Bulletin of Insectology ***61*** *(2): 349-354, 2008*
ISSN 1721-8861

Influence of Chrysanthemum yellows phytoplasma on the fitness of two of its leafhopper vectors, *Macrosteles quadripunctulatus* and *Euscelidius variegatus*

Romina D'AMELIO[1,2], Simona PALERMO[2], Cristina MARZACHÌ[1], Domenico BOSCO[2]
[1]*Istituto di Virologia Vegetale, CNR, Torino, Italy*
[2]*Di.Va.P.R.A., Entomologia e Zoologia Applicate all'Ambiente "C. Vidano", Università di Torino, Grugliasco, Italy*

Abstract

Chrysanthemum yellows phytoplasma (CY) is a strain of aster yellows, which is classified as '*Candidatus* Phytoplasma asteris' (16 SrI-B). The amounts of CY cells as genome units per ng of insect DNA was measured in individuals of *Macrosteles quadripunctulatus* (Kirschbaum) (Rhynchota Cicadellidae) and *Euscelidius variegatus* (Kirschbaum) (Rhynchota Cicadellidae), using PCR on total DNA followed by real-Time PCR. The effects of CY infection in terms of longevity and fecundity were also measured. CY was propagated in daisy (*Chrysanthemum carinatum* Schousboe) where the third and the fourth instar nymphs were fed. Healthy leafhoppers and those fed on daisy were maintained on oat (*Avena sativa* L.). After emergence as adults, individuals from populations of infected insects were assayed for CY presence. All the ten tested specimens of *M. quadripunctulatus* were found to carry CY at high titres (on average 70,000 cells per ng of insect DNA) whereas nine out of 10 *E. variegatus* sampled were infected at considerably lower levels (on average 3,700 cells per ng of insect DNA). Survival times of both male and female *M. quadripunctulatus* were reduced by CY infection but no significant effect was found with *E. variegatus*. CY infection of *M. quadripunctulatus* females did not affect their fecundity, whereas that of *E. variegatus* females was slightly increased.

Key words: phytoplasma vectors, longevity, fecundity, pathogenicity, real-time PCR quantification.

Introduction

Phytoplasmas are wall-less, non-helical prokaryotes that colonize plant phloem and insects. They are associated with more than 600 diseases, some of great economic importance, in several hundred plant species (Seemüller *et al*., 1998; McCoy *et al*., 1989), with symptoms such as witches' broom, proliferation, flower malformation (virescence, phyllody), stunting and general decline. Currently, phytoplasma classification is mainly based on 16S rRNA gene grouping (Seemüller *et al*., 1998; Lee *et al*., 1998), but several strains have gained "*Candidatus*" species status (Bertaccini, 2007).

Phytoplasmas are transmitted by phloem-sucking insects in the families Cicadellidae, Cixiidae, Cercopidae, Derbidae, Delphacidae and Psyllidae in a persistent, propagative manner (Weintraub and Beanland, 2006). Phytoplasma colonisation of the vector involves passage into the midgut lumen, adhesion to midgut epithelium cells, passage between or through midgut cells, and subsequent invasion of haemolymph with transport to different organs and tissues, including salivary glands (Marzachì *et al*., 2004). Phytoplasmas multiply in the insect vector (Liu *et al*., 1994; Marzachì *et al*., 2004) and are injected into the plant phloem with saliva. There are several reports on the transovarial transmission of phytoplasmas to the progeny by infected females (Alma *et al*., 1997; Kawakita *et al*., 2000; Tedeschi *et al*., 2006). The intimate relationship between phytoplasmas and vectors suggests that some pathogenic effects on the insect could occur. However, the reported effects of phytoplasmas on insect hosts are conflicting, ranging from beneficial to harmful (Marzachì *et al*., 2004; Weintraub and Beanland, 2006). Reduced fitness in leafhoppers infected by X-disease and "flavescence dorée" phytoplasmas was reported (Whitcomb and Williamson, 1979; Bressan *et al*., 2005a; 2005b) whereas some insect hosts infected with phytoplasmas may show improved overwintering and increased fertility and longevity (Beanland *et al*., 2000; Ebbert and Nault, 2001).

For chrysanthemum yellows phytoplasma (CY), an aster yellows strain belonging to the '*Candidatus* Phytoplasma asteris' species (16 SrI-B), the interaction of the phytoplasma with its vector leafhoppers *Macrosteles quadripunctulatus* (Kirschbaum) and *Euscelidius variegatus* (Kirschbaum) have been studied to describe acquisition and transmission efficiencies, transmission pattern over time, latent period in the vector, effect of source plant species and insect life stage on CY acquisition and transmission (Bosco *et al*., 1997; 2007; Palermo *et al*., 2001). CY membrane proteins putatively involved in the adhesion to the insect cells have also been investigated (Galetto *et al*., 2007). The aim of this study was to investigate the influence of CY on the fitness of these two vectors, a feature that was not addressed so far.

Materials and methods

Phytoplasma strain, source plants and insect species

Chrysanthemum yellows phytoplasma (CY, 16SrI-B), a strain of '*Candidatus* Phytoplasma asteris' (Lee *et al*., 2004) was used. It was originally isolated from *Argyranthemum frutescens* (L.) Schultz-Bip in Liguria, Italy, and maintained by vector transmission in daisy, *Chrysanthemum carinatum* Schousboe. Healthy colonies of the leafhoppers *M. quadripunctulatus* and *E. variegatus*, vectors of CY (Bosco *et al*., 1997), were maintained in

oat (*Avena sativa* L.) inside plastic and nylon cages in growth chambers at 25 °C, photoperiod L16:D8 and checked by PCR assays to verify phytoplasma absence.

Longevity experiments

Third and fourth instar nymphs of *M. quadripunctulatus* and *E. variegatus* were taken from healthy colonies on oat and transferred to CY-infected daisy plants for an acquisition access period (AAP) of 7 days. Healthy nymphs were also caged on healthy daisy for the same period as a control. Following the AAP all nymphs were transferred to an oat plant until adult emergence. The newly emerged adults were collected at 3-day intervals and caged in groups on new oat plants. To test for survival, cages were checked daily and dead insects removed and sexed. Quartiles, i.e., time from adult emergence to 75% (t_{75}), 50% (t_{50}) and 25% (t_{25}) survival, were determined for each leafhopper survival distribution.

Fecundity experiments

Third and fourth instar nymphs from healthy colonies of each insect species were allowed to acquire CY as described above, and then maintained on healthy oat plants until emergence. The newly emerged females, together with some males to ensure that females were mated, were collected at 3-day intervals, counted, and caged in groups on healthy oat plants for oviposition. After two weeks the females were transferred to a new oat plant for a new oviposition period of two weeks. The cages were checked daily and the number of dead females recorded. Plants with eggs were maintained in a rearing chamber at 25 °C and a photoperiod of L16:D8 to allow hatching of the eggs and development of nymphs. After 40 days from the start of oviposition, progeny nymphs were counted. Fecundity was recorded as number of hatched nymphs, to avoid difficulty in counting endophytic eggs. The mean number of nymphs per female per day was calculated by dividing the total number of progeny nymphs (obtained in a two week oviposition period) by the total number of female/days of life.

Detection of CY in the leafhoppers

DNA extraction and PCR assays

To check the proportion of leafhoppers that acquired CY, phytoplasmas were assayed in single insects. Ten adults of each species were sampled from the CY-infected populations 3 weeks after the AAP. Negative controls were taken from the healthy colonies. For DNA extraction, the method of Marzachì *et al*. (1998) was used.

Group-specific primers R16(I)F1/R1 (Lee *et al*., 1994) were used to amplify CY in insect samples. Reaction and cycling conditions were as detailed in the original paper but the primers were used for direct, instead of nested, amplification from total DNA. DNA from leafhoppers fed on healthy plants and water instead of template DNA were used as controls. Aliquots of 7.5 µl PCR products were separated in 1% agarose gel, buffered in TBE (90 mM Tris–Borate, 2 mM EDTA, pH 8.3), stained with ethidium bromide and visualized under ultraviolet (UV) light.

Quantification of CY DNA in insects by quantitative-real time PCR

After detection of CY in the leafhoppers, phytoplasma DNA was quantified in relation to insect DNA using quantitative real-time PCR (qR-PCR) (Marzachì and Bosco, 2005). Reactions were performed in Platinum Quantitative Supermix-UDG (Invitrogen, UK) with 300 nM primers (for CY, CYS2Fw/Rv; for insect, MqFw/Rv) and 200 nM probes (for CY, CYS2Probe; for insect, MqProbe) (Marzachì and Bosco, 2005), together with 5 µL of the corresponding template, in a final volume of 25 µL. Total DNA extracted from 20 CY-infected insects (5 males and 5 females for each species) was measured with a Hoefer DyNA Quant 200 fluorimeter (Amarsham Pharmacia Biotech, U.S.). Diluted samples (1ng/µL in sterile double-distilled water) were analysed in triplicate by qR-PCR assays. Reactions were run in 96-well plates in a BioRad iCycler (BioRad) using described cycling conditions (Marzachì and Bosco, 2005). DNA from healthy insects of the two species and PCR mix devoid of template were used as negative controls. Threshold cycles and standard curves were automatically calculated by the BioRad iCycler software, version 3.06070. CY DNA from the samples was measured as femtogram (fg) of phytoplasma DNA per ng of insect DNA and then transformed into the number of CY genome units (GU) per ng of vector DNA as described in Marzachì and Bosco (2005). CY and host DNA from the same sample were quantified in the same plate. Absolute quantification of CY was achieved by comparison of threshold cycles (CTs) of the samples with those of four dilutions (2 ng, 20 pg, 200 fg, and 2 fg) of the plasmid, pOP74, containing CY 16S rDNA target sequence. Absolute quantification of insect DNA was achieved by comparison of the CTs of the samples with those of four dilutions (50, 10, 5 ng, and 50 pg) of the corresponding healthy insect DNA. To compare the phytoplasma titres in individual specimens of the two species, the t-test was applied to CY quantities measured by qR-PCR assays. For all the analyses, raw data (CY GU/ng of daisy DNA) were transformed into logarithms, since the standard deviation appeared a function of the mean.

Results

CYP detection and quantification

When tested singly by PCR, all the *M. quadripunctulatus* and 9 out of 10 *E. variegatus* samples gave the 16SrDNA I-B specific amplification product of 1100 bp (not shown) proving that leafhoppers fed on phytoplasma-source plants were actually infected.

In qR-PCR assays, calibration using known amounts of serially diluted plasmid containing the phytoplasma 16S rDNA target sequence gave a straight line, confirming reliability of phytoplasma quantification. One fg of this plasmid corresponds to 97 CY genome units (GU) (Marzachì and Bosco, 2005). In *M. quadripunctulatus* the quantity of CY GU was estimated as 69,642 ± 12,799 (mean ± S.E.), while in *E. variegatus* it was 3,713 ± 1,094 GU per ng of insect DNA. Data on CY

and insect DNA quantification are shown in table 1. The mean phytoplasma titre was significantly different in the two species ($t = 7.274$; $P < 0.001$). CY quantities measured in males and females leafhoppers of both species did not differ.

Longevity

CY-infected and healthy leafhopper survivals were compared. Reduced male and female longevity was observed in CY-infected *M. quadripunctulatus* but not in *E. variegatus*, as shown in table 2. For *M. quadripunctulatus* mean survival times (in days ± SD) were significantly different between healthy and CY-infected males ($t = 3.238$; $P = 0.002$), and females ($t = 3.031$; $P = 0.003$). For *E. variegatus*, slightly reduced longevity was recorded for females only, but the difference was not significant ($t = 1.796$; $P = 0.078$).

Healthy *E. variegatus* males and females had a longer life span compared to healthy *M. quadripunctulatus* (males, $t = 4.877$, $P < 0.001$; females, $t = 7.333$, $P < 0.001$). Moreover, healthy females of both species lived longer than males (*M. quadripunctulatus*, $t = -3.515$; $P \leq 0.001$; *E. variegatus* $t = -2.103$; $P = 0.041$).

Table 1. Quantification of chrysanthemum yellows phytoplasma DNA (genome units, GU) in relation to leafhopper DNA by real time PCR. Leafhoppers were analysed 21 days post-acquisition.

no. insect	*E. variegatus* GU/ ng insect DNA	*M. quadripunctulatus* GU/ ng insect DNA
1	10,602	32,243
2	4,223	72,246
3	5,430	70,305
4	3,280	61,207
5	1,240	123,615
6	5,863	74,509
7	347	21,494
8	555	90,162
9	1,877	136,667
10	Not detected	13,981
Mean ± S.E.	3,713 ± 1,094	69,642 ± 12,799

Fecundity

Fecundity data are summarised in table 3 and expressed as the average number of nymphs hatched from eggs laid per adult female per day of life. The average number of *M. quadripunctulatus* offspring was similar between CY-infected and healthy females in both oviposition periods. CY-infected *E. variegatus* females laid a slightly higher number of eggs compared to uninfected ones, especially in the second oviposition period when latency was completed and CY phytoplasmas had already colonised the whole body.

Discussion

The present study shows that CY significantly reduced the longevity of *M. quadripunctulatus* but not the one of *E. variegatus*. CY-infection reduced the life-span of both males and females of both species (figures 1 and 2); a weak reduction in longevity of *E. variegatus* females

Table 2. Quartiles of survival distribution (t_{75}, t_{50} and t_{25}) of the two leafhopper species fed on healthy or CY-infected daisy plants.

	parameters	Healthy	Infected
M. quadripunctulatus male (healthy n=65; infected n=33)	t_{75}	29.3	15.6
	t_{50}	34.8	29.2
	t_{25}	44.2	33.5
M. quadripunctulatus female (healthy n=74; infected n=41)	t_{75}	33.2	27.8
	t_{50}	44.6	33.4
	t_{25}	53.5	40.9
E. variegatus male (healthy n= 23; infected n=36)	t_{75}	35.1	50.2
	t_{50}	55.2	59.2
	t_{25}	70.8	70.5
E. variegatus female (healthy n=23; infected n=39)	t_{75}	55.6	59.4
	t_{50}	76.6	67.4
	t_{25}	92.3	76.6

Table 3. Fecundity expressed as the average number of nymphs hatched from eggs laid per adult female per day of life.

Species	Oviposition period (Days after emergence)	N° nymphs/female/day (n*) Infected	Healthy
M. quadripunctulatus	1-14	1.31 (n = 23)	1.35 (n = 28)
"	15-28	1.05 (n = 20)	0.86 (n = 23)
E. variegatus	1-14	1.02 (n = 21)	0.83 (n = 23)
"	15-28	1.33 (n = 17)	1.09 (n = 19)

*number of ovipositing females.

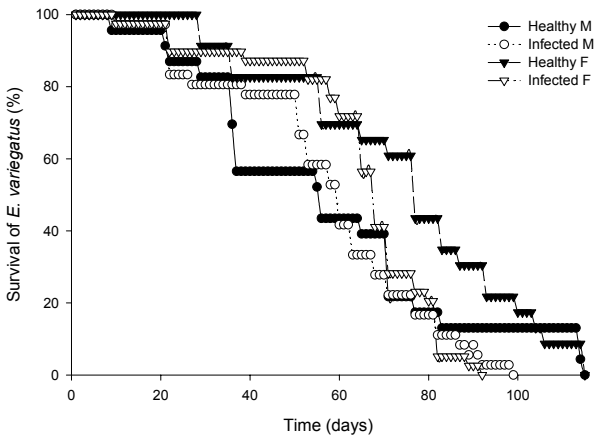

Figure 1. Survival of healthy and CY-infected males (M) and females (F) *E. variegatus*. Days were counted from adult emergence.

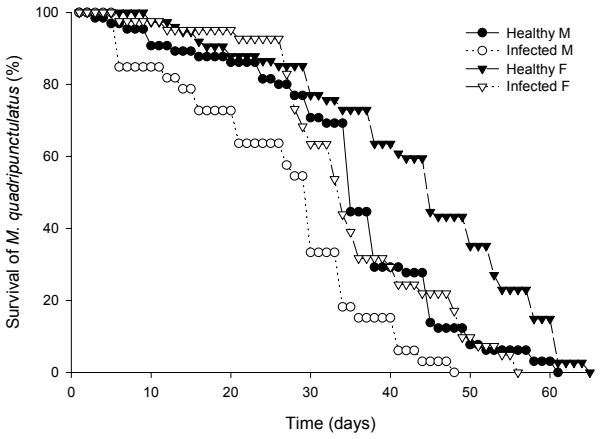

Figure 2. Survival of healthy and CY-infected males (M) and females (F) *M. quadripunctulatus*. Days were counted from adult emergence.

cannot be excluded. *M. quadripunctulatus* life was short than the one of *E. variegatus*, irrespective of CY infection; therefore, *E. variegatus*, once infected, can act longer as a vector. On the other hand, *M. quadripunctulatus* transmits CY with 100% efficiency while *E. variegatus* is less efficient, about 80%, under the same experimental conditions, as also shown by Bosco *et al*. (2007).

The fecundity of both vector species seems not be affected by CY. For *E. variegatus,* a slight increase in the number of eggs laid by infected females was recorded, although in our experimental set-up (oviposition by grouped females) the data could not be statistically analysed. For *M. quadripunctulatus* no differences in the number of eggs was found between infected and healthy females during the first four weeks after emergence; however, infected females are likely to lay fewer eggs because of their shorter life span.

Beanland *et al*. (2000) found that *Macrosteles quadrilineatus* (Forbes) infected with both bolt and severe strains of aster yellows (AY) lived longer than healthy ones, but only bolt-infected females showed improved prolificity. The association between *Dalbulus maidis* (DeLong et Wolcott) and maize bushy stunt phytoplasma was also found to be mutualistic, and leafhopper survival improved after exposure to the pathogen (Ebbert and Nault, 2001). For *D. maidis* infected with each of 3 strains of AY (Dwarf, Severe and Tulelake *sensu* Freitag, 1964) the increased longevity was likely due to the superior nutritional quality of the infected *Callistephus chinensis* Nees rather than to the direct effect of the phytoplasma on the insect (Purcell, 1988). Furthermore, corn stunt spiroplasma affects *D. maidis* survival only in association with predation by *Gonatopus bartletti* Olmi (Moya-Raygoza *et al*., 2008). In contrast, pathogenic effects on the vectors have been observed for "flavescence dorée phytoplasma" (FD), 16SrV-C subgroup, in the natural vector *Scaphoideus titanus* Ball (Bressan *et al*., 2005a) and in the laboratory vector *E. variegatus* (Bressan *et al*., 2005b); two strains of FD decreased the fitness of their vectors reducing adult life span, fecundity and egg hatching rate. Similarly, X-disease phytoplasma is clearly pathogenic to its vectors *Colladonus montanus* (Van Duzee) (Jensen, 1958), *Colladonus geminatus* (Van Duzee) (Jensen, 1959) and *Paraphlepsius irroratus* (Say) (Garcia-Salazar *et al*., 1991). The pathogenicity of X-disease phytoplasma to his vector *C. montanus* results from direct effects of phytoplasma on the host salivary, neural, adipose and alimentary tract tissues (Whitcomb *et al*., 1966; 1967; 1968) and not from indirect effects due to the altered physiology of diseased host plants (Jensen, 1959).

Under our experimental conditions, leafhoppers were allowed to feed on infected plants for 7 days only, therefore we suggest that a direct effect of CY, rather than physiological or nutritional alterations of the infected source plant, may account for the reduction of longevity in *M. quadripunctulatus*. Furthermore, in this latter species, CY titre was very high and this can be the reason of its pathogenic effect.

Phytoplasmas can affect leafhopper longevity in a temperature-dependent manner (Murral *et al*., 1996; Moya-Raygoza and Nault, 1998). Although we did not study the influence of temperature on longevity, CY multiplication is not significantly affected between 20 and 30 °C, while at 15 °C CY multiplies slowly (unpublished data). It is therefore likely that at lower temperatures, CY is less pathogenic.

The different results obtained with different pathogen-insect interaction, can be explained by different times of evolutionary interaction between phytoplasma and vector in the different associations. Low pathogenicity or mutualistic effects are likely to occur only in well-adapted vectors, and this can explain the situation of *M. quadrilineatus*, which has probably co-evolved with some American aster yellows strains for a long time. However, the presence of many aster yellows strains as well as of many vector species of the same phytoplasma provides possibilities for different phytoplasma-vector interactions, from the mutualistic to the pathogenic (Marzachì *et al*., 2004). For the maize feeder *D. maidis* the association with maize bushy stunt (MBS) was found to be mutualistic, and survival of leafhoppers im-

proved after exposure to the pathogen (Ebbert and Nault, 2001) but for some *Dalbulus* species not associated with maize, the MBS infection caused a clear reduction in longevity (Madden *et al*., 1983).

Our study shows that CY multiplies faster in *M. quadripunctulatus* compared to *E. variegatus* (Bosco *et al*., 2007). Therefore, the longevity reduction suffered by the former vector could be related to the higher and faster phytoplasma multiplication rate. This finding strongly suggests that multiplication and pathogenicity are correlated. Rapid multiplication of CYP in *M. quadripunctulatus* is required for transmission of the pathogen by this short-living vector under natural conditions.

References

ALMA A., BOSCO D., DANIELLI A., BERTACCINI A., VIBIO M., ARZONE A., 1997.- Identification of phytoplasmas in eggs, nymphs and adults of *Scaphoideus titanus* Ball reared on healthy plants.- *Insect Molecular Biology*, 6: 115-121.

BEANLAND L., HOY C. W., MILLER S. A., NAULT L. R., 2000.- Influence of aster yellows phytoplasma on the fitness of aster leafhopper (Homoptera: Cicadellidae).- *Annals of the Entomological Society of America*, 93: 271-276.

BERTACCINI A., 2007.- Phytoplasmas: diversity, taxonomy, and epidemiology.- *Frontiers in Bioscience*, 12: 673-689.

BOSCO D., MINUCCI C., BOCCARDO G., CONTI M., 1997.- Differential acquisition of chrysanthemum yellows phytoplasma by three leafhopper species.- *Entomologia Experimentalis et Applicata*, 83: 219-224.

BOSCO D., GALETTO L., LEONCINI P., SARACCO P., RACCAH B., MARZACHÌ C., 2007.- Interrelationships between "Candidatus phytoplasma asteris" and its leafhopper vectors (Homoptera: Cicadellidae).- *Journal of Economic Entomology*, 100 (5): 1504-1511.

BRESSAN A., GIROLAMI V., BOUDON PADIEU E., 2005a.- Reduced fitness of the leafhopper vector *Scaphoideus titanus* exposed to Flavescence dorée phytoplasma.- *Entomologia Experimentalis et Applicata*, 115: 283-290

BRESSAN A., CLAIR D., SÉMÉTEY O., BOUDON-PADIEU É., 2005b.- Effect of two strains of Flavescence dorée phytoplasma on the survival and fecundity of the experimental leafhopper vector *Euscelidius variegatus* Kirschbaum.- *Journal of Invertebrate Pathology*, 89 (2): 144-149.

EBBERT M. A., NAULT L. R., 2001.- Survival in *Dalbulus* leafhopper vectors improves after exposure to maize stunting pathogens.- *Entomologia Experimentalis et Applicata,* 100: 311-324.

FREITAG J. H., 1964.- Interactions and mutual suppression among three strains of aster yellows virus.- *Virology*, 24: 401-413.

GALETTO L., BOSCO D., FLETCHER J., MARZACHÌ C., 2007.- Production of polyclonal and monoclonal antibodies specific against membrane proteins of "*Candidatus* Phytoplasma asteris", chrysanthemum yellows isolate.- *Bulletin of Insectology*, 60 (2): 211-212.

GARCIA-SALAZAR C., WHALON M. E., RAHARDJA U., 1991.- Temperature-dependent pathogenicity of the X-disease mycoplasma-like organism to its vector, *Paraphlesius irroratus* (Homoptera: Cicadellidae).- *Environmental Entomology*, 20: 179-184.

GUNDERSEN D. E., LEE I. M., REHNER S. A., DAVIS R. E., KINGSBURY D. T., 1994.- Phylogeny of mycoplasma like organisms (phytoplasmas) – a basis for their classification.- *Journal of Bacteriology*, 176: 5244-5254.

JENSEN D. D., 1958.- Reduction in longevity of leafhoppers carryng peach yellow leaf roll virus.- *Phytopathology*, 48: 394.

JENSEN D. D., 1959.- A plant virus lethal to its insect vector.- *Virology*, 8: 164-175.

KAWAKITA H., SAIKI T., WEI W., MITSUHASHI W., WATANABE K., SATO M., 2000.- Identification of mulberry dwarf phytoplasmas in the genital organs and eggs of Leafhopper *Hishimonoides sellatiformis.*- *Phytopathology*, 90: 909-914.

LEE I. M., GUNDERSEN D. E., HAMMOND R. W., DAVIS R. E., 1994.- Use of mycoplasma-like organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed MLO infection in a single host plant.- *Phytopathology*, 84: 559-566.

LEE I. M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M. 1998.- Revised classification scheme of phytoplasmas based an RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

LEE I. M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BOTTNER K. D., MARCONE C., SEEMULLER E., 2004.- 'Candidatus Phytoplasma asteris' a novel phytoplasma taxon associated with aster yellow and related disease.- *International Journal of Systematic and Evolutionary Microbiology*, 54: 1037-1048.

LIU H. W., GOODWIN P. H., KUSKE C. R., 1994.- Quantification of DNA from the aster yellows mycoplasma like organism in aster leafhoppers (*Macrosteles fascifrons* Stål) by a competitive polymerase chain reaction.- *Systematic and Applied Microbiology*, 17: 274-280.

MADDEN L. V., NAULT L. R., 1983.- Differential pathogenicity of corn stunting mollicutes to leafhopper vectors in Dalbulus and Baldulus species.- *Phytopathology*, 73: 1608-1614.

MARZACHÌ C., BOSCO D., 2005.- Relative quantification of chrysanthemum yellows (16Sr I) phytoplasma in its plant and insect host using Real Time PCR.- *Molecular Biotechnology*, 30: 117-127.

MARZACHÌ C., VERATTI F., BOSCO D., 1998.- Direct PCR detection of phytoplasmas in experimentally infected insects.- *Annals of Applied Biology*, 133: 45-54.

MARZACHÌ C., MILNE R. G., BOSCO D., 2004.- Phytoplasma-plant-vector relationships.- *Recent Research Developments in Plant Pathology*, 3: 211-241.

MCCOY R. E., CAUDWELL A., CHANG C. J., CHEN T. A., CHIYKOWSKI L. N., COUSIN M. T., DALE J. L., DE LEEUW G. T. N., GOLINO D. A., HACKETT K. J., KIRKPATRICK B. C., MARWITZ R., PETZOLD H., SINHA R. C., SUGIURA M., WHITCOMB R. F., YANG I. L., ZHU B. M., SEEMULLER E., 1989.- Plant diseases associated with mycoplasma-like organisms, pp. 546-640. In: *The Mycoplasmas. Vol. 5* (WHITCOMB R. F., TULLY J. G., Eds).- Academic Press, New York, USA.

MOYA-RAYGOZA G., NAULT L. R., 1998.- Transmission biology of maize bushy stunt phytoplasma by the corn leafhopper (Homoptera: Cicadellidae).- *Annals of the Entomological Society of America*, 91: 668-676.

MOYA-RAYGOZA G., CHACÓN-TORRES N. M., PALOMERA-AVALOS V., BECERRA-CHIRON I. M., BERNAL J. S., 2008.- Effects of corn stunt spiroplasma on survival of *Dalbulus maidis* (Hemiptera: Cicadellidae) parasitized or preyed upon by *Gonatopus bartletti* (Hymenoptera: Dryinidae) or infected by *Metarhizium anisopliae*.- *Annals of the Entomological Society of America*, 101 (1): 113-118.

MURRAL D. J., NAULT L. R., HOY C. W., MADDEN L. V., MILLER S. A., 1996.- Effects of temperature and vector age on transmission of two Ohio strains of aster yellows phytoplasma by the aster leafhopper (Homoptera: Cicadellidae).- *Journal of Economic Entomology*, 89: 1223-1232.

NICOLAISEN M., BERTACCINI A., 2007.- An oligonucleotide microarray-based assay for identification of phytoplasma 16S ribosomal groups.- *Plant Pathology*, 56: 332-336.

PALERMO S., ARZONE A., BOSCO D., 2001.- Vector-pathogen-host plant relationships of chrysanthemum yellows (CY) phytoplasma and the vector leafhoppers *Macrosteles quadripunctulatus* and *Euscelidius variegatus*.- *Entomologia Experimentalis et Applicata*, 99: 347-354.

PURCELL A. H., 1988.- Increased survival of *Dalbulus maidis* Delong & Wolcott, a specialist on maize, on non host plants infected with mollicute plant pathogens.- *Entomologia Experimentalis et Applicata*, 46:187-196.

SCHNEIDER B., MARCONE C., KAMPMANN M., RAGOZZINO A., LEDERER W., COUSIN M. T., SEEMULLER E., 1997.- Characterization and classification of phytoplasmas from wild and cultivated plants by RFLP and sequence analysis of ribosomal DNA.- *European Journal of Plant Pathology*, 103: 675-686.

SEEMÜLLER E., MARCONE C., LAUER U., RAGOZZINO A., GÖSCHL M., 1998.- Current status of molecular classification of the phytoplasmas.- *Journal of Plant Pathology*, 80: 3-26.

TEDESCHI R., FERRATO V., ROSSI J., ALMA A., 2006.- Possible phytoplasma transovarial transmission in the psyllids *Cacopsylla melanoneura* and *Cacopsylla pruni*.- *Plant Pathology*, 55:18.

WEINTRAUB P. G., BEANLAND L., 2006.- Insect Vectors of Phytoplasmas.- *Annual Review of Entomology*, 51: 91-111.

WHITCOMB R. F., WILLIAMSON D. L., 1979.- Pathogenicity of mycoplasmas for arthropods.- *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, 245 (1-2): 200-221.

WHITCOMB R. F., SHAPIRO M., RICHARDSON J., 1966.- An Erwinia like bacterium pathogenic to leafhoppers.- *Journal of Invertebrate Pathology*, 8: 299-307.

WHITCOMB R. F., JENSEN D. D., RICHARDSON J., 1967.- The infection of leafhoppers by Western X-disease virus. III. Salivary, neural, and adipose histopathology.- *Virology*, 31: 539-549.

WHITCOMB R. F., JENSEN D. D., RICHARDSON J., 1968.- The infection of leafhoppers by western X-disease virus : IV. Pathology in the alimentary tract.- *Virology*, 34: 69-78.

Authors' addresses: Domenico Bosco (corresponding author, domenico.bosco@unito.it), Simona PALERMO, Università degli Studi di Torino, Di.Va.P.R.A. Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy; Romina D'AMELIO, Cristina MARZACHÌ, Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, 10135Torino, Italy.

Received July 22, 2008. Accepted October 24, 2008.