

SHORT COMMUNICATION

OCCURRENCE OF *ERWINIA NIGRIFLUENS* ON ENGLISH WALNUT (*JUGLANS REGIA*) IN THE VENETO REGION (NORTHERN ITALY)

A. Saccardi, V. Bonetti, A. Melegatti and M. Cristanini

Regione Veneto, Servizio Fitosanitario, Osservatorio per le Malattie delle Piante, Lungadige Capuleti 11, I-37122 Verona, Italy

SUMMARY

In the period 1995-1997, in the Padua province of the Veneto region, bark cankers were observed on 15-20 year old walnut trees (*Juglans regia* L.) and young walnut trees in the nursery. On the older trees the cankers on the trunks and on the proximal part of the scaffold branches consisted of brownish areas that often produced drops of exudate. On young trees the cankers caused longitudinal grooves, often brownish and severe deformation of the trunks. Gram-negative bacteria isolated from both types of canker and purified were identified as *Erwinia nigrifluens* based on phenotypic traits (including fatty acid profiles) and pathogenicity. The symptoms caused on young trees were unusual for *E. nigrifluens*. This was the first report of *E. nigrifluens* in Italy.

RIASSUNTO

PRESENZA DI *ERWINIA NIGRIFLUENS* SU NOCE (*JUGLANS REGIA*) NELLA REGIONE VENETO (ITALIA SETTENTRIONALE). Nel periodo 1995-1997 cancri corticali di alberi di noce (*Juglans regia* L.) di 15-20 anni di età e di giovani astoni di vivaio sono stati osservati in provincia di Padova nella regione Veneto. Nei vecchi alberi i cancri su tronco e parte prossimale delle branche principali consistevano in aree brune da cui colavano spesso gocce di liquido. Sui giovani alberi i cancri causavano scanalature longitudinali, spesso brune e gravi deformazioni dei fusti

Batteri gram-negativi isolati da entrambi i tipi di cancri e purificati sono stati identificati come *Erwinia nigrifluens* per caratteri fenotipici (inclusi i profili degli acidi grassi) e patogenetici.

I sintomi causati sui giovani alberi sono inusuali per *E. nigrifluens*. Per la prima volta è stata segnalata la presenza di *E. nigrifluens* in Italia.

Key words: walnut, bark canker, *Erwinia rubrifaciens*, Gram-negative, Po valley.

During 1995, in the Padua province of the Veneto region, bark cankers were observed on the trunks and scaffold branches of 15-20 year old walnut trees (*Juglans regia* L.), and on the trunks of 2-3 year old walnut trees in the nursery. On the main branches, only the proximal parts were affected. The same type of canker was observed in the two following years in other nurseries and in walnut orchards and timber stands.

On young trees, the cankers first appeared as longitudinal streaks, slightly sunken, approximately 5-20 cm long. Removal of the surface bark revealed blackish longitudinal streaks, discontinuous at each end (Fig. 1), that did not normally affect the deeper layers of the bark and cambium. Spreading and merging of the streaks led to the appearance of longitudinal grooves on the surface, often brownish below underneath, that caused severe local distortion of the trunk (Fig. 2). Beneath the surface layer of the bark, there were longitudinal pockets of blackened tissue, that at times reached a depth of approximately 2/3 the bark; in more severe cases, these pockets also reached the cambium. As the trunk grew, occasionally cracks appeared at the bottom of the grooves, that produced small quantities of clear or brownish, watery exudate. This exudate was also released when the bark over the pockets was perforated. For this reason, these lesions are called 'fistulas' by the local farmers. The growth of the trunk also led to enclosure of the black pockets within the wood, so that a cross-section of the trunk revealed characteristic lobes of wood corresponding with the initial distribution of the pockets around the circumference.

The cankers on the trunks of 15-20 year old trees appeared as brownish oval areas, 4 or 5 cm long, frequently producing drops of liquid (Fig. 3). Removal of the outer bark exposed blackened oval areas (Fig. 4). The cankers on the trunks of old trees, over the years, were masked by the accumulation of ritidoma, becoming difficult to recognise.

The lesions on the trunks of the English walnuts were similar in appearance and localisation to those of



Fig.1. Cankers caused by *E. nigrifluens*, observed on the trunk of a 15 year old English walnut tree. Traces of exudate can be seen.



Fig.2. Intracortical view of a trunk canker on a 15 year old English walnut tree.



Fig.3. Severe distortion of the trunk of a young English walnut tree in the nursery. Longitudinal grooves can be seen.

shallow-bark canker caused by *Erwinia nigrifluens*, described by Wilson, Starr and Berger (1957) in California. Laboratory tests were performed to confirm this diagnosis.

For isolation, pieces of tissue were collected from the edges of the bark pockets placed in test tubes with (5 ml) sterile water and left to soak for 30 minutes at 20-27°C. After vigorous shaking of the tubes, 30 ml of suspension was streaked on Bacto EMB agar (Difco) plates. After 3 days of incubation at 28°C, colonies had formed that were circular, dark purple with metallic green sheen, whole margins, not mucoid, reaching a diameter of 2-4 mm after 6 days. The isolates from the



Fig.4. Intracortical view of the upper section of a pocket under a longitudinal groove in a young English walnut tree in the nursery.

15-20 year old trees and the nursery trees were selected and 4 pure cultures were prepared for identification (Lelliott and Stead, 1987). The pure cultures were rod-shaped, Gram-negative, oxidase-negative, facultatively anaerobic, did not produce levan-positive colonies on nutrient agar with 5% sucrose, or fluorescent pigment on KB agar and did not hydrolyse starch. They did not induce a hypersensitive reaction on tobacco leaves, did not show pectolytic activity on pectic gels (Cuppels and Kelman, 1974), and did not hydrolyse gelatine; they were urease positive, and produced acid without gas from glucose, but not from maltose.

For the pathogenicity test, a drop of bacterial suspension, with a concentration of approximately 10^8 cells ml^{-1} , was injected with a syringe into the bark of an English walnut (*J. regia*). The hole was then covered for 24 hours with transparent plastic film. Eight injections were made in the same tree, 4 with bacterial suspension and 4 with sterile water, as a control. The inoculations were performed on June 4th 1996 and the trees were kept under observation up to October when the symptoms were observed and attempts were made to re-isolate the bacteria. After 5 months, drops of brownish liquid were noted at the injection sites. When the outer part of the bark was removed, damp, blacken areas

were exposed, with a diameter of approximately 2.5 cm. There were no exudates or black areas in the bark at the control injection sites. The bacteria were successfully reisolated from the black tissue of the experimental cankers and showed the same morpho-physiological features as the bacteria used for the inoculations.

Comparative analysis of the fatty acid methyl ester profiles of two English walnut strains and the two reisolates was made by the Central Science Laboratory at Harpenden (UK) using as reference the profiles of strains NCPPB 564, NCPPB 565 and NCPPB 566 of *E. nigrifluens* from the library of the National Collection of Plant Pathogenic Bacteria. Statistical analysis made it possible to include the two English walnut strains and the two reisolates in the same group as the three *E. nigrifluens* strains with Euclidean distances of less than three units (Stead, personal communication).

On the basis of all these traits, the English walnut strains were identified as *E. nigrifluens* Wilson, Starr and Berger, thus confirming the symptomatic diagnosis.

Shallow-bark canker of English walnut, described for the first time in California by Wilson *et al.* (1957), generally only affects the outer layers of the bark. It rarely reaches the cambium and affects trees more than 10-15 years old (Wilson *et al.*, 1957). This pattern of canker development was only observed in some cases from the Veneto region. Indeed, in the more severe cases, necrosis reached the cambium and the most active and damaging cankers were found on young trees in the nursery. The greater depth of the cortical cankers in the Veneto cases resembles the so-called deep-bark cankers caused by *E. rubrifaciens* on English walnut (Wilson *et al.*, 1967). On the basis of some symptomatic aspects (*e.g.* absence of dark brown pits on the sapwood, type and colour of the exudate) (Sinclair *et al.*, 1987; Ogawa and English, 1991) of the natural infections and, above all, some phenotypic traits of the isolates, it was possible to exclude the presence of *E. rubrifaciens* in the cases of the Veneto region. In fact, infections of *E. rubrifaciens* can be associated with *E. nigrifluens* on the same tree (Ogawa and English, 1991). It is possible that in the Po Valley the *E. nigrifluens* strains are more virulent and/or the genotypes of *J. regia* more susceptible. This is the

second report of *E. nigrifluens* on English walnut in Europe, following that of Lopez *et al.* (1994) in Spain.

The damage caused by *E. nigrifluens* on English walnut can affect walnut orchards and timber stands, but damage to production of walnut orchards is negligible. However, in the nursery, the young walnut trees can be severely damaged and unsuitable for sale. In walnut timber stands, deep bark canker in young trees can seriously alter the pattern of the veins and thus affect quality of the wood.

REFERENCES

- Cuppels D., Kelman A., 1974. Evaluation of selective media for isolation of soft-rot bacteria from soil and plant tissue. *Phytopathology* **64**: 468-475.
- Lelliott R.A., Stead D.E., 1987. Methods for the diagnosis of bacterial diseases of plants. Blackwell Scientific Publications, Oxford, UK.
- Lopez M.M., Marti R., Morente C., Oreliana N., Ninot T., Aleta N., 1994. Phytopathogenic bacteria identified in walnut in Spain. Fuera de Serie No. 2, 307-314. Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia, Spain.
- Ogawa J.M., Harley English, 1991. Diseases of temperate zone tree fruit and nut crops. University of California, Division of Agriculture and Natural Resources, Oakland, Ca, Publication 3345, USA.
- Sinclair W.A., Lyon H.H., Johnson W.T., 1987. Diseases of trees and shrubs. Comstock Publishing Associates, Cornell University Press, Ithaca and London, USA.
- Wilson E.E., Fakhery M., Zeitoun F.M, Fredrickson D.L., 1967. Bacterial phloem canker, a new disease of Persian walnut tree. *Phytopathology* **57**: 618-621.
- Wilson E.E., Starr M.P., Berger J.A., 1957. Bark canker, a bacterial disease of the Persian walnut tree. *Phytopathology* **47**: 669-673.

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