

ORIGINAL ARTICLE

Serodetection Of *Citrus Tristeza Closterovirus*(Ctv) In Algeria

BelkahlaHadjira, LarbiDjamila, BouafiaLylia, MoudoudRazika, GuettoucheFadhila and BouzidiSaida

University SaadDahleb of Blida, Faculty of Science Agrovétérinaire, Department of Agronomy, Virology laboratory.

BelkahlaHadjira, LarbiDjamila, BouafiaLylia, MoudoudRazika, GuettoucheFadhila and BouzidiSaida:
Serodetection Of *Citrus Tristeza Closterovirus*(Ctv) In Algeria

ABSTRACT

Surveys on the *Citrus Tristeza Closterovirus*(CTV) and their vectors were carried out in Algerian citrus fruit areas of Mitidja [station of department of agronomy of Blida (1), Guerrouaou (2), Hadjout (3), Boufarik (4), Mouzaia (5) and Soumaa (6)]. *Aphis spiraecola* was present in three exploitations of citrus fruit areas of Mitidja (2), (5), and (1). Whereas *Toxoptera aurantii* was detected only in two exploitations of citrus fruit areas of Mitidja (1), and (5). In most areas CTV-like symptoms i.e. complete deterioration of the trees, yellowing of leaves were observed. Serological tests were done on these crops using DAS-ELISA. CTV was prevalent only in five exploitations of citrus fruit areas of Mitidja (6) (5) (2) (1) (4).

Key words: *Tristeza, Citrus* fruits, *Aphis spiraecola, Toxoptera aurantii*, ELISA,

Introduction

The *citrus Tristeza virus* (CTV) is present in most of the citrus area (Bové *et al* 1983), and is recognized as the most dangerous disease of citrus known as a “rapid decline”. The movement of the infected material and its transmission according to the semi-persistent, provided by several species of aphids particularly the kind *Toxoptera* and *Aphis* (Lee 2000), are the cause of spread of this virus in the world.

In Algeria the presence of CTV has been recorded (Bayoud, 2001). During 2011-2012 season high aphid populations and severe expression of CTV were observed in different citrus fruit crop areas. The objectives of this work were to study the distribution of Algerian aphid populations of the potential vectors *Aphis spiraecola* (Patch) and *Toxoptera aurantii* (Boyer de fonscolombe), and to identify the virus infecting these citrus fruit crops.

Materials and Methods

Prospected sites Symptoms and aphid populations of CTV:

Surveys were carried out from the end of March to the end of May in six exploitations of citrus fruit areas of Mitidja [station of department of agronomy of Blida (1), Guerrouaou (2), Hadjout (3), Boufarik (4), Mouzaia (5) and Soumaa (6)].

The percentage of trees expressing symptoms or inhabited by aphids was directly estimated from 100 to 200 trees randomly collected in different part of trees. Aphid populations were estimated only once and at the near flowering stage. A tree was considered infested when at least one nymph or an adult aphid was found. Determination of aphid species was done in the laboratory.

Trees sampling:

Fifty or fifty-five trees showing or no symptoms and eventually infested or no by aphids were collected in 6 areas of Mitidja (Gottwald *et al* 2000).

Storage of leaf samples:

Field samples were dried at room temperature and stored at 4°C for 2 to 4 months before testing.

Corresponding Author: BelkahlaHadjira, University SaadDahleb of Blida, Faculty of Science Agrovétérinaire, Department of Agronomy, Virology laboratory.
E-mail: hbelkahla@yahoo.fr, Mob. 00213771900789

Serological test:

Dried samples were ground in five volumes of phosphate buffered saline containing polyvinylpyrrolidone (1%) and bovine serum albumin (0, 1%). Virus-free citrus fruit were used as controls. Control samples were ground in a roller in the presence of five volumes of the same buffer. DAS-ELISA (Clark and Adams 1977) kits for detection of CTV were obtained from Biorad (France). In DAS-ELISA microplate was first coated with polyclonal antibody (PC), and incubated at 30°C for 4h. Then leaf extracts were incubated overnight at 4°C in duplicate wells (100 µL extract per well). Then 5h at 30°C with rabbit PC conjugated to alkaline phosphatase. After 2h substrate incubation at room temperature, for alkaline phosphatase reaction absorbance values of well were measured at 405nm with a microplate reader. Samples were considered as positive when O.D. (optical density) values were greater than three times the means of the resultsuninfected control leaves.

Results:

Distribution of CTVof the aphid vector populations:

The main aphid vector species of CTV (*A.spiraecola* and *T.aurantii*) were found in Algerian citrus fruit cultivation (Fig. 1).During spring 2011-2012 *A. spiraecola* was prevalent in three exploitations of citrus fruit areas of Mitidja (2), (5), and (1).] (Fig.2). *T. aurantii*was less frequent and detected only in two exploitations of citrus fruit areas of Mitidja (1), and (5)(Fig.2).



Fig. 1: *Aphispiraecola*(Patch) (A) and *Toxopteraaurantii* (Boyer de fonscolombe)(B)

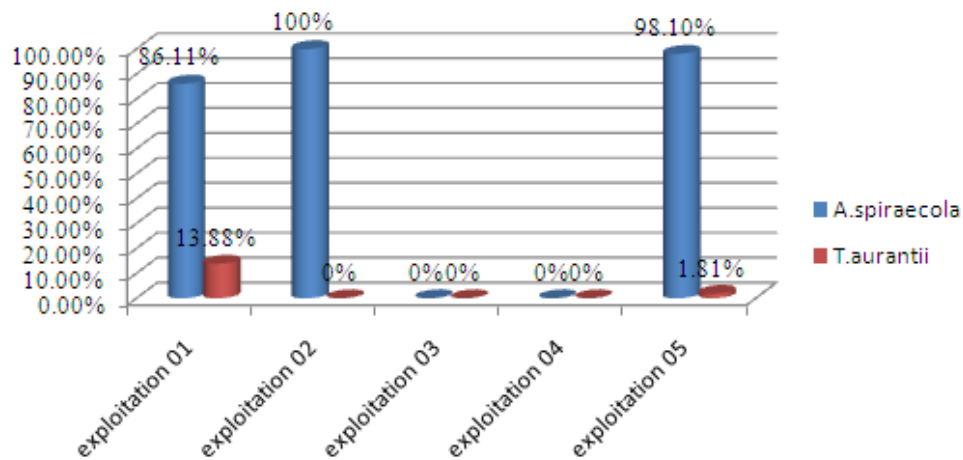


Fig. 2: Frequencies of aphid-infested citrus fruits crop areas in Algeria

Distribution and impact of CTV-like symptoms:

CTV-like symptoms were observed in all exploitations of citrus fruit areas of Mitidja. The most frequent symptom was the complete deterioration of the trees and yellowing (Fig.3).

Identification and distribution of CTV:

Only 25.18% (173/687) of the trees tested were positive with a specific antisera tested (Fig.4). The exploitation (6) is infected followed by the exploitation (5) and (2). Whereas the rate of infection of the exploitations (1) and (4) is very weak.



Fig. 3: Symptom of CTV in citrus fruit crop areas in Algeria
(A) complete deterioration of the trees (B) yellowing of leaves

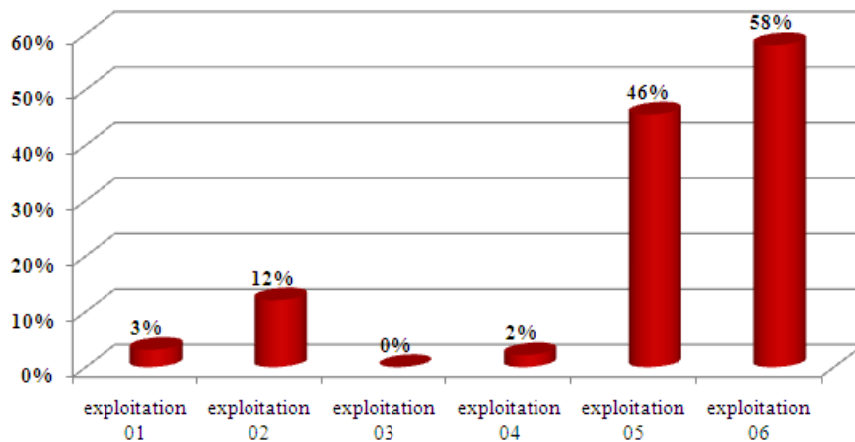


Fig. 4: Frequencies of the CTV in citrus fruit crop areas in Algeria

Discussion:

Surveys conducted in the main citrus fruit areas of Algeria during 2011-2012 seasons during the near flowering stage of citrus fruit showed the general presence of high aphid populations. In all areas *Aphis spiraecola* was prevalent and was frequently associated with yellowing of most leaves on all trees. The survey was limited to six exploitations and therefore gives only preliminary data on the distribution of the different aphid species. Despite the high percentage of trees showing CTV-like symptoms, ELISA tests to detect CTV were negative for (3/4) of the samples.

References

- Bayoud, H., 2001. Sérédetection du virus de la tristeza des citrus et du phytoplasme dans les parcs à bois et la cage d'isolement des agrumes de l'ITAFV. Mem. Ing.Blida, pp: 89.
- Bové, J.M.et R. Vogel, 1981. "Description and illustration of virus and virus-like diseases of citrus", Setco-IRFA, Paris, France
- Clark, M.F.et A.N. Adams, 1977. "Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruse", *Journal of General Virology*, 34: 475-483.
- Gottwald, T.R., G. Hughes, 2000. "A new survey method for Citrus tristeza virus disease assessment", Fourteenth IOCV Conference, pp: 77-87.
- Lee, R.F. and M. Bar-Joseph, 2000. Tristeza. In Timmer L.W., Gasney S.M., Graham J.H. Eds. *Compendium of citrus Diseases*. APS Press, St Paul M.N.