CSIRO PUBLISHING
www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2009, **38**, 437–443

Epidemiological aspects of garlic decline disease caused by a phytoplasma in Asiatic and Argentinean garlic cultivars

Ernestina Galdeano[A,F], *Luis R. Conci*[B], *Orlando González*[C], *Susana Paradell*[D], *Julio A. Di Rienzo*[E], *Claudia Nome*[B] *and Vilma C. Conci*[B,F,G]

[A]Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina.
[B]Instituto de Fitopatología y Fisiología Vegetal INTA, Córdoba, Argentina.
[C]Estación Experimental Agropecuaria INTA, Pocito, San Juan, Argentina.
[D]División Entomología, Facultad de Ciencias Naturales y Museo, La Plata, Argentina.
[E]Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina.
[F]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
[G]Corresponding author. Email: vconci@correo.inta.gov.ar

Abstract. Garlic decline is a disease that has been detected in most of the garlic-growing areas of Argentina. The associated pathogen has been identified as a 16SrIII-group phytoplasma. Little is known, however, about epidemiological aspects of the disease. Incidence and prevalence of the disease were analysed during 3 consecutive years in fields of an Asiatic garlic cultivar (Chino) and two Argentinean cultivars (Blanco and Colorado) from the principal garlic-growing areas of Argentina. Although low incidence was registered (0.03–0.78%), disease prevalence was high in both sampled regions (23–100% fields had at least one diseased plant). Cultivars Chino and Blanco were more susceptible to the disease since incidence and prevalence were significantly higher than in cv. Colorado. The pathogen was detected by polymerase chain reaction (PCR) in a high proportion of cloves from infected plants and in plants derived from potentially infected cloves, showing that the phytoplasma can be transmitted from a crop cycle to the following by planting infected cloves. The phytoplasma was detected by transmission electronic microscopy, PCR and dot blot immunoassay in all parts of the symptomatic plants, and the highest pathogen concentration was found in root tissues. Although 22 leafhopper species were found associated with the garlic crop, insect populations were low during the whole crop cycle.

Additional keywords: *Allium sativum*, 'tristeza del ajo'.

Introduction

Garlic (*Allium sativum*) is an Asiatic crop which has spread to other continents and is now broadly cultivated. This species is highly valued for its medicinal properties as well as a food spice worldwide. China is by far the largest producer of garlic, with over 75% of world tonnage (Food and Agricultural Organization of the United Nations 2005), and is the main world exporter. Although the people of Argentina are not great consumers of garlic, Argentina is the second largest world exporter of garlic. Of the nearly 130 000 t of garlic produced per year in Argentina, 64% is destined for exportation. Approximately 80% of the area where garlic is cultivated corresponds to the provinces of Mendoza and San Juan, in the Cuyo region (Argentina's central Andes) (Portela 2007). Blanco and Colorado are cultivars traditionally planted in Argentina whereas the Chino cultivar was introduced from Asia within the last 20 years, and represents a high percentage of the garlic cultivated area at this time.

Although garlic is an annual crop, its exclusively vegetative reproduction favours the propagation of systemic diseases through successive crop cycles. For example, chronic infections with a virus complex from the recurring plantation of infected cloves would generate great yield losses unless virus-free plants were periodically introduced (Walkey and Antill 1989; Lot *et al*. 1998; Conci *et al*. 2003; Cafrune *et al*. 2006).

The exchange of genetic material is a frequent practice among garlic producing countries. This has facilitated the spread of a great number of systemic pathogens like viruses and possibly phytoplasmas. In 1992, a disease known as garlic decline, 'tristeza del ajo', was reported in Argentina. It was associated with the presence of pleomorphic wall-less organisms in sieve cells observed by transmission electronic microscope (TEM) (Conci *et al*. 1992). Phytoplasmas were detected later by polymerase chain reaction (PCR) and serological tests using a polyclonal antiserum produced against China-tree decline (ChTDIII) phytoplasma (Conci *et al*. 1998). The garlic decline phytoplasma (GDIII) has been associated with the X-disease group (16SrIII) subgroup J by PCR-restriction fragment length polymorphism and sequence analysis of the 16S ribosomal gene (Galdeano *et al*. 2004). Typical symptoms of garlic decline are leaf reddening or

 10.1071/AP09019 0815-3191/09/040437

yellowing, depending on the genotype, and wilt and general plant decline, which then lead to plant death. Severely infected bulbs are often darker than non-infected ones, and have a dehydrated or mummified appearance.

Since phytoplasmas are transmitted to new plant hosts in a circulative and persistent manner by phloem-feeding insects (Fletcher *et al*. 1998), the population and transmission efficiency of the vector is a relevant component of the disease epidemiology. All identified phytoplasma vectors correspond to the Order *Hemiptera*; among them, leafhoppers Cicadomorpha (*Cicadellidae*) represent the largest group, followed by planthoppers Fulgoromorpha (*Fulgoroidea*), one cixiid (*Cixiidae*) and two genera of psyllids (*Psyllidae*) (Weintraub and Beanland 2006). The vector for the GDIII phytoplasma and the closely related ChTDIII phytoplasma is unknown.

In preliminary observations of the garlic decline field behaviour, plants with different degrees of decline severity have been found. Among them, there were plants that only showed leaf colour change symptoms by the end of the crop cycle. Such plants had been able to form bulbs that were suspected to be carrying the phytoplasma (Conci *et al*. 1998). However, there is no information regarding GDIII phytoplasma survival and transmission via seed cloves. This might have an important impact on the disease epidemiology because it represents an inoculum source present in the field from the beginning of the crop cycle.

This paper reports epidemiological components of the garlic decline disease, including the detection of the phytoplasma in clove seeds and the transmission of the phytoplasma through them. The results of a 3-year survey performed in garlic fields of the two main production provinces of Argentina are reported, including the incidence, prevalence and distribution of garlic decline in Asiatic (Chino) and Argentinean (Blanco and Colorado) cultivars. The occurrence of *Hemiptera* species present in garlic fields during the surveys, which may represent potential phytoplasma vectors, was also determined.

Materials and methods

Field surveys

Surveys were conducted in Mendoza and San Juan provinces during three crop cycles (2000, 2001 and 2002). Disease incidence was estimated in each field by visual symptom inspection in five randomly distributed plots of 200 plants each, and was expressed as the percentage of GDIII phytoplasma symptomatic plants. The plants with ambiguous symptoms were analysed by PCR to confirm the diagnosis. In each sampling site, fields cultivated with garlic cultivars Blanco, Chino and Colorado were evaluated. The number of fields corresponding to each cultivar was proportional to the planted area in each region, during each year. Disease prevalence was calculated as the percentage of fields with at least one diseased plant.

Since preliminary data had shown that symptom expression in infected plants occurs from September and that such plants die 30–45 days later, two surveys were conducted per year, in early September and early November, and the cumulative disease incidence for a crop cycle was calculated by adding both datasets. Approximately 720, 220 and 330 ha were surveyed during the years 2000, 2001 and 2002, respectively.

Data were analysed by ANOVA and Tukey's multiple comparison test using the statistics program InfoStat (2003) Professional version 1.5 following arcsin-transformation of percentages. The goodness-of-fit test of Poisson and negative binomial distributions were performed for the number of diseased plants per field, grouped by year and site.

Distribution of GDIII phytoplasma in garlic plants

The occurrence of GDIII phytoplasma was tested in different plant organs using serological (nitrocellulose dot blot immunoassay), molecular (PCR), and TEM analyses. Plants with symptoms of leaf reddening but not decline were collected from the field 30 days before harvest: 10 plants of garlic cv. Colorado and 25 plants of garlic cv. Blanco. Tissue samples were obtained from leaves (the youngest, second and basal), pseudostem, bulb protective leaves, one random clove and roots. Asymptomatic samples were used as negative controls.

Immunoassays were performed on nitrocellulose [Bio-Rad Trans-Blot transfer medium, 0.45-μm pore (Bio-Rad, Hercules, CA)] using the polyclonal antiserum produced from the ChTDIII phytoplasma (ChTDIIIas), able to react against related phytoplasmas (Gómez *et al*. 1996). The plant samples were homogenised in 0.02 M TRIS-HCl buffer + 0.5 M NaCl, and three serial dilutions were used (1 : 20, 1 : 40, 1 : 80). The antiserum was preadsorbed with healthy plant sap to avoid reaction against normal plant proteins.

For the PCR reactions, total DNA was purified from different tissue samples of five plants of each garlic cultivar. Plant tissue from roots, cloves, bulb protective leaves, pseudostem, and leaves were ground separately with a sterile pestle and mortar using liquid air, and total DNA was extracted with 2% hexadecyltrimethil-ammonium bromide following the protocol from Doyle and Doyle (1990). DNA was also extracted from the same tissues of healthy plants used as negative controls. For PCR detection, phytoplasma universal primers that amplify a 1.2-kb fragment of the 16S rRNA gene (R16F2/R16R2, Lee *et al*. 1993) were used. The PCR reactions were performed according to Galdeano *et al*. (2004). The amplification results were evaluated by 1% agarose gel electrophoresis, stained with ethidium bromide and observed under ultraviolet light.

Samples of 2 × 3 mm, taken from the different garlic organs, were fixed in 2.5% glutaraldehyde – 2.5% paraformaldehyde, postfixed in 1% osmium tetroxide and contrasted in 0.5% uranyl acetate. The samples were dehydrated in acetone and embedded in Spurr low viscosity resin. Ultrathin sections were prepared with a diamond knife and contrasted with lead citrate and 2% uranyl acetate. The sections were observed on a TEM (Jeol JEM 1220 EXII, Tokyo, Japan at 4000–10 000×).

Survival of GDIII phytoplasma in garlic bulbs

To evaluate whether the phytoplasma survived in the cloves from one cycle to the next, bulbs from previously tested

diseased plants were harvested from the field and stored in a cool room (16°C) until they were analysed. Since some cloves from each diseased bulb could not completely develop, probably as a consequence of the phytoplasma infection, all malformed or mummified cloves were discarded from the analysis. Vernalised viable cloves from eight bulbs were analysed by PCR for the presence of phytoplasma in the storage leaf and sprout.

At the same time, in order to evaluate the persistence of the phytoplasma in plantlets grown from potentially infected cloves and the natural infection of plants, a 2-year field experiment was performed. Symptomatic and asymptomatic garlic plants were harvested, and phytoplasmal infection was confirmed by PCR analysis from leaf tissues. The bulbs originated from PCR-positive plants were selected for the diseased treatment while the negative ones were used as controls. During the first year, nine bulbs of cv. Blanco were assayed for each treatment. The following year, cloves from six bulbs of cv. Blanco and six of cv. Chino were planted. Plantlet emergence was evaluated 20 days after planting. Plant symptoms were registered and leaf samples were analysed by PCR for phytoplasma detection at monthly intervals.

Insect collection

In order to obtain a preliminary list of the potential vectors of GDIII phytoplasma, insects were trapped from garlic fields located in San Juan during cycles 2000, 2001 and 2002. Between 6 and 10 yellow sticky traps (Sticky Strips, Olson Products, Medina, OH) were installed on two fields each year, on wooden sticks 20 cm above canopy, varying the altitude according to the crop growth. The number of insects captured during the 2000 crop cycle was notably low because of the regular insecticide applications. Therefore, during years 2001 and 2002, assays were carried out in an organic field and also in a field using conventional management. Traps were harvested and replaced every 15 days during the crop cycle (from June to November). The insects that had adhered to the traps were removed using an organic solvent, and leafhoppers were separated and stored in 70% ethanol for taxonomic identification. Insect species were identified by specialists of the Museo de Ciencias Naturales (MLP), La Plata, Argentina, and insect voucher specimens were deposited in the MLP entomological collection.

Results

Garlic decline incidence and prevalence

Garlic decline incidence was low in both sampling areas (Table 1). The difference of incidence percentage for each sampling date between San Juan and Mendoza was not significant, except for November 2001. The highest accumulated mean disease incidence (0.78%) was registered during 2000 in Mendoza whereas the lowest (0.03%) corresponded to 2001, ranging from 0.03 to 0.23% of diseased plants in Mendoza and San Juan, respectively. However, the lowest disease prevalence was registered in 2001 in Mendoza (4.7%), in concurrence with the lowest incidence percentages. The highest disease prevalence corresponded to San Juan province during 2000 and 2002 (100 and 95%, respectively). Regardless of the geographical origin, disease incidence and prevalence in garlic cv. Colorado was significantly lower than in cvv. Chino and Blanco. The lowest values registered were 0.01% diseased plants and 18% fields with diseased plants for cv. Colorado whereas the highest recorded values for cv. Blanco were 1.17% incidence and 96% prevalence (Table 2).

The number of diseased plants recorded for each field, grouped by year and by site (San Juan, Mendoza) fitted a negative binomial distribution, but not a Poisson distribution, showing an aggregated spatial pattern. This type of distribution implies that, even within the low incidence registered, some fields had a high number of diseased plants although others had very low or no diseased plants. However, the line plot of the disease prevalence against the disease incidence showed that high disease prevalence (higher than 80%) corresponded to less than 1% incidence percentages (Fig. 1).

Table 1. Garlic decline incidence and prevalence in San Juan and Mendoza provinces during years 2000, 2001 and 2002

Year	Month	Sampling site	No. of fields	Partial incidence (%)[A]	Accumulated incidence (%)[B]	Prevalence (%)
2000	September	Mendoza	14	0.26	–	71.4
	September	San Juan	[C]	–	–	–
	November	Mendoza	29	0.52	0.78	89.6
	November	San Juan	21	0.67	0.67	100.0
2001	September	Mendoza	25	0.03	–	40.0
	September	San Juan	16	0.08	–	43.7
	November	Mendoza	21	0.003a	0.03	4.7
	November	San Juan	6[D]	0.15b	0.23	83.3
2002	September	Mendoza	23	0.21	–	69.5
	September	San Juan	21	0.37	–	95.2
	November	Mendoza	22	0.28	0.49	72.7
	November	San Juan	16	0.32	0.69	88.2

[A]Different letters within the same month and year represent significant difference according to Tukey's multiple comparison test ($P < 0.05$).
[B]Accumulated disease incidence for each year, resulting from the addition of September and November disease incidence mean.
[C]No data available.
[D]Only six fields were sampled in San Juan on November 2001 because many garlic fields had been harvested earlier.

Table 2. Average incidence and prevalence of garlic decline in fields of garlic cvv. Blanco, Chino and Colorado in Mendoza and San Juan provinces during 2000, 2001 and 2002

Year	Garlic cultivar	Number of fields	Incidence (%) Mean[A]	Incidence (%) Accumulated[B]	Prevalence (%)
2000	Blanco	27	0.71b	1.17	96.3
	Chino	12	0.68b	1.17	91.6
	Colorado	25	0.24a	0.37	84
2001	Blanco	26	0.07b	0.14	42.3
	Chino	20	0.03ab	0.04	40
	Colorado	22	0.01a	0.03	18.2
2002	Blanco	25	0.38b	0.76	92
	Chino	34	0.36b	0.74	88.2
	Colorado	23	0.10a	0.19	60.9

[A]Different letters within the same year represent significant difference according to Tukey's multiple comparison test ($P < 0.05$).
[B]Accumulated disease incidence for each cultivar and year, resulting from the addition of September and November disease incidence mean.

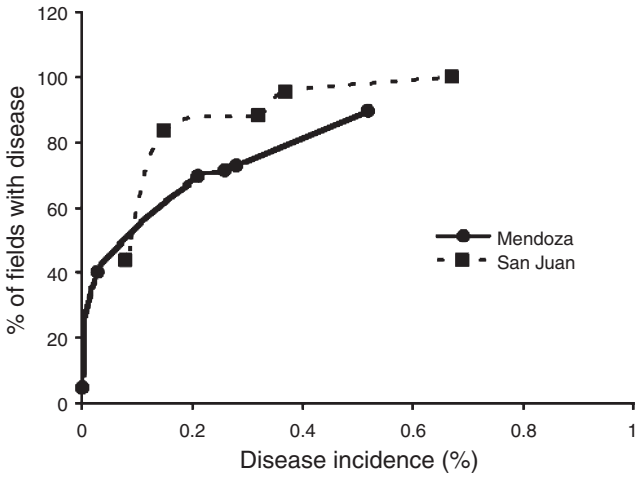

Fig. 1. Distribution of garlic decline prevalence with respect to the incidence percentage registered in fields of garlic cvv. Blanco, Chino and Colorado in Mendoza and San Juan provinces during 2000, 2001 and 2002.

Distribution of GDIII phytoplasma in garlic plants

In the symptomatic plants, the immunoassays on nitrocellulose were positive for all the samples tested from roots, cloves, protective leaves, pseudostem, and young and adult leaves. However, a stronger colour reaction was observed in root samples from 26 out of 35 plants (Fig. 2*a*). Analysis by PCR (Fig. 2*b*) and observations by TEM (Fig. 2*c*, *d*) confirmed the presence of phytoplasmas in all the assayed tissues. Results of TEM observations are only presented for roots and leaves.

Survival of GDIII phytoplasma in garlic bulbs

PCR analysis confirmed the presence of phytoplasmas in potentially infected garlic cloves. Phytoplasmas were detected in the sprout and storage leaf of the 22 cloves tested of garlic cv. Chino and 24 (86%) of the 28 cloves tested of cv. Blanco. Field assays showed that phytoplasmas were able to persist during sprouting since PCR-positive plants originated from infected cloves of cv. Blanco. However, the proportion

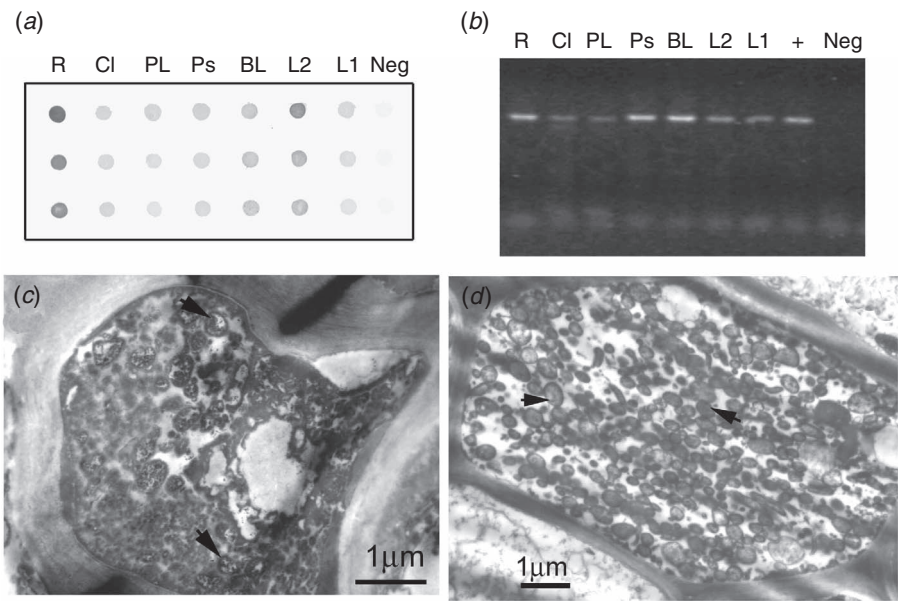

Fig. 2. Examples of garlic decline phytoplasma distribution within infected garlic plants of cv. Blanco. (*a*) Nitrocellulose dot blot immunoassay with China-tree phytoplasma antibodies. (*b*) Agarose gel electrophoresis of PCR products using primers R16F2/R16R2. R, root; Cl, clove; PL, protective leaves; Ps, pseudostem; BL, basal leaf; L2, second leaf; L1, first leaf; Neg, negative control; +, positive control. (*c*) and (*d*) Transmission electronic micrograph of phytoplasmas (black arrows) in phloem cells of garlic leaf (*c*) and root (*d*).

of infected plants derived from a potentially infected bulb (29.9%) was lower than the proportion of PCR-positive cloves (Table 3). Most infected cloves of cv. Chino (diseased treatment) died before sprouting, and the low number of plantlets produced died shortly after emergence. In contrast, cloves of the control treatment grew normally, suggesting that bulblet and plant death would be related to the phytoplasma infection. Phytoplasmas were not detected in asymptomatic plants nor was natural infection observed in control treatments of both cultivars.

Insect collection

Approximately 380 leafhoppers were collected during the 3-year survey, most of them from the organic garlic fields. They belonged to 22 species of Family *Cicadellidae*, included in the subfamilies *Cicadellinae*, *Agallinae*, *Gyponinae*, *Xerophloeinae*, *Deltocephalinae* and *Typhlocibinae* (Table 4). *Bucephalogonia xanthophis* was the most abundant species, followed by *Empoasca curveola* and *Syncharina punctatissima*. Several species were only collected at the organic field studied during year 2002, which was implanted within a vineyard, suggesting that such species might be related to the presence of grapevines.

Discussion

Garlic decline distribution analysed during three consecutive crop cycles in the main garlic production areas of Argentina showed that the disease spread in both regions with high prevalence but low incidence percentages. The results obtained from the PCR analyses and field assays indicate that the GDIII phytoplasma can be transmitted through planting material. In this way, the phytoplasma is transmitted from one crop cycle to the next by the planting of infected cloves, ensuring the presence of diseased plants into the newly implanted crop, and this may explain the high prevalence observed in the garlic-growing regions that were surveyed. However, the field assay showed that the number of infected plants derived from infected cloves was low (29.9%) in cv. Blanco and none were derived from cv. Chino, and this may explain the low disease incidence in the garlic fields that were surveyed in each region. In addition, plants infected early in the crop cycle die before the harvest and are not able to produce bulbs, reducing the number of bulblets potentially infected for the next season.

The low incidence of infected plants in each field might also result from some management techniques commonly used by garlic growers. Among these techniques the selection of cloves with good external aspect and large size to be used as seeds can reduce the initial inoculum since infected cloves are generally smaller and, in some cases, the bulbs have a mummified appearance and different colour. Another common practice is the elimination by roguing of plants with symptoms of decline.

Epidemiological studies on other phytoplasma diseases reported variations of disease incidence related mainly to the presence of efficient insect vectors. In vineyards from different regions of Spain infected with grapevine yellows, for example, incidence values between 3 and 80% were registered. The highest incidence percentage corresponded to regions with a high population of infective insects (Batlle *et al*. 2000). In this study, the dispersion of GDIII phytoplasma in garlic fields by means of vector transmission may be limited, as only low numbers of insects were collected in the garlic fields, providing an additional explanation for the low incidence of disease. However, the dispersion of GDII phytoplasma in garlic fields by a vector, even at low levels, cannot be discounted.

The distribution of the number of diseased plants per field showed an aggregative pattern, which might be explained by differences in the vector population and/or management practices among farmers. The accumulation of diseased plants in some fields may correspond to a higher vector population

Table 3. The rate of transmission of garlic decline phytoplasma through infected cloves of cvv. Blanco and Chino

	Garlic cultivar	
	Blanco	Chino
PCR-positive cloves per bulb (%)[A]	24/28 (86%)	22/22 (100%)
PCR-positive plants derived from potentially infected cloves (%)[A]	22/77 (29.9%)	–[B]

[A]Average percentage. Bulbs corresponded to plants that tested positive for polymerase chain reaction (PCR) from leaf tissues. All cloves from control bulbs tested negative and generated non-infected plants.
[B]No plants grown from potentially infected cv. Chino cloves.

Table 4. Leafhopper species and the number of individuals captured on yellow sticky traps in garlic fields from San Juan province during the crop cycles (June–November) 2000, 2001 and 2002

Leafhopper species	Year		
	2000	2001	2002
Subfamily *Cicadellinae*			
Bucephalogonia xanthophis	17	85	72
Ciminius platensis	–	5	2
Scopogonalia subolivacea	–	–	29
Syncharina punctatissima	–	14	1
Subfamily *Agallinae*			
Agalliana ensigera	–	12	4
Subfamily *Gyponinae*			
Curtara pagina	–	1	2
Curtara samera	–	–	3
Subfamily *Xerophloeinae*			
Xerophloea viridis	–	–	2
Subfamily *Deltocephalinae*			
Amplicephalus dubius	–	3	4
Atanus sp.	1	6	3
Atanus angustus	–	–	3
Atanus viridis	–	–	2
Baclutha rosea	1	–	–
Chlorotettix sp.	1	–	4
Exitianus obscurinervis	–	–	1
Fusanus griseostriatus	–	–	1
Paratanus exitiosus	–	7	12
Spangbergiella vulnerata	–	1	1
Unerus sp.	–	–	1
Subfamily *Typhlocibinae*			
Empoasca curveola	4	4	55
Empoasca manubriata	1	–	2
Empoasca punena	–	–	1

in those fields. Alternatively, the level of seed selection and diseased plants elimination done by each farmer could be reflected in the disease incidence of each field.

In this study we report the first list of leafhoppers associated with garlic crops in Argentina. Although insects were not assayed for the phytoplasma presence, some of the collected species should be considered for further studies as potential vectors of the garlic decline phytoplasma. Among them, *Paratanus exitiosus* is known to be vector of the sugar beet yellow wilt phytoplasma in Chile (Nielson 1979). Phytoplasmas have been detected in some species of genera *Empoasca* and *Agalliana* captured in alfalfa fields affected with alfalfa witches' broom in Oman (Khan *et al*. 2002), and *E. decedens* has been suggested as a probable vector of the European stone fruit yellows phytoplasma in Italy (Pastore *et al*. 2004).

According to the results from the surveys, garlic cv. Colorado was less affected by the garlic decline than the other garlic cultivars sampled, as it had the lowest incidence and prevalence during all the years assayed. These results suggest that cv. Colorado could be less susceptible to the phytoplasma. This is not the first case of differential response to phytoplasmal infection within a crop species, which has been previously reported among varieties of grapevines, *Stylosanthes* and *Cocos nucifera* genotypes (Harries 1995; De La Rue *et al*. 2003; Constable *et al*. 2004). However, cv. Chino was the genotype most affected by the phytoplasma since all plants derived from cv. Chino diseased cloves died and the bulbs assayed had the highest proportion of infected cloves. Taking into consideration that GDIII phytoplasma would have evolved in South America (Galdeano *et al*. 2004), and that cv. Chino is a genotype introduced into Argentina in recent times, the better performance shown by garlic genotypes with a longer culture history, such as cv. Colorado and cv. Blanco, might be a consequence of the successive selection of better adapted material in regions were the disease has long been present.

The host colonisation and pathogen distribution patterns within the plant vary depending on the phytoplasma strain and host species. In periwinkle plants, graft inoculated dwarf aster yellows and severe aster yellows phytoplasmas moved towards meristematic tissues, leaves, stems and finally to the roots (Kuske and Kirkpatrick 1992). By immunohistochemical analysis and nested PCR, it was observed that the onion yellows phytoplasma migrated from the inoculated leaf to the apex and later distributed to the roots and remaining leaves, originating a systemic infection 21 days after inoculation (Wei *et al*. 2004). However, Lherminier *et al*. (1994) studied the 'flavescence dorée' phytoplasma distribution within *Vicia faba* plants and observed that, after inoculation, phytoplasmas multiplied in the roots showing higher titres than the other organs. In lethal yellowing diseased palms, less phytoplasma cells were registered in mature leaves (Parthasarathy 1974) and it was not possible to detect the Australian papaya dieback phytoplasma in adult leaves (Siddique *et al*. 1998). Such results are consistent with those obtained in this work, where a stronger reaction was obtained with the diseased garlic roots in the immunoassays on nitrocellulose compared with other parts of the plants, indicating an uneven distribution and titre of the GDIII phytoplasma within the garlic plants. Uneven distribution and titre might also explain the low proportion (29.9%) of infected plants derived from potentially infected cloves of cv. Blanco. It is possible that the low proportion of infected plants may result from an uneven distribution of the phytoplasma in the bulb, resulting in a proportion of uninfected cloves. Alternatively, the phytoplasmas may not have spread systemically at the time of testing or the phytoplasmas were present but below the level of detection. However, the difference between the proportion of diseased plants (29.9%) and infected cloves (86%) suggests that disease remission might have occurred during cloves sprouting. Further studies should be undertaken to examine these possibilities.

Acknowledgements

We thank Natalia Meneguzzi and Pablo Lunello for their help during the field samplings. This work was supported by PICT 08–03792, PICT 08–15219, PICT 08–12914 from the Agencia Nacional de Promoción Científica y Técnica, and Instituto Nacional de Tecnología Agropecuaria (INTA).

References

Batlle A, Martinez MA, Laviña A (2000) Occurrence, distribution and epidemiology of grapevine yellows in Spain. *European Journal of Plant Pathology* **106**, 811–816. doi: 10.1023/A:1008794905178

Cafrune E, Perotto MC, Conci VC (2006) Effect of two *Allexivirus* isolates on garlic yield. *Plant Disease* **90**, 898–904. doi: 10.1094/PD-90-0898

Conci V, Nome SF, Docampo DM (1992) Micoplasmas asociados a la 'tristeza' del ajo en Argentina. In 'Proceedings VI Congreso Latinoamericano de Fitopatología VI Congreso Nacional de la Sociedad Española de Fitopatología, Torremolinos, Spain'. (Eds F Romero Muñoz, A Gómez Barcina) p. 58.

Conci VC, Gómez GG, Docampo DM, Conci LR (1998) Phytoplasma associated with symptoms of 'Tristeza del ajo' (Garlic decline) in garlic (*Allium sativum* L.). *Journal of Phytopathology* **146**, 473–477. doi: 10.1111/j.1439-0434.1998.tb04608.x

Conci VC, Canavelli A, Lunello P, Di Rienzo J, Nome SF, Zumelzu G, Italia R (2003) Yield losses associated with virus-infected garlic plants during five successive years. *Plant Disease* **87**, 1411–1415. doi: 10.1094/PDIS.2003.87.12.1411

Constable FE, Jones J, Gibbs KS, Chalmers YM, Symons RH (2004) The incidence, distribution and expression of Australian grapevine yellows, restricted growth and late season leaf curl diseases in selected Australian vineyards. *The Annals of Applied Biology* **144**, 205–218. doi: 10.1111/j.1744-7348.2004.tb00335.x

De La Rue SJ, Hopkinson R, Foster S, Gibb KS (2003) Phytoplasma host range and symptom expression in the pasture legume *Stylosanthes. Field Crops Research* **84**, 327–334. doi: 10.1016/S0378-4290(03)00099-6

Doyle J, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus (San Francisco, Calif.)* **12**, 13–15.

Fletcher J, Wayadande A, Melcher U, Ye F (1998) The phylogenetic mollicute-insect vector interface: a closer look. *Phytopathology* **88**, 1351–1358. doi: 10.1094/PHYTO.1998.88.12.1351

Food and Agricultural Organization of the United Nations (2005) Economic and Social Department: The Statistical Division. Countries by commodity. Garlic. http://www.fao.org/es/ess/top/commodity.html

Galdeano E, Torres L, Meneguzzi N, Guzmán F, Gómez GG, Docampo DM, Conci LR (2004) Molecular characterization of 16S ribosomal DNA and phylogenetic analysis of two X-disease group phytoplasmas affecting China-tree (*Melia azedarach* L.) and garlic (*Allium sativum* L.) in Argentina. *Journal of Phytopathology* **152**, 174–181. doi: 10.1111/j.1439-0434.2004.00822.x

Gómez GG, Conci LR, Ducasse DA, Nome SF (1996) Purification of the phytoplasma associated with China-tree (*Melia azedarach* L.) decline and the production of a polyclonal antiserum for its detection. *Journal of Phytopathology* **144**, 473–477. doi: 10.1111/j.1439-0434.1996.tb00327.x

Harries HC (1995) The genetics of durable resistance to lethal yellowing disease. In 'Lethal yellowing: research and practical aspects'. (Eds C Oropeza, F Howard, G Ashburmer) pp. 149–171. (Kluwer Academic Publishers: Dordrecht, the Netherlands)

InfoStat (2003) InfoStat, version 1.5, Manual del Usuario. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. 1st edn. Brujas, Argentina.

Khan AT, Bolt S, Al-Suthi AM, Gundersen-Rindal DE, Bertaccini AF (2002) Molecular identification of a new phytoplasma associated with alfalfa witches'-broom in Oman. *Phytopathology* **92**, 1038–1047. doi: 10.1094/PHYTO.2002.92.10.1038

Kuske CR, Kirkpatrick BC (1992) Distribution and multiplication of Western aster yellows mycoplasmalike organisms in *Catharanthus roseus* as determined by DNA hybridization analysis. *Phytopathology* **82**, 457–462. doi: 10.1094/Phyto-82-457

Lee IM, Hammond RW, Davis RE, Gundersen DE (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. *Phytopathology* **83**, 834–842. doi: 10.1094/Phyto-83-834

Lherminier J, Courtois M, Caudwell A (1994) Determination of the distribution and multiplication sites of Flavescence Dorée mycoplasma-like organisms in the host plant *Vicia faba* by ELISA and immunocytochemistry. *Physiological and Molecular Plant Pathology* **45**, 125–138. doi: 10.1016/S0885-5765(05)80071-2

Lot H, Chovelon V, Souche S, Delecolle B (1998) Effects of onion yellow dwarf and leek yellow stripe viruses on symptomatology and yield loss of three French garlic cultivars. *Plant Disease* **82**, 1381–1385. doi: 10.1094/PDIS.1998.82.12.1381

Nielson MW (1979) Taxonomic relationships of leafhopper vectors of plant pathogens. In 'Leafhopper vectors and plant disease agents'. (Eds K Maramorosch, KF Harris) pp. 3–27. (Academic Press: New York)

Parthasarathy MV (1974) Mycoplasma-like organisms associated with lethal yellowing disease of palms. *Phytopathology* **64**, 667–674.

Pastore M, Raffone E, Santomastaso H, Priore R, Paltrimieri S, Bertaccini A, Simeone AM (2004) Phytoplasma detection in *Empoasca decendens* and *Empoasca* spp. and their possible role as vectors of European stone fruit yellows (16SrX-B) phytoplasma. *Acta Horticulturae* **657**, 507–511.

Portela JA (2007) Ajo argentino. Pautas de cultivo para la Region Andina Central Argentina. La Consulta, INTA EEA La Consulta, Mendoza, Argentina. pp. 88. (Ediciones INTA)

Siddique ABM, Guthrie JN, Walsh KB, White DT, Scott PT (1998) Histopathology and within-plant distribution of the phytoplasma associated with Australian papaya dieback. *Plant Disease* **82**, 1112–1120. doi: 10.1094/PDIS.1998.82.10.1112

Walkey DGA, Antill DN (1989) Agronomic evaluation of virus-free and virus infected garlic (*Allium sativum* L.). *Journal of Horticultural Science* **64**, 53–60.

Wei W, Kakizawa S, Suzuki S, Nishigawa H-Y, Miyata S, Oshima K, Ugaki M, Hibi T, Namba S (2004) In planta dynamic analysis of onion yellows phytoplasma using localized inoculation by insect transmission. *Phytopathology* **94**, 244–250. doi: 10.1094/PHYTO.2004.94.3.244

Weintraub PG, Beanland L (2006) Insect vectors of phytoplasmas. *Annual Review of Entomology* **51**, 91–111. doi: 10.1146/annurev.ento.51.110104.151039

Manuscript received 29 September 2008, accepted 5 March 2009