Phytopathol. Mediterr. (2010) 49, 414–416

NEW OR UNUSUAL DISEASE REPORTS

Shoot necrosis of olive caused by *Phoma incompta*, a new disease of olive in Croatia

DARIO IVIC, ANTE IVANOVIC, TIHOMIR MILICEVIC and BOGDAN CVJETKOVIC

Department of Plant Pathology, University of Zagreb, Faculty of Agriculture, Svetosimunska cesta 25, 10 000 Zagreb, Croatia

Summary. Reddish-brown lesions on young shoots, withering of leaves, cankers on older shoots and shoot necrosis were observed on some olive trees in southern Croatia. The fungus *Phoma incompta* was identified as the causal agent of the disease. Pathogenicity of *P. incompta* isolates was confirmed by inoculating young olive plants in a greenhouse; these plants developed symptoms similar to those observed in the field.

Key words: *Olea europea*, wood canker.

Olive (*Olea europea* L.) is an important crop in the Mediterranean part of Croatia. In recent years, a significant number of new olive orchards has been established, supported and co-financed by the government and the local authorities. However, olive diseases are still rather poorly studied in Croatia, with most research focusing on practical aspects of leaf spot (*Spilocaea oleaginea*) and on olive knot (*Pseudomonas savastanoi*) management.

In 2008 and 2009, symptoms of an unknown olive disease were seen on the island of Brac in southern Dalmatia. The disease was particularly severe in orchards where the local variety Pastrica was grown. Reddish-brown lesions occurred on one-year-old shoots, with a clear margin between discoloured and asymptomatic wood (Figure 1a). Affected plant tissue was sometimes slightly raised, with longitudinal cracks (Figure 1b). Lesions started from around the buds, leaf scars or from the point where young shoots started to grow. Shoots withered, with greyish-brown leaves still attached to some shoots, whereas other shoots were dry and without leaves. Brown necrosis beyond the outer canker zone was visible under the bark of symptomatic wood, with a strict dividing line between necrotic and apparently healthy inner plant tissue (Figure 1c). Cankers and cracks were also evident in the older wood. A significant number of trees in several orchards was affected, and losses has become important. According to the growers, multiple sprayings with the copper-based fungicides during the last few seasons did not slow down disease development.

Symptomatic shoots were sampled and analysed in a laboratory. One-year old shoots did not exhibit fungal fruiting bodies, but pycnidia with small *Phoma*-like conidia occurred around the cankers in the older wood. To isolate the fungus from the lesions, one-year-old shoots were washed,

Corresponding author: D. Ivic
Fax: +385 1 2390 730
E-mail: divic@agr.hr

surface-sterilised in 0.1% NaOCl, and wood slices were cut from the area between symptomatic and asymptomatic tissue. Slices were inoculated on water agar amended with streptomycin and incubated in darkness at 20°C. Fungal colonies started to grow from inoculated plant tissue, and the hyphal tips were transferred to malt extract agar (MEA) to obtain pure cultures. The majority of the cultures on MEA developed pycnidia with conidia characteristic of *Phoma*. Fifteen isolates were collected and identified according to Boerema *et al*. (2004). Colonies were slow growing, some sporulating abundantly with only a little whitish aerial mycelium (Figure 1e), while others sporulated less abundantly with much more aerial mycelium. Pycnidia were subglobose, dark, always covered with mycelial hairs, mostly confluent, solitary usually in the outer part of the colonies. Conidia were aseptate, without guttules and very small (average 3×1 μm). By their shape the isolates were identified as *Phoma incompta* Sacc. & Martelli. To confirm the identification, isolates were compared to the reference isolate, CBS 467.76, from the Centraalbureau vor Schimmelcultures (Utrecht, The Netherlands). The MEA cultures of six of the isolates were very similar to the reference isolates, while others differed somewhat in the abundance of their aerial mycelium. The size, colour and distribution of the pycnidia and the conidial morphology of all isolates were similar to those of the reference isolate.

Pathogenicity tests were performed in a heated greenhouse on two-year-old olive plants, cv. Oblica and cv. Leccino. Six isolates were chosen for inoculations. Mycelial plugs approximately 5×5 mm were cut from the sporulating part of the MEA colonies, placed on cuts of similar size made with a sterile scalpel on the olive shoots, and sealed with parafilm. Each isolate was inoculated on two shoots of the two cultivars. Sterile MEA was applied to the control. The first symptoms appeared on both cultivars approximately one month after inoculation. Reddish-brown flecks became apparent on the upper leaves of some of the inoculated shoots. Upper leaves soon turned brown and rolled inwards, dried and fell off. A reddish discoloration appeared on the inoculated shoots, which was especially evident when shoots were soaked with water (Figure 1d). After six months, four of the 24 inoculated shoots were dead and dried, and 15 were symptomatic. No apparent differences were visible between the two cultivars. No pycnidia were seen on the symp-

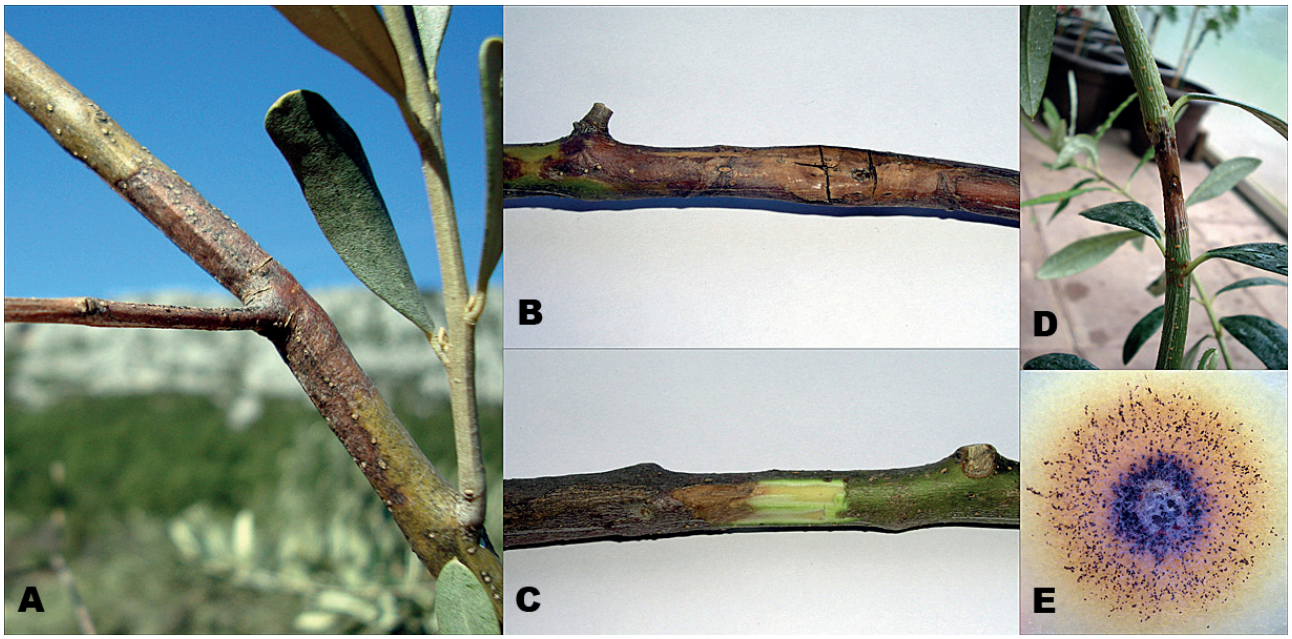

Figure. 1. (A) Lesions on olive shoots; (B) cracks on symptomatic tissue; (C) dividing line between healty and necrotic inner tissue; (D) symptoms on inoculated olive shoot; (E) an isolate of *Phoma incompta* on MEA.

tomatic inoculated shoots, but the fungus was readily re-isolated from the lesion edges. Control shoots inoculated with uncolonized agar did not develop symptoms, and callus formation was observed on the wounds.

So far, shoot necrosis caused by *P. incompta* has been recorded and described in Greece (Malathrakis, 1979) and Italy (Tosi and Zazzerini, 1994). Local epidemics and significant damage to olive trees from the disease were reported from Umbria (Tosi and Zazzerini, 1994), Sicily (Scarito and Salamone, 2004) and Crete (Malathrakis, 1979). To our knowledge, this is the first report of *Phoma incompta* on olive in Croatia.

Literature cited

Boerema G.H., J. de Gruyter, M.E. Noordeloos and M.E.C. Hamers, 2004. *Phoma Identification Manual: Differentiation of Specific and Infra-specific Taxa in Culture*. CABI Publishing, CAB International, Wallingford, UK, 470 pp.

Malathrakis N.E., 1979. *A Study of an Olive Tree Disease Caused by the Fungus* Phoma incompta *Sacc. et Mart*. PhD Thesis, Agricultural College, Athens (in Greek).

Scarito G. and A. Salamone, 2004. Gravi alterazioni su olivo in Sicilia causate da *Phoma incompta* Sacc. et Mart. In: *Atti del 2° Congresso Nazionale: Piante Mediterranee. Valorizzazione delle Risorse e Sviluppo Sostenibili*, Ottobre 7–8, 2004, Agrigento, Italia, 197–199.

Tosi L. and A. Zazzerini, 1994. *Phoma incompta* a new olive parasite in Italy. *Petria* 4, 161–170.

Accepted for publication: August 2, 2010