Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: corrado.cariddi@agr.uniba.it

A specific pair of primer (E3-E4) amplifying a single DNA fragment of 110 bp from plasmid pEA29 was designed to identify, detect, and quantify *Erwinia amylovora* by real-time PCR. Primer E3 was modified to obtain a Scorpion probe for detecting the specific 110 bp amplicon by florescence emitted from a fluorophore through a self-probing PCR assay. Specificity of primers and probe was assessed by means of BLAST analysis, to exclude the presence of similar sequences among available DNA databases (GenBank) and by using genomic DNA from a large number of *E. amylovora* isolates and other bacteria from several hosts and different geographic areas. In Scorpion-PCR, with a 10-fold dilution series of *E. amylovora* DNA, the limit of detection was 1 pg ml^{-1}. A high and significant correlation (r^2 = 0,995) was obtained between target DNA quantity and cycle threshold (Ct). Combining two sequential amplifications with conventional reported primers (PEANT1-PEANT2) and Scorpion primers (E3 Scorpion-E4) the detection limit was 1 fg μl^{-1} (nested Scorpion-PCR). Using serial dilution of bacterial suspensions the limit of detection was 10^4 CFU ml^{-1} in Scorpion PCR and 10^2 CFU ml^{-1} in nested Scorpion PCR. Real-time PCR combined with simple, rapid, and effective procedures for DNA extraction enabled the detection and the quantification of the epiphytic population of *E. amylovora* in the washings of flowers and leaves of artificially inoculated pear. A significant correlation (r^2 = 0.91) was found between pathogen CFU on semi-selective media and the corresponding target DNA concentration evaluated by real time PCR.

CHARACTERISATION OF SARDINIAN ISOLATES OF *CITRUS TRISTEZA VIRUS* BY SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS OF THE COAT PROTEIN GENE. A. Schiaffino, R.I. Pinna and F. Marras. *Dipartimento di Protezione delle Piante – Sezione di Patologia Vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39. 079. 229316; E-mail: fmarras@uniss.it*

Citrus tristeza virus (CTV) was detected in Sardinia since 2001. Lately the infection was found in three different sites on *Citrus* spp., mainly on symptomless plants of orange (*C. sinensis*) and mandarin (*C. reticulata*) grafted on sour orange (*C. aurantium*) rootstocks. CTV detection was carried out by DAS-ELISA and confirmed by means of RT-PCR using primers for the coat protein gene of the Florida B3 isolate of the virus. Positive samples showed an amplification product of 670 bp, which was confirmed to be the CTV coat protein gene by digestions with the restriction endonucleasis *Bst*EII and *Cla*I. Isolates were compared for variation in their coat protein gene sequence by single-strand conformation polymorphism analysis (SSCP). Six distinct SSCP profiles were found for the isolates examined. Samples from the province of Oristano showed a common SSCP profile which was different from the five mobility patterns found among isolates in the province of Cagliari. In particular one pattern was common to most samples from two different sites in this area: S. Vito and Muravera. The remaining four mobility patterns were characteristic of four isolates, two from each site. These results suggest introduction in Sardinia of CTV from different origins.

***BISCOGNIAUXIA NUMMULARIA* PRIMARY PATHOGEN ON BEECH. A. Sidoti[1] and G. Granata[2].** *[1]Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Strutturali, Servizio IV, UO 21-OMP di Acireale, Corso Umberto 114, 95024 Acireale, Italy. E-mail: asidoti@omp-acireale.org. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: granatag@unict.it*

Some beech (*Fagus sylvatica* L.) stands in the Nebrodi Mountains and Mt. Etna are known to be affected by a decline associated with the fungus *Biscogniauxia nummularia* (Bull. Fr.) O. Kuntze since 1990. The disease determines yellowing, leaf drop, cankers and tree death. A similar decline condition has also been observed in the Ferdinandea beech wood (Calabria, Italy). Artificial field inoculations were performed to assess the effective pathogenic capacity of Sicilian and Calabrian isolates, and whether the *B. nummularia* populations are heterogeneous and if they possess a different degree of virulence. Field trials showed that *B. nummularia* has a primary pathogenic role under the environmental conditions studied. Isolates from the declining beech woods in Sicily and Calabria were heterogeneous in characteristics and pathogenic behaviour. The repeated periods of drought and high temperatures, the soil conditions that do not favour good water retention and the effects of coppice management may have determined stress and reduced the resistance of trees to the fungus. Reduced ammonification and nitrogen fixation processes in the soil under declining trees confirmed the existence of environmental degradation. It was also observed that the fungus can adopt endophyte-like behaviour and show its pathogenic capacity on stressed trees. Felling suckers and infected stumps are necessary for controlling the spread of the disease. On the contrary, the disappearance of vegetation may determine soil degradation, erosion and desertification.

A MOLECULAR ASSAY TO INVESTIGATE THE POSSIBLE ASSOCIATION BETWEEN THE CHESTNUT WEEVIL *CURCULIO PROPINQUUS* AND THE BLACK ROT FUNGUS *RACHODIELLA CASTANEAE*. A.M. Vettraino, S. Speranza, B. Paparatti, C. Pucci and A. Vannini. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: vettrain@unitus.it*

Black rot, induced by *Rachodiella castaneae* Pyr. [teleomorph: *Sclerotinia pseudotuberosa* Rehm.; syn. *Ciboria batschiana* (Zopf) Buchw.], and the chestnut weevil [*Curculio propinquus* (Desbr.)] are among the most serious phytosanitary problems of the chestnut (*Castanea sativa* Mill.) fruit industry in Italy, causing relevant economic losses at harvest and during storage. Symptoms of black rot are frequent in nuts infested by *C. propinquus*, and hence an association between the fungus and the insect has been hypothesised. To verify this hypothesis, clusters of immature burrs from three trees in a chestnut area in Central Italy were covered with a net and treated as follow: (i) infested with *C. propinquus* adults that had been artificially contaminated with *R. castaneae*; (ii) infested with *C. propinquus* uncontaminated adults; (iii) uninfested negative control. At harvest time, the nets were removed and the adults of the insects and chestnut fruits were collected. Due to the inefficacy of traditional diagnostic methods, the detection *R. castaneae* in *C. propinquus* and in chestnut fruits was performed by PCR. Two sequences specific for *R. castaneae* were identified in the ITS region of rDNA, and two species-specific primers (RAC1 and RAC2) were designed. *R. castaneae* was detected in more than 90% of the chestnut weevils analysed, regardless the treatment, and in 77, 73, and 92% of nuts following the treatments (i), (ii) and (iii), respectively. These findings suggest an endophytic habitus of *R. castaneae* in chestnut fruits and that *C. propinquus* is a potential vector of the pathogen.

A TWO-YEAR MONITORING OF AN EPIDEMIC OF *TOMATO SPOTTED WILT VIRUS* IN SPECIALIZED LETTUCE PRODUCTION AREA. M. Tessitori, A. Reina, S. Rizza, P. Roggero and R. La Rosa. *Dipartimento di Scienze e Tecnologie Fi-*

Volume 85 (4, Special issue) December 2003

Journal of Plant Pathology

Formerly *Rivista di patologia vegetale* established in 1892

EDIZIONI ETS, Pisa, Italy
Spedizione in a.p. art. 2 comma 20/B L. 662/96 - N. 1043 del 03/06/2002 - Pisa.

ISSN 1125-4653