Calculating the expression of those genes is enough for over a 60 %-accuracy for disease status determination.

O14.012 Transmission of chestnut yellow crinkle phytoplasma by leafhopper *Parabolopona ishihari* Webb (Homoptera: Cicadellidae)

C.L. Lin[1], G.Z. Tian[1], X.Q. Zhu[2], Z.P. Li[2], C.S. Song[1], Z.G. Ren[1], Y. Li[1] and C.G. Piao[1]
[1]Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry/Key Laboratory of Forest Protection of State Forestry Administration, Beijing 100091, P. R. China; [2]Huairou Forest Pest and Disease Control and Quarantine Station, Beijing, 101400, P. R. China
Email: lincl@caf.ac.cn

Chinese chestnut (*Castanea mollissima* BL.), a deciduous tree native to China, belongs to the family *Fagaceae* and is widely cultivated in eastern Asia. Recently Chinese chestnut trees planted in a suburb of Beijing, China developed symptoms including yellowing, leaf crinkling, little leaf, shortened internodes, and empty burrs. Transmission electron microscopy revealed the presence of phytoplasma cells in phloem sieve elements of the symptomatic chestnut trees. Molecular cloning and sequence analysis of PCR-amplified near-full length 16S rRNA gene indicated that the phytoplasma associated with the Chinese chestnut yellow crinkle (CnYC) disease is closely related to Japanese chestnut witches' -broom phytoplasma. Eleven insect species with piercing-sucking mouthparts were collected in chestnut plantation with symptomatic trees. CnYC phytoplasma was detected in *Parabolopona ishihari* Webb by PCR amplification of 16S rRNA gene using CnYC phytoplasma specific primer pairs. Nymphs and adults of *P. ishihari* Webb collected from chestnut trees infected with phytoplasmas were feed with healthy periwinkle (*Catharanthus roseus*) growing in green house. The symptoms of yellowing, leaf crinkling, little leaf and shorten internodes were observed and the CnYC phytoplasma was also detected by PCR amplification in periwinkles at 30 days post inoculation. These results suggested that CnYC phytoplasma could be transmitted from Chinese chestnut to other plant by *P. ishihari* Webb.

P14.001 Effects of environmental temperature on the corn stunt spiroplasma disease

E.O. Sabato, S.R. Galvão and E.C. Landau
Embrapa, Rod. MG 424, Km 65 C.P. 151 35701970 Sete Lagoas, MG, Brazil
Email: elizabeth.o.sabato@embrapa.br

The corn stunt spiroplasma (CSS) is caused by *Spiroplasma kunkelii*, transmitted by the leafhopper *Dalbulus maidis*. The objective of this study was to verify the spiroplasma transmission by *D. maidis*, and the development of CSS symptoms in maize, under acclimated chambers and screen-house conditions. The maximum and minimum temperatures (^{0}C) and amplitude, respectively, were: chamber I (27.35; 23.58; 3.76), II (27.22; 18.53; 8.69), III (24.8; 22.33; 2.48), IV (28.4; 23.29; 5.1), screen-house (32.29; 18.33; 12.96). In each condition, 24 spiroplasma-infective, and 6 healthy leafhoppers were confined on maize seedlings, for 6 days (one per seedling). After that, half of these seedlings were cultivated inside the chambers, and the other half was cultivated in the screen-house. More than 80% of the plants submitted to spiroplasma inoculation, and cultivated in the screen-house, showed CSS symptoms, indicating no effect of that temperatures on this pathogen transmission. Some plants without CSS symptoms could be due the death of the infective leafhopper, before spiroplasma transmission. For the maize seedlings submitted to spiroplasma inoculation, and cultivated under the five temperature conditions, only in the chamber II none plant presented CSS symptoms, until 60 days age. These asymptomatic plants were transferred to the screen-house where, in few days, the CSS symptoms appeared, indicating that bland temperature condition can stop the spiroplasma growth in maize.

P14.002 Effect of the corn stunt spiroplasma disease on maize production

E.O. Sabato, E.C. Landau and A.M. Coelho
Embrapa, Rod. MG 424, Km 65 C.P. 151 35701970 Sete Lagoas, MG, Brazil
Email: elizabeth.o.sabato@embrapa.br

The effect of corn stunt spiroplasma (CSS) on maize development and production was evaluated in the screen-house, with spiroplasma inoculation, and in the field, using one maize cultivar and one popcorn cultivar. In the screen-house 20 seedlings of each cultivar were submitted or not to spiroplasma inoculation, using one spiroplasma-infective or healthy leafhoppers *Dalbulus maidis* confined for six days in each eight-days-seedling. The CSS symptoms were detected on 40% and 60% of the maize and the popcorn cultivar plants that had the development drastically reduced by this disease, in relation to the healthy plants. Each cultivar was sowed in 10 lines (10m each one) in three different areas at Embrapa experiment station, Sete Lagoas, MG, Brazil, and, in each area, 10 plants with and 10 plants without CSS symptoms were marked for grain production evaluation. The averages of the corn stunting diseases symptoms incidence were 21.9%; 24.6%; 15.7% and 65.6%; 77.5%; 74.2% for the maize and the popcorn cultivar, respectively, with CSS symptoms predominance. The periodic insect sampling showed *D. maidis* leafhoppers presence since 15 days after sowing. The averages of CSS reductions on the maize and the popcorn cultivars grain production were, respectively: 84.07%; 75.40%; 76.80%; and 63.17%; 72.62%; 60.66%. These results indicate

that damage by CSS on the field maize crop can be severe.

P14.003 Identification of sesame phyllody phytoplasmas and incidence of sesame phyllody disease in Antalya, Turkey

Z. Özdemir, H. Topuz, and M. İ. Çağırgan
Department of Plant Protection, Adnan Menderes University, Aydın 09100 Turkey
Email:zahide_ozdemir@yahoo.com

Sesame phyllody is one of the major diseases of sesame (*Sesamum indicum*) reducing the yield significantly, in Antalya, Turkey. In 2011 and 2012, surveys conducted to sesame fields and incidence was recorded in three to four different times per growing season. In 2011, through the end of growing season, disease was found in all of the sesame fields and incidence was ranged from 0.7 to 11 % in 112 da survey area. In 2012, at the end of the season in 73 percent of the fields surveyed, disease was present and incidence ranged from 0.2 to 11% in 99 da survey area. Phytoplasmas detected from genomic DNA of diseased plants collected from surveys by the amplification of 16SrDNA using nested PCR with primer pairs P1/P7 and R16F2n/R16R2. An amplification product of 1.24 kb band was detected by nested PCR. Amplified 1.24 kb product of 16SrDNA were cloned and sequenced from seven phytoplasma isolates from different survey locations. According to BLAST search, six of the isolates were identified as Peanut Witches Broom (16SrII) and one isolate was identified as Clover Proliferation (16SrVI) phytoplasma group. Previously sesame phyllody phytoplasmas reported from Eastern Mediterranean region of Turkey were grouped in Clover Proliferation (16SrVI) phytoplasma group. Identification of more of the phytoplasma isolates from different locations is being carried out.

P14.004 An update on Awka wilt disease of coconut caused by Phytoplasma In Nigeria

I.J. Osagie, E.E. Ojomo and F. Pilet
CIRAD, UMR RPB (CIRAD/IRD/UM2), F-34398 Montpellier, France;NIFOR, PMB 1030, Benin City, Nigeria
Email: fabian.pilet@cirad.fr

Lethal yellowing disease (LYD) caused by phytoplasma is the most damaging threat to coconut in Africa. Nigeria is the first African country where the disease (Awka wilt) was reported in 1917. The symptoms of the disease are similar to those reported in other West and East African countries. In West Africa, LYD is caused by a phytoplasma belonging to the 16SrXXII group, and the disease is still very active in Nigeria and Ghana. In early 1990s, sequencing of the 16SrDNA of one Ghanaian and one Nigerian isolate confirmed the implication of a very similar but not identical phytoplasma strains in the two countries. In 2012, a new survey for coconut LYD covering most of the coastal Nigerian States was done. Symptomatic coconut trees were observed in all the States surveyed. Stem samples were collected from symptomatic trees from three Eastern States around Awka. From each sample, DNA was extracted and the 16SrDNA was amplified by PCR using P1/P7 primers. The PCR products were sequenced and compared with each other and with the LDN sequence published 20 years ago. A homology of 100% was observed between the 16SrDNA sequence previously published and the sequences of our samples. Based on the known conserved 16SrDNA sequences, this result suggests a relative stability of the LYD phytoplasma populations in Eastern Region of Nigeria and absence of introduction of the "Ghanaian strain". A similar investigation for symptomatic coconut from the Western Region and by using more variable genes is necessary.

P14.005 Dissecting the role of *NPR1* in Defense against periwinkle leaf yellowing phytoplasma in *Catharanthus roseus* using virus-induced gene silencing

Y.C. Sung, C.P. Lin and J.C. Chen
Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, China; Institution of Biotechnology, National Taiwan University, Taipei, Taiwan, China
Email: R00633002@ntu.edu.tw

Phytoplasmas are prokaryotic plant pathogens causing considerable loss in many economical crops globally. Previously, we found that periwinkles (*Catharanthus roseus*) infected with periwinkle leaf yellowing (PLY) phytoplasma contained both symptomatic and non-symptomatic shoots. The expressions of *CrPR1* genes were up-regulated in both symptomatic and non-symptomatic shoots, indicating a systemic resistant machinery might be activated after PLY phytoplasma infection. Therefore, we aimed to investigate on *NPR1* gene, a critical gene in SAR activation, to realize the effects of SAR on phytoplasma pathogenesis. Because an analysis for gene functions was lacking for periwinkle, we aimed first to develop an effective virus-induced gene silencing system in periwinkle. *Tobacco rattle virus* (TRV)-based VIGS system was tested in periwinkle, and several potentially influential factors (temperature, plant age, and leaf age) were analyzed to optimize the VIGS system. The results show that high temperature had negative effects on VIGS efficacy and virus accumulation. Plant age showed no significant differences, while newborn leaves had better VIGS efficacy and virus accumulation compared to other leaves. Moreover, silencing effects and phytoplasma symptoms were able to coexist, indicating that the VIGS system we established can be used for functional analysis. Using this system, I generated