-nettle), *Solanum sisymbriifolium* Lam. (wild tomato, sticky nightshade), *Datura stramonium* L. (*D*. *ferox* Nees von Esenbeck*)* (fierce thorn-apple), *Lycium* L. sp., and *Lycopersicum puberulum* (Desneux et al. 2010). Other wild hosts may include: *Solanum chenopodioides* Lam. (= *S. gracilis* Herter*) (black nightshade), Solanum pseudocapsicum* L. (Jerusalem cherry), *Solanum viride* Spreng (= *S. puberulum* Nutt. ex Seem) (green nightshade), *Solanum aculeatissimum* Jacq. (Dutch eggplant, love-apple), *Solanum americanum* Mill. (American black nightshade), and *Lycium chilense* (coralillo),

2.5 Pest distribution

To aid in early detection of this pest, general surveillance strategies including gathering information from other sources, should be used (ISPM 6: 1997). Pest distribution lists may be useful to alert the NPPO of the importing country as well as the importing facilities to the potentially higher risk of a certain commodity.

The following countries or areas are currently considered infested with *Tuta absoluta*: Albania, Algeria, Argentina, Austria, Bahrain, Belgium, Bolivia, Brazil, Bulgaria, Cayman Islands, Chile, Colombia, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Ethiopia, Finland, France, Germany, Greece, Hungary, Iran, Iraq, Ireland, Israel, Italy, Jordan, Kosovo, Kuwait, Latvia, Lebanon, Libya, Lithuania, Luxembourg, Malta, Morocco, Netherlands, Palestinian Authority (West Bank), Panama, Paraguay, Peru, Poland, Portugal (including the Azores), Qatar, Romania, Russia, Saudi Arabia, Senegal, Slovakia, Slovenia, Spain (including the Canary Islands), Sudan, Sweden, Switzerland, Syria, Tunisia, Turkey, United Kingdom (all regions), Uruguay, Venezuela, and Western Sahara.

The website, www.tutaabsoluta.com, provides unofficial up-to-date information on world distribution of the pest. For confirmation, please consult with the NPPO of the country of interest.

2.6 Pathways for introduction

Tuta absoluta could be introduced to North America by the importation of infested tomato fruit, tomato plants, and used tomato crates or packing boxes.

A pest risk analysis carried out by the Plant Protection Service of The Netherlands (Ministry of Agriculture, Nature and Food Quality of the Netherlands, 2010) identified some pathways in which *T. absoluta* can be introduced and spread, as per the following:

- Seedling
- Tomato fruit and eggplant
- Production facilities and outdoor markets
- Farm equipment and transportation vehicles.

3. Detection Surveys

3.1 Purpose

Guidelines for detection surveys are provided for member country implementation to help in the early detection of *Tuta absoluta* should it arrive in North America (Canada, Mexico, and USA).

3.2 Target life stages

The detection surveys described in this standard are targeted to adult moths (trapping); complementary surveillance for additional life stages, especially larvae, may also be useful.

3.3 Timing and duration

Tuta absoluta can have 10 or more continuous generations in a year under optimal conditions and can be present year round where suitable environmental conditions exist. For tomato production sites, timing for the *T. absoluta* survey is directly linked to the tomato production cycle. This survey should be implemented as soon as the tomato production cycle starts (whether under protected cultivation or in open field) and should be concluded 30 days after tomato harvest or after the crop residues are removed. In packing/receiving facilities not under a production cycle, traps should be placed as soon as product is available. Trapping may be stopped after packing activities have concluded.

3.4 Survey locations

Tuta absoluta could be introduced to North America primarily by the importation of infested tomato fruit, tomato plants, and used tomato crates/packing boxes.4. Site selection should be prioritized according to the volume of importation and risk of host plants.

Facilities importing tomato products from the countries where *Tuta absoluta* is present should be considered for trapping. High risk locations listed in Table 2 should be targeted.

Other potential survey locations may also include market stalls selling tomatoes, or high risk sites such as: nurseries selling tomato seedlings, tomato farms, border crossings, customs inspection area, truck waiting yards, sea ports, and airports.

Table 2: Suggested trap placement sites based on potential high risk locations for the introduction of *Tuta absoluta*

Survey location	Trap placement sites
Tomato seedling nursery	Production area Compost area
Tomato farm	Production area Sorting and packing area Waste disposal area
Composting plant (using plant waste from tomato farms)	Raw plants reception area Composting area Compost storage area
Wholesale vegetable markets	Truck loading area Stores of bulk tomato
Vegetable repacking and distribution centres	Truck loading area Washing and packing lines
Food processing / salad packing / tomato processing plants	Truck loading area Washing and processing area

3.5 Trap placement sites

Once a location is selected for survey, trap sites are then determined and traps are placed within each location.

Trap placement sites should include reception area in packing houses, sorting and packing areas, truck loading areas, waste disposal areas, composting facilities using plant waste from tomato farms, raw plants reception areas, composting areas, compost storage areas, wholesale vegetable markets, reception areas of incoming trucks, stores of bulk tomato, vegetable repacking and distribution centers, washing and packing lines, food processing/ salad packing/ tomato processing plants, and washing and processing areas (Table 2).

Grid maps of the survey area may also be prepared and areas identified to be high risk locations selected by their proximity to the grid point. Sampling points are then established. As this is a pheromone -based survey, the sample point is the same as the trap placement site.

4. Delimiting Surveys

4.1 Purpose

Delimiting surveys may be used to establish the boundaries of an area considered to be infested by *Tuta absoluta*. A delimiting survey involves looking at a pest infestation and investigating the extent to which a pest has spread from the initial point of detection.

4.2 Target life stages

The target life stage of a delimiting survey is primarily adult moths; however, if the survey has been limited to a facility, larval stages may also be included.

4.3 Timing

The timing will be set by the date of the first detection that was observed and how quickly a survey can be planned and organized.

4.4 Target areas and site selection

4.4.1 Description of the area that is to be surveyed

The delimiting survey area could be an officially defined country, part of a country or all or parts of several countries (ISPM 5); for example - all of Mexico or the State of Colima, MX or the Region of Peel, Mississauga, CA or Dade County, FL, USA. The area may be described using political or natural geographic boundaries, or as specific places/sites of production.

4.4.2 Delimiting survey procedure

Delimiting surveys are conducted in an ever-increasing radius from the initial detection at locations and sites that may have had or currently contain a suitable host(s) and a conducive climate or environment for the pest to establish and reproduce.

Delimiting survey locations should be defined as suggested in 3.5.

5. Survey Methodology

5.1. Sampling procedures

5.1.1 Pheromone lure

Pheromone type: Tomato leaf miner lure (includes (3E,8Z,11Z)-3,8,11-tetradecatrien-1-yl acetate and (3E,8Z)-tetradecadien-1-yl acetate) loaded on a rubber septum at a dosage of 0.5 mg. Depending on the environmental conditions the pheromone should last up to six weeks. Please refer to the country NPPO for approved suppliers.

Handling and storage: All pheromones should be stored in sealed containers at temperatures below 0^{o} C. Only one pheromone component should be stored per container (do not mix with other types of pheromones). Opened storage containers should be re-sealed after use. Pheromones can be stored for a maximum of two years if refrigerated properly. During transportation to the field, the pheromones should be kept cool and out of direct sunlight (in a cooler).

The pheromone should be installed before assembling the trap by laying the release device with the lure directly on the center of the sticky surface, ensuring that air passes through it.

Disposable gloves should be worn at all times when handling pheromones, and a new pair of gloves between types of pheromone. This avoids cross-contamination of the volatile compounds and possible interference with their attractiveness. Gloves should not be disposed of in the vicinity of traps.

5.1.2 Trap

Two types of Delta traps are available: cardboard delta triangle covered with sticky surface or another option with a removable liner. Either one is suitable for the current purposes, although Delta traps with non-drying sticky liners are preferred. Traps are available from several suppliers in multiple colors, and all should be considered equivalent for this survey. Dry touch liners can capture and better preserve insects for examination than liners using soft glue which tend to wick through the specimen and ruin moth scale patterns.

Folding the trap is facilitated by breaking all creases and perforations. The end of the sides should not be folded in, to enhance moth capture. The peak of the trap should be stapled and an attachment wire threaded through the perforation.

NPPO contact information should be placed on the trap.

5.1.3 Trap placement and density

One trap should be placed at each trap placement site except where trap placement sites are very large, ie. greater than 5000 m^2. It is recommended that for each additional 5000 m^2 the area should be treated as a separate trap placement site and another trap should be placed.

The delimiting survey may also be done by pheromone trapping using Delta traps at predetermined distances of 2 to 5 kilometres (this can be modified as needed) at the rate of two traps per hectare, in a circle from the initial site of infestation. Once another positive trap catch occurs within this predetermined distance, additional two to three traps are placed per site at shorter intervals, 30 to 100 meters (for example).

Permission must be obtained from the owner or manager prior to initiating any trapping or survey.

Tuta absoluta does not fly very high and prefers young shoots so traps should be placed at an initial height of approximately 0.40m. They should be maintained at the approximate height of the canopy and raised as the plant grows, but where they do not interfere with normal activities. Traps may be attached to planting stakes or other objects such as irrigation piping if the objects are in close proximity to the site. While it may be preferable to have traps within a planting row, these will receive pesticide applications and will remain inaccessible during re-entry intervals after applications. Traps at alternative locations may also be inaccessible, so it is important to communicate with the field manager immediately before entering the field.

In facilities that handle product which may be either sealed (for example in plastic wrap) or non sealed (for example in trays or cardboard boxes), traps should be placed near non sealed products because there will likely be higher plant volatiles present which would

increase attraction of *T. absoluta* to the area and could increase the trap detection efficiency.

5.1.4 Servicing the trap

Gloves should be used to handle traps and liners in order to prevent their contamination. Traps and lures should be checked every two weeks and lures replaced every four to six weeks. After a positive detection is made, the delimitation survey is initiated for which the servicing interval should be shortened to give the survey more precision and to allow for quicker response in determining the true extent of the population.

- Traps should be replaced if missing, damaged, or if the sticky surface has been compromised by debris.
- Traps with suspect moths must be collected for diagnostic submission and replaced with a new trap and lure.
- Liners should be replaced at the two week servicing if they are covered with dust or insects; the lure should be transferred to the new insert with gloves or forceps.
- Old liners (older than 4-6 weeks) should be checked for pheromones lures, replacing any that may be missing.
- Old lures can remain in the trap for one servicing interval, and then they should be removed.
- At the end of the survey, if the trap is empty, it should be collapsed, squashed flat and placed in a suitable container for disposal.

5.1.5 Data collection

Records should be maintained that provide data which at a minimum should be precise location of every trap (the GPS coordinates), sketch map of local trap location, name of the facility or land owner, the name of the trapper, the day the trap was placed at a particular site, servicing dates, when the trap was decommissioned and the results of each trap, whether negative, positive or suspicious. Positive results also should include the day the pest was detected and how many target pests were in the trap. Records of infested tomato plants or alternate hosts also should be collected and maintained. Site numbers will be assigned that should generally conform to the following: two letter pest code - one letter for region - site number - trap letter (Ex: TA - D - 0001 -a) (See figure 2 of Appendix 1).

5.1.6 Data storage

Existing country databases may be used to store all survey data. The files should be updated at least weekly or as new information is available. Further guidance on recordkeeping may be obtained in ISPM 6: 1997.

5.2 Diagnostic procedures

5.2.1. Biosecurity precautions

When visiting facilities, surveyors should make sure they have taken steps to reduce the spread of pests such as removing soil and debris from clothing and footwear and washing

hands with soap or an approved antimicrobial. Where targeted facilities have biosecurity procedures in place, surveyors should become aware of the procedures and follow them.

5.2.2 Sample handling and laboratory submission

If there are no moths in the trap or if there are only medium to large moths, with body length greater than 1.0 cm, the trap or trap liner should not be removed from the field as the size would preclude *T. absoluta*.

If moths with body length less than 1.0 cm are present, they should be examined with a hand lens in the field. A field screening aid is available at:
(http://caps.ceris.purdue.edu/screening/tuta_absoluta)

If suspect moths or other life stages are present, or if there is uncertainty in the field regarding the species, the suspect moths must be submitted for identification by a trained taxonomist.

5.2.3 Sample preparation

The sample should be carefully packaged and sent overnight to the designated identifier or office in the respective country. NPPO guidelines should be followed for sample preparation. Details on suggested procedures for packaging and shipping may be found in Appendix 1.

5.2.4 Labelling samples for shipping

“Tomato Leaf Miner Survey” should be indicated on the sample container. All samples should be shipped by express courier to the closest official NPPO diagnostic laboratory. Laboratory notification through email is suggested to ensure sample is tracked and properly handled.

5.2.5 Reporting procedures

For information on responsibilities of and requirements for contracting parties in reporting the occurrence, outbreak and spread of pests in areas, please refer to ISPM 17: 2002.

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Tuta absoluta information network www.tutaabsoluta.com.

Appendix 1: Suggested procedure for preparation, packaging and shipping of samples for identification of *Tuta absoluta*.

The following are suggested procedures only. Each country may define their particular guidelines according to their requirements.

1. When sending whole traps

Traps with suspect *T. absoluta* must be removed from the site and the entire trap must be placed into a paper bag for submission. A paper label should be inserted into the paper bag with the trap. The paper label must contain the sample number or trap number, if applicable, collector's name, location, host and date of collection written in HB pencil. Trap(s) and packing material should be packaged to prevent shifting within a sturdy box that will resist crushing during shipping and be submitted as soon as possible after collection.

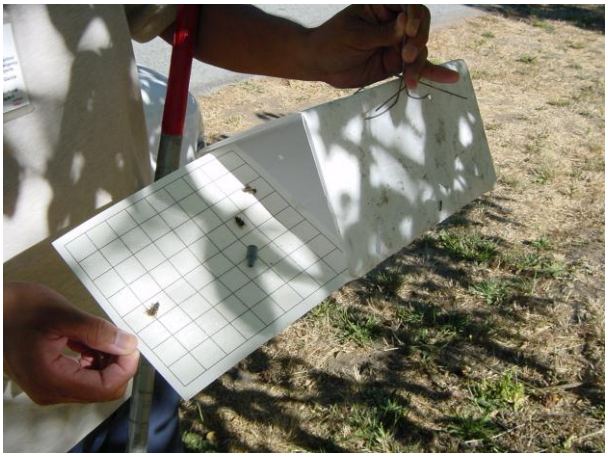

Figure 1. Delta trap with liner.

2. When sending trap liners

Trap liners from Delta traps with suspect moths should be carefully packed prior to shipping. The following are the suggested steps.

Ensure that trap number, the date the trap was installed and removed, and the number of suspect moths is recorded on the bottom of the liner, preferably with permanent ink (Figure 2).

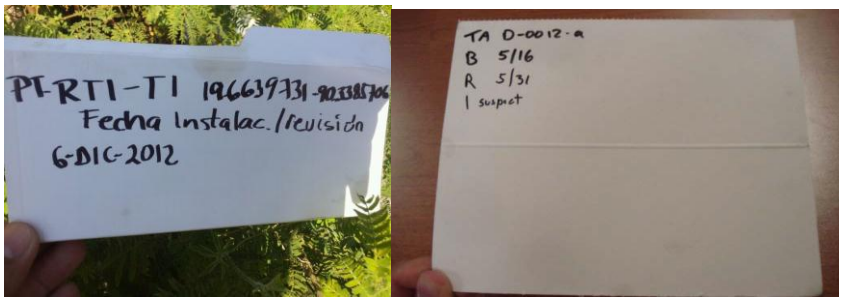

Figure 2. Examples of information included on trap

3. Fold the liner over so that it forms a “C” shape. For the liner to form a "C" shape it needs to be rolled against (=90 degrees, or perpendicular) the original fold of the trap; otherwise, the trap will fold flat and the specimens could be damaged. Secure the liner with two elastic bands. Ensure that the edges do not come in contact with each other.

Figure 3. Folded trap

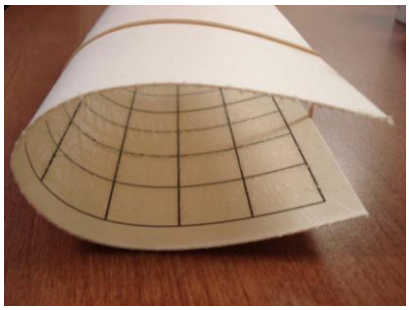

Figure 4. Folded trap showing “C” shape

4. Place the liner into a sealable bag. Sealing air in the bag will cushion the liner and help protect it from getting crushed.

Figure 5. Placement of trap in sealed bag.

5. Place the first sealable bag and a form (designed to record details of the trap as trap number, installation date, servicing date, number of suspect moths) into another bag. Sealing air in the bag will cushion the liner and help protect it from getting crushed.

Figure 6. Placement of form and sealed bag with trap into second sealed bag.

6. Place the liners into a sturdy box for shipping to ensure that they will not be crushed. Place other material (packing peanuts, bubble wrap, etc.), if necessary, in the box to ensure that the liners will not move around.

7. Send an email to the office/identifier receiving the box with information about the trap liner being shipped and details entered on the form that is accompanying the trap liner.