DISEASE NOTE

THE HORSE CHESTNUT POWDERY MILDEW CAUSED BY ERYSIPHE FLEXUOSA (SYN. UNCINULA FLEXUOSA) IN ITALY

C. Nali

Dipartimento di Coltivazione e Difesa delle Specie Legnose “Giovanni Scaramuzzi”, Università di Pisa, Via del Borg hetto 80, 56124 Pisa, Italy

In late spring 2006, a powdery mildew outbreak was observed on horse chestnut plants (Aesculus hippocastanum) growing in the Botanical Garden of Pisa and in a boulevard of Lucca (Tuscany, central Italy). Whitish fungal colonies were present on the upper surfaces of both young and old leaves. The hyaline mycelium had abundant ellipsoid conidia, 20 to 40 µm in length and 10 to 15 µm in width, produced singly on 2-3 celled conidiophores. Cleistothecia were globose, 100 to 120 µm diameter, dark brown, with undulate appendages and contained several asci (up to 10), each comprising 6-8 hyaline, round, and single-celled ascospores. The pathogen was identified as Erysiphe flexuosa (Peck) U. Braun et S. Takamatsu (syn. Uncinula flexuosa), a North American powdery mildew species recently introduced in Europe (Kiss et al., 2004; Milevoj, 2004). To the best of my knowledge, this is the first report of E. flexuosa in Italy.


Corresponding author: C. Nali
Fax: +39.050.2210559
E-mail: cnali@agr.unipi.it

Received July 12, 2006
Accepted July 27, 2006

DISEASE NOTE

FIRST REPORT OF PHOMOPSIS VEXANS ON ILEX CRENATA THUNB. var. CONVEXA IN CHINA

B.G. Lou1, W.J. Chen1 and X.D. Zheng2

1 Institute of Biotechnology, Zhejiang University, Hangzhou 31002, People’s Republic of China
2 Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310029, People’s Republic of China

Ilex crenata Thunb. var. convexa is an ornamental plant, which is widely planted in the gardens of Zhejiang province (China). Blight symptoms were observed on twigs and branches in summer 2002 and 2005 which consisted of small water-soaked discoloured spots that rapidly turned into irregular lesions, with a brown outer edge and a dark brown sunken central area. When lesions encircled a branch, leaves wilted and dried up, dieback ensued which was soon followed by the death of the trees. Numerous pycnidia were observed in the dead cortex of twigs and branches. A fungus isolated on potato dextrose agar (PDA) from symptomatic tissues had a white floccose mycelium and produced black, globose to irregular pycnidia up to 290 µm in diameter. Alpha-conidia were one-celled, hyaline, and ellipsoidal (5.5 to 9.05 µm long × 1.9 to 2.3 µm wide); beta-conidia were one-celled, hyaline, filiform, and straight or curved (19.9 to 28.2 µm long × 0.95-1.32 µm wide). The fungus was morphologically identical to Phomopsis vexans (Sacc. et Syd.) Harter (Punithalingam et al., 1972).

A suspension of spores (10^6 spores/ml) collected from PDA cultures was used to spray-inoculate potted Ilex crenata Thunb. var. convexa plants that were kept for 48 h under a polyethylene sheet cover, and grown at 22-25°C in a greenhouse. After 5 to 10 days, inoculated trees showed symptoms resembling those seen in nature. The same fungus used for inoculum was re-isolated from the margins of necrotic tissues of inoculated plants but not from controls sprayed with sterile water. Ph. vexans has been previously recorded on eggplant (Solanum melongena) in China (Liu et al., 1998), but this is the first report of its occurrence on I. crenata var. convexa.

Punithalingam E., Holliday P., 1972 Phomopsis vexans. IMI Descriptions of Fungi and Bacteria. 34:338.


Corresponding author: X.D. Zheng
Fax: +44.571.86971628
E-mail: bg lou@zju.edu.cn

Received July 17, 2006
Accepted July 27, 2006
**DISEASE NOTE**

**FIRST REPORT OF SHOOT BLIGHT CAUSED BY *DIPLODIA SCROBICULATA* ON *PINUS RADIATA* TREES IN ITALY**

B.T. Linaldeddu, L. Maddau and A. Franceschini

Dipartimento di Protezione delle Piante, sezione di Patologia vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy

Monterey pine (*Pinus radiata*) was introduced in Sardinia (Italy) at the beginning of the 1960's to meet the paper industry demand. In spring and summer 2004, branch dieback and shoot blight were observed in an artificial stand of central Sardinia. A fungus identified morphologically as *Diplodia scrobiculata* J. de Wet, B. Slippers et M.J. Wingfield was isolated from symptomatic shoots and needles. On potato-dextrose-agar (PDA) fungal colonies showed a suppressed white mycelium that turned mouse-black after 4-6 days at 25°C. Within 10-14 days pycnidia appeared on sterile *P. radiata* needles placed on the PDA surface. Two spore types were observed: hyaline cylindrical microconidia (2.6 ± 1.0 µm), and dark brown macroconidia (26.3)-29.5-(34.1) × (9.8)-11.2-(12.7) µm. Spores were either aseptate or showed 1 to 3 septa. Pathogenicity tests were made by placing 20 µl of a conidial suspension (1 to 3 septa. Pathogenicity tests were made by placing 20 µl of sterilized twigs of declining trees. On potato-dextrose-agar (PDA) at 25°C *B. obtusa* isolates developed grey fluffy colonies and produced pycnidia on sterile cork oak twigs placed on the surface of PDA within one month. The brown, oblong, straight and aseptate conidia measured (21)-24.8-(29) × (8.5)-9.9-(11.5) µm. *B. obtusa* identity was confirmed by analysis of the nucleotide sequences of the internal transcribed spacer (ITS) from the rRNA repeat, and the translation elongation factor 1-alpha (EF1-a). Representative sequences of both regions were deposited in GenBank (ITS: DQ487159; EF1-a: DQ 487160). For pathogenicity tests, seven 2-year-old cork oak seedlings were inoculated with mycelial plugs (3-4 mm²) from the infection point, from which the fungus was consistently reisolated. The results prove the endophytic occurrence of *B. obtusa* in living bark of declining cork oak trees and confirm its weak pathogenicity. *B. obtusa* is a cosmopolitan weak pathogen of several plant species including *Quercus* spp. (Frisullo et al., 2000). This is the first record of *B. obtusa* on cork oak trees.


**Corresponding author:** A. Franceschini
Fax: +39.079.229316
E-mail: afran@uniss.it

Received July 17, 2006
Accepted July 27, 2006

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**DISEASE NOTE**

**OCCURRENCE OF *BOTRYOSPHERIA OBTUSA* IN DECLINING CORK OAK TREES IN ITALY**

B.T. Linaldeddu¹, J. Luque² and A. Franceschini¹

¹ Dipartimento di Protezione delle Piante, sezione di Patologia vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy
² Dipartiment de Protecció Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, Centre de Cabrils, Ctra. de Cabrils s.n., E-08348 Cabrils, Barcelona, Spain

In the last decades cork oak decline was observed in several natural stands in Sardinia, Italy (Franceschini et al., 1999). Affected trees exhibit crown thinning, leaf yellowing, branch dieback, bark necrotic lesions, production of epicormic shoots, and exudates on branches and trunk. Surveys conducted in 2003 in a stand of Northern Sardinia, disclosed the consistent presence of *Botryosphaeria obtusa* (Schwein.) Shoemaker in symptomatic and symptomless twigs of declining trees. On potato-dextrose-agar (PDA) at 25°C *B. obtusa* isolates developed grey fluffy colonies and produced pycnidia on sterile cork oak twigs placed on the surface of PDA within one month. The brown, oblong, straight and aseptate conidia measured (21)-24.8-(29) × (8.5)-9.9-(11.5) µm. *B. obtusa* identity was confirmed by analysis of the nucleotide sequences of the internal transcribed spacer (ITS) from the rRNA repeat, and the translation elongation factor 1-alpha (EF1-a). Representative sequences of both regions were deposited in GenBank (ITS: DQ487159; EF1-a: DQ 487160). For pathogenicity tests, seven 2-year-old cork oak seedlings maintained in a greenhouse at 14-26°C were inoculated with mycelial plugs (3-4 mm²) from the margin of an actively growing colony of *B. obtusa* strain CBS 119936, placed in a shallow wound on the basal part of the stem of each plant. Sterile PDA plugs were placed into similar wounds on three control plants. After 4 weeks, only the plants inoculated with *B. obtusa* showed a small (1.4±0.1 cm) brown and sunken lesion around the infection point, from which the fungus was consistently reisolated. The results prove the endophytic occurrence of *B. obtusa* in living bark of declining cork oak trees and confirm its weak pathogenicity. *B. obtusa* is a cosmopolitan weak pathogen of several plant species including *Quercus* spp. (Frisullo et al., 2000). This is the first record of *B. obtusa* on cork oak trees.


**Corresponding author:** A. Franceschini
Fax: +39.079.229316
E-mail: afran@uniss.it

Received July 17, 2006
Accepted July 27, 2006

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**Corresponding author:** A. Franceschini
Fax: +39.079.229316
E-mail: afran@uniss.it

Received July 17, 2006
Accepted July 27, 2006


**DISEASE NOTE**

**FIRST REPORT OF TAPHRINA INSITITIAE ON SLOE FRUITS IN KOSOVA**

L.R. Susuri¹ and H. Sh. Susuri²

¹Kosova Academy of Sciences and Arts, Street Emin Duraku 1, Pristina, Kosova
²Ministry of Agriculture, Food and Rural Development, Street Mother Theresa No 35, Pristina, Kosova

During the last 5-6 years, widespread outbreaks of a serious disease of sloe (Prunus spinosa) were observed in Kosova. Symptoms were primarily visible on the fruits, which were deformed and hypertrophic, thus much larger than those of healthy plants, whitish-green in colour at first, then bright pink or red. With time, infected fruits shrivelled and eventually fell to the ground. Based on morphological observations, the causal agent of the disease was identified as the ascomycete Taphrina insititiae (Sadebeck) Johansson. The plentiful asci present on fruits were elongated and had an average size of 11.1 × 46.6 mm (8.8-13.5 × 35.3-59.8 mm). Ascospores were ovoid, one-celled, and measured 5.7 × 7.8 mm (5.0-7.5 × 3.8-10 mm in diam.). Infections of T. insititiae produce large amount of inoculum, which is responsible for heavy attacks to plums (Bondoux 1988). Small “wiches’ brooms” on the branches of Prunus pennsylvanica are caused by Exaoscus insititiae Sadb, a fungus which, according to Mix (1949), is the same as Taphrina pruni, whereas due to the shape and size of asc, is considered as a distinct species by Booth (1998). This is the first record of T. insititiae in Kosova. Whether this fungus represents a threat to the plum industry of this region remains to be ascertained.


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**DISEASE NOTE**

**FIRST REPORT OF PEACH LATENT MOSAIC VIROID AND HOP STUNT VIROID IN PRUNUS IN SLOVENIA**

M. Virscek-Marn¹, I. Mavric Plesko¹, G. Urek¹, A. Myrta² and I. Zezlina³

¹Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana, Slovenia
²Instituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy
³KGZ Nova Gorica, Pri brastu 18, 5000 Nova Gorica, Slovenia

To test for Peach latent mosaic viroid (PLMVd) and Hop stunt viroid (HSVd) on Prunus hosts in Slovenia, surveys were carried out in 2003 and 2005, collecting 240 samples altogether in commercial orchards and home gardens. In September 2003, 117 plum (Prunus domestica), 24 peach (P. persica), 14 mahaleb cherry (P. mahaleb), 6 blackthorn (P. spinosa) and 3 apricot (P. armeniaca) samples were tested. In September 2005, 44 samples of ornamental Prunus species (P. cistena, P. glandulosa, P. nigra and 8 cultivars of P. laurocerasus) were taken in a nursery. Also, three samples of bird cherry (P. padus), 28 of blackthorn, 62 of plum, 25 of apricot and 14 of peach were collected. Eight mother trees of peach cv Iris Rosso for graftwood production were also tested in 2005. Sampled mahaleb cherry, bird cherry and blackthorn plants grew wild. None of the 240 sampled plants showed symptoms of viroid diseases. “Tissue printing” hybridisation was according to Pallás et al. (2003), pressing freshly cut ends of leaf petioles onto Hybond N+ nylon membrane and hybridizing at 55°C with full-length cRNA digoxygenin-labelled probes (Shamloul et al., 1995). In 2003 HSVd was detected in one home garden apricot and PLMVd in a peach hybrid. In 2005, PLMVd was found in 7 of 8 trees of cv Iris Rosso, five of which contained also HSVd. PLMVd was also detected in four peaches of an unknown variety from a commercial orchard. To our knowledge this is the first report of PLMVd and HSVd in Prunus species in Slovenia.


DISEASE NOTE
FIRST REPORT OF SHARKA IN THE ÇUKUROVA REGION OF TURKEY
G. Koç and S. Baloglu
Department of Plant Protection and Plant Pathology, Faculty of Agriculture, University of Çukurov, 01330, Adana, Turkey

For the last couple of years, plum trees (Prunus domestica) showing various patterns of chlorotic mottling, line patterns and deformation of the leaves and fruits were repeatedly observed in a number of orchards of Adana and Mersin (Çukurova region, Turkey). These symptoms resembled very much those of Sharka, the most serious disease of stone fruits, elicited by the potyvirus Plum pox virus (PPV). Leaf samples collected at random from different trees were tested by DAS-ELISA using a commercial polyclonal antiserum (Bioreba, Reinach, Switzerland). A clear-cut positive reaction was obtained with samples from three trees, whereas samples from three additional trees gave inconsistent and doubtful responses. Mechanical inoculation of sap extracted from symptomatic leaves, induced chlorotic/necrotic local lesions in Nicotiana benthamiana. Total nucleic acids from leaves of the six trees tested by ELISA were extracted according to Spiegel et al. (1996) and subjected to RT-PCR using the protocol and primers described by Wetzel et al. (1991). The expected 234 bp fragments were obtained only from the three clearly ELISA-positive samples. Analysis of the amplicons exposed to the enzymes AluI and RsaI yielded restriction patterns consistent with those reported by Wetzel et al., (1991) for PPV strain M. These results confirm the already known existence of Sharka in Turkey and provide evidence for its previously unreported presence in the Çukurova region.


DISEASE NOTE
SEVERE OUTBREAK OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* ON NEW APRICOT CULTIVARS IN CENTRAL ITALY
M. Scortichini
C.R.A.-Istituto Sperimentale per la Frutticoltura, Via di Fioranello, 52, 00134 Roma, Italy

Symptoms of severe twig dieback and death of whole plants were observed in one-year-old apricot (Prunus armeniaca) orchards planted in the province of Rome (Italy) with newly introduced cultivars Lilycot, Mangocot, Orangecot, and Sweetcot. The incidence of completely wilted plants was up to ca 30%. To isolate the pathogen, tissue from lesion margins was ground in a mortar containing sterile saline; 0.1 ml aliquots of serial ten-fold dilutions were plated on medium B of King et al. (1954), and incubated at 25-27°C for two days. The resulting fluorescent colonies were analysed with biochemical and pathogenicity tests and by rep-PCR using BOX and ERIC primers. All isolates were levan-positive and tobacco hypersensitivity-positive. They were all oxidase-, potato soft rot and arginine dehydrolase-negative (LOPAT tests, group Ia). In addition, they had an oxidative metabolism of glucose and did not reduce nitrates. Upon rep-PCR, the isolates showed, with both primers, high similarity with some representative *Pseudomonas syringae* pv. *syringae* van Hall strains obtained from apricot and previously characterized with the same technique (Scortichini et al., 2003). Pathogenicity tests were carried out on apricot twig, lilac and pear leaves and lemon fruits. All isolates induced necrotic lesion on lemon fruits as well as on lilac and pear leaves. In addition, they caused wilting on inoculated apricot twigs one month after inoculation. Re-isolations yielded the same colony type as in the primary isolates. The conclusion is that the observed dieback and wilting of young apricot trees is caused by *P. s. pv. syringae*. It is likely that the severe outbreak was due to the very high susceptibility of apricot cultivars recently introduced in the area.


Corresponding author: M. Scortichini
Fax: +39.06.79340138
E-mail: mscortichini@yahoo.it
Received September 5, 2006
Accepted September 29, 2006

Corresponding author: G. Koç
Fax: +90.322.3386437
E-mail: gkc@cu.edu.tr
Received October 29, 2006
Accepted November 20, 2006
A survey was done during spring 2006 in several stone fruit orchards of Egypt for infections by *Apricot latent virus* (ApLV) and Plum bark necrosis stem pitting-associated virus (PBNSPaV). The orchards were in the new reclaimed and old lands of the governorates of Fayoum, Qalyoubia, Giza, Menoufia, Beharya and Behaira. Samples from 29 apricots, 5 European plums, and 5 peaches were collected randomly for RT-PCR analysis. DNA polymerase kits (Invitrogen, Carlsbad, California, USA). A duplex RT-PCR was done using sets of specific primers as described by Sánchez-Navarro et al. (2005). Fragments of amplified DNA of 717 or 270 bp, were derived from RNA of ApLV and PBNSPaV, respectively. RT-PCR results were confirmed by uniplex RT-PCR using other primer sets specific for ApLV (Nemchinov and Hadidi, 1998) or PBNSPaV (Amenduni et al., 2005). Of the tested samples, 4 plums and 2 peaches were infected by PBNSPaV, an apricot was infected by ApLV, and 2 plums were infected by both viruses. These infections were not associated with any particular field symptoms. All infected trees belonged to imported cultivars, except for a native apricot cv. Balady, that was infected with ApLV. To our knowledge, this is the first report of infections by ApLV and PBNSPaV in Egypt.


In early spring of 2006, stunted plants were observed in more than 30-year-old Satsuma mandarin trees (*Citrus unshiu*) grafted on *Poncirus trifoliata* in the Eastern Black Sea region of Turkey. Young shoots were collected from 43 such trees in different commercial groves and home gardens and were analyzed by DAS-ELISA and DTBIA using a commercial kit for detecting *Citrus tristeza virus* (CTV) (Loewe, Germany). Five trees gave a positive reaction. The presence of CTV in these trees was confirmed by using RT-PCR of total RNA extracts using primers specific for the CTV CP gene (Pappu et al., 1993). This yielded the expected 672 bp DNA fragment. When budwood from the five CTV-positive trees was grafted onto Mexican lime (*Citrus aurantifolia*) seedlings, symptoms (vein-clearing and small-sized leaves) were induced, that were similar to those elicited by CTV (Garneyse et al., 1987). The results showed that some Satsuma mandarins were infected by CTV. While CTV and one of its vectors, *Aphis gossypii*, have been found previously in the Mediterranean and Aegean regions (Cinar et al., 1993), the presence of this virus has not been reported elsewhere in Turkey. To our knowledge, this is the first report of CTV on Satsuma mandarins in the Eastern Black Sea region of Turkey.


DISEASE NOTE

FIRST RECORD OF FIG LEAF MOTTLE-ASSOCIATED VIRUS 1 IN TUNISIA

S. Nahdi1, T. Elbeaino1, M. Digiaro1 and G.P. Martelli2

1 Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano-Bari, Italy
2 Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, sezione di Bari, via Amendola 165/A, 70126 Bari, Italy

Fig (Ficus carica) orchards in four different areas in the north and south of Tunisia were surveyed in autumn 2005 for the presence of mosaic disease (FMD). Diseased plants were very common and showed a wide array of symptoms, especially on the leaves, which were variously deformed and discoloured. A total of 156 samples of symptomatic leaves, were collected from FMD plants and analyzed in Bari. Double-stranded RNA (dsRNA) was extracted from the main veins of 40 samples and total nucleic acids (TNAs) from the same tissues of all samples. TNAs were tested by RT-PCR using the protocol and the primers specific for Fig leaf mottle-associated virus 1 (FLMaV-1), described by Elbeaino et al. (2006). dsRNAs with a maximum size of about 18 kbp were recovered from 9 of the tested extracts, whereas a product of the expected size (ca. 350 bp) was amplified from 45 of the tested samples (c. 30%). FMD had already been recorded from Tunisia by Martelli et al. (1993) who observed the enveloped virus-like particles (double-membraned bodies) thought to be the putative agent of the disease, in cells of symptomatic leaf tissues. The occurrence of the hitherto unreported FLMaV-1 in a fairly high number of samples, now suggests that a multiplicity of viruses may be involved in the aetiology of FMD.


Corresponding author: G.P. Martelli
Fax: +39.080.5442911
E-mail: martelli@agr.uniba.it

Received November 25, 2006
Accepted December 13, 2006

DISEASE NOTE

GLOMERELLA CINGULATA CAUSING LEAF SPOT ON ZONAL AND IVY GERANIUM IN ARGENTINA

S. Larran1 and S.M. Wolcan1,2

1 Centro de Investigaciones de Fitopatología, Facultad de Ciencias Agrarias y Forestales, UNLP, 60 y 119, (1900) CC 31 La Plata, Buenos Aires, Argentina
2 Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina

Zonal geranium (Pelargonium x hortorum Bailey) and ivy geranium [P. peltatum (L.) L’Hér. ex Ait.] are ornamental plants cultivated in gardens and baskets or pots in balconies and patios. Since 2001, in nurseries and gardens of La Plata, Buenos Aires, both ornamentals have shown a disease characterized by necrotic spots larger at the edge of the leaves and narrower towards the petiole, so as to assume a V shape. These spots are light to dark brown, surrounded by a chlorotic halo and may coalesce, covering most of the leaf surface. Under humid conditions, orange spore masses arise from acervuli. Nursery plants may die and mature plants can be seriously defoliated. Isolation from diseased tissues from both hosts on potato dextrose agar produced cultures with mycelium and spores typical of Colletotrichum gloeosporioides (Penz.) Sacc. (Sutton, 1980) [teleomorph: Glomerella cingulata (Stoneman) Spauld. et H. Schrenk]. Cross inoculations were conducted using spore suspensions with isolates from zonal and ivy geranium separately. Plants sprayed with distilled water were used as controls. Plants were covered with plastic bags for 48 h and kept at 20-25°C. After 12 days, symptoms were observed only on the leaves of both hosts and C. gloeosporioides was recovered from the inoculated plants. This is the first record of C. gloeosporioides causing leaf spot on Pelargonium x hortorum and P. peltatum in Argentina.


Corresponding author: S. Larran
Fax: +54.221.4252346
E-mail: silvinalar@yahoo.com.ar

Received June 6, 2006
Accepted June 18, 2006