

MINIREVIEW

APICAL NECROSIS OF PERSIAN (ENGLISH) WALNUT (*JUGLANS REGIA*):
AN UPDATEC. Moragrega¹ and H. Özaktan²¹ *Institute of Food and Agricultural Technology, University of Girona, Campus Montilivi s/n., 17071 Girona, Spain*² *University of Ege, Faculty of Agriculture, Department of Plant Protection, 35100 Bornova-Izmir, Turkey*

SUMMARY

In recent years, a new disease of Persian (English) walnut (*Juglans regia* L.) causing premature fruit drop and important yield losses has been observed in orchards of Mediterranean walnut-growing regions. Characteristic disease symptoms consist of an apical necrosis originating at the stigmatic end of the hull. In early stages of fruit development, differentiation of “apical necrosis” from “blight”, another walnut disease, requires a detailed observation of externally and internally affected tissues. Different approaches have been used to characterize disease symptoms in affected walnut-growing areas and to identify the causal agents and predisposing factors. Recent studies indicate that the bacterium *Xanthomonas arboricola* pv. *juglandis* is the microorganism most frequently associated with apical necrosis and may produce the initial infections in young nuts. *Fusarium* spp. and *Alternaria* spp. seem also to be involved in the induction of apical necrosis, causing secondary infections, or growing as saprophytes on bacterial-infected tissues, thus enhancing disease symptoms and severity. Nutritional problems and soil characteristics could be partially related to apical necrosis, in addition to microbial infection. Many aspects of apical necrosis are, however, still unknown and management strategies cannot be defined, so the disease is not readily controlled. Etiological and epidemiological studies on apical necrosis are discussed in this review in order to integrate information and contribute to a better understanding of the disease and its predisposing factors.

INTRODUCTION

In recent years “apical necrosis”, a new disease of Persian (English) walnut (*Juglans regia* L.) that causes premature fruit drop and important yield losses has been observed in Mediterranean walnut-growing regions. In Spain, the disease was first observed in 1997 in

Extremadura following an intense early fruit drop and was found to affect the main walnut production areas (Aletà and Ninot, 2002; Arquero *et al.*, 2005). In years when the disease incidence was high, the whole fruit production was lost, as it occurred in some commercial walnut orchards of Tarragona (north-east Spain). In France, a premature walnut fruit drop has also been reported, that reduced nut production in commercial orchards of the south-east part of the country (Garcin and Duchesne, 2001; Bouvet, 2005). Important yield losses caused by premature fruit dropping have also been recorded in the last years in the Marmara Region, an important walnut production area in north-west Turkey (Özaktan *et al.*, 2009). In 1998 a severe fruit drop that reduced yield up to 20% was observed on walnut in northern Italy. The disease was called brown apical necrosis or BAN (Belisario *et al.*, 2001) and, according to Belisario (2002), it was observed in 1999 in France and again in Italy in 2002, where it affects walnut orchards since the late 90s (Belisario *et al.*, 2004).

DISEASE SYMPTOMS

Symptoms on dropped fruits in the different geographic areas consist of apical necrosis originating at the stigmatic end of the nuts. In the early fruit development stages, external apical necrosis may be confused with walnut blight apical infections caused by the bacterium *Xanthomonas arboricola* pv. *juglandis* (*Xaj*). When nuts are infected by this pathogen during the prebloom or blooming periods, the lesions are typically located at the apical or blossom end, and the first macroscopic symptoms consist in the appearance of small, tendentially circular, water-soaked areas in the bracts and the apical end of the hulls. Later, the water-soaked black lesions become depressed acquiring the characteristics morphology of walnut blight (Miller and Bollen, 1946). Also walnut blight can cause premature fruit dropping, resulting in a strong reduction of the yield. In fact, preliminary studies led to consider apical necrosis as a particular manifestation of walnut blight combined with physiological alterations (Garcin and Duchesne, 2001). However, recent investigations focused to characterize the

external and internal symptoms of apical necrosis, conducted to its differentiation from bacterial blight (Belisario *et al.*, 2002; Moragrega *et al.*, 2008). So, in the early stages of nut development, differentiation of apical necrosis and true walnut blight symptoms requires a detailed observation of the external and internal tissues.

Information from several years of studies on apical necrosis in Spanish and Turkish walnut orchards promoted the description of the external and internal symptoms of affected fruits that remain attached to the tree or drop to the soil (Moragrega *et al.*, 2008; Özaktañ *et al.*, 2009). Initial external symptoms appear after fruit set as small dark brown or blackish non water-soaked lesions located at the apical end of the nuts, sometimes restricted to the stigmata and surrounding area. As the fruit grows, symptoms become more evident, consisting of brown lesions, often circular and with regular margins, 2 to 15 mm in size. Lesions can become larger and turn brown or red-brown with more irregular margins (Fig. 1A, B). The external progress of infection in infected fruits that remain attached to the trees is less extensive than in dropped fruits, in which external necrosis can reach the equatorial zone of the nuts. In fruits on the ground, old apical infections are sometimes covered by white superficial fungal mycelium. The infection progresses internally from the epicarp to the mesocarp and can reach the seed (Fig. 1C, D). The epicarp and mesocarp lesions are dry and hard but rot develops when the seed is affected. After shell hardening, lesions remain restricted to epicarp and mesocarp. Apical necrosis in dropped fruits is more severe and extended to all internal tissues. Sometimes the extent of external and internal lesions is not correlated, and small external necrosis can be associated with severe infection affecting all internal fruit tissues. These symptoms coincide with those described for brown apical necrosis affecting Italian walnut orchards, corresponding to brown to dark-brown patch originating exclusively at the blossom end of the hulls and a brown to blackish rot of the inner tissues (Belisario *et al.*, 2002).

The similarity of external and internal symptoms of apical necrosis observed in the different Mediterranean walnut production areas suggests that the same disease is affecting walnuts and causing premature fruit drop and important yield reduction.

CAUSAL ORGANISMS

Etiological studies on brown apical necrosis affecting Italian walnut orchards led to consider it as a complex disease in which *Fusarium* species can play an important role. Different *Fusaria* were isolated alone or associated with other fungi (*Alternaria* spp. or *Colletotrichum* spp.) from affected walnut fruits, whereas *Xaj* was isolated only sporadically (Table 1). It was observed that *Fusarium* species differed in relation to the area and time of sampling, as it occurred with other plant species, such as sugarbeet (Nitschke *et al.*, 2009), cassava (Bandyopadhyay *et al.*, 2006) and cereals (Bottalico, 1998). Additionally, a complex of morphologically diverse, small-spored catenulate *Alternaria* spp. could also be involved in the genesis of brown apical necrosis (Belisario *et al.*, 2004). Similarly, the involvement of *Alternaria* spp. was reported in complex diseases of other hosts, including citrus (Simmons, 1990), pear (Simmons and Roberts, 1993), almond (Teviotdale *et al.*, 2001) and apple (Serdani *et al.*, 2002). *Alternaria* species isolated from BAN and pathogenic to walnut belonged to the *A. arborescens*, *A. tenuissima* and *A. alternata* groups (Belisario *et al.*, 2004, Hong *et al.*, 2006). However, until now, a role of *Xaj* in apical necrosis of walnut affecting Italian walnuts has not been ascertained. On the other hand, preliminary studies in Spanish walnut orchards showed that *Xaj* is the most frequently isolated microorganism from fruits affected by apical necrosis, alone or in association with *Fusarium* and *Alternaria* species (Arquero *et al.*, 2005). These results agree with field studies carried out over 10 years in northern Spanish walnut orchards, in which *Xaj* was the microorganism most fre-

Table 1. Incidence of microorganisms isolated from walnut fruits affected by apical necrosis in Mediterranean walnut orchards.

Microorganisms recovered from fruits (%)				
Country	<i>X. a. pv. juglandis</i>	<i>Alternaria</i> spp.	<i>Fusarium</i> spp.	Reference
Spain	60-90 ^a	10-30 ^a	10-20 ^a	Arquero <i>et al.</i> , 2005; Moragrega <i>et al.</i> , 2008
Turkey	30	7	3	Özaktañ <i>et al.</i> , 2009
Italy	sporadically	10-15	16	Belisario <i>et al.</i> , 2002
France	sporadically	8-15	20	Belisario <i>et al.</i> , 2002

^a Percentages may vary depending on orchards and fruit origin (trees or soil).

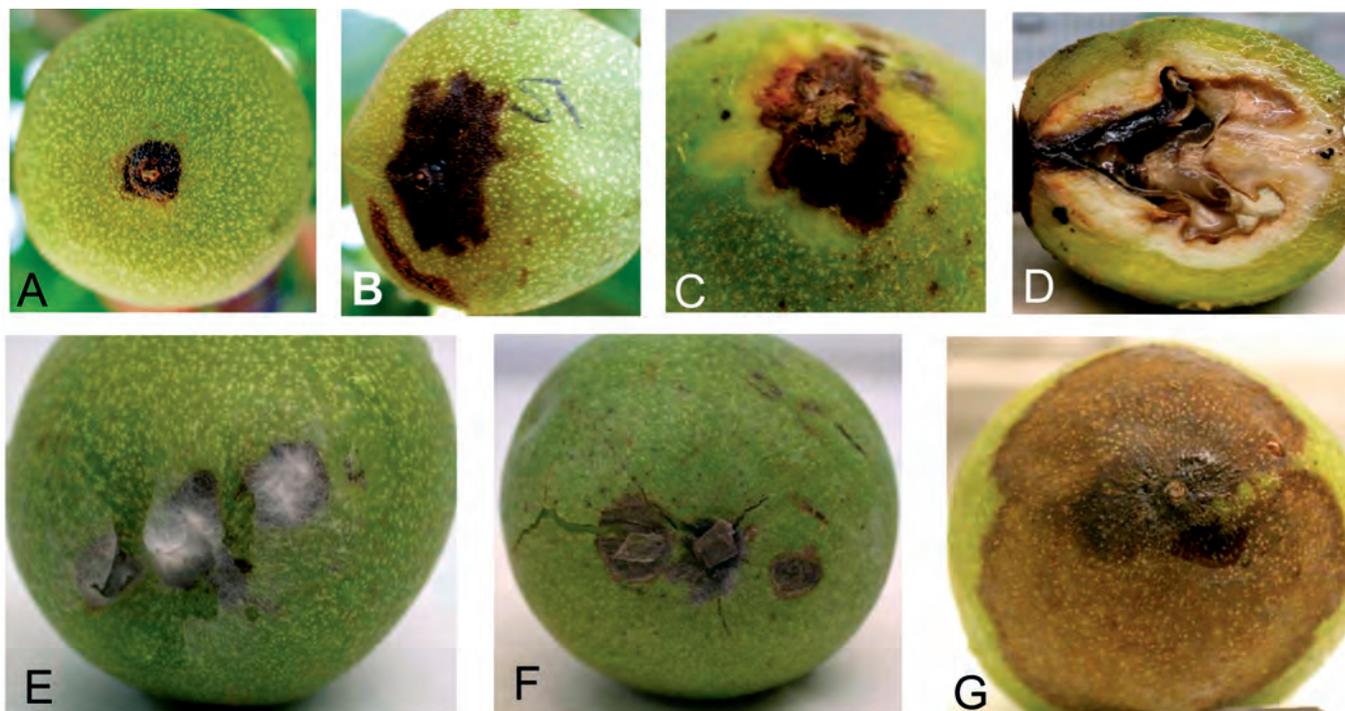


Fig. 1. External (A, B) and internal (C, D) symptoms of “apical necrosis” on cv. Hartley fruits. External symptoms on young cv. Chandler fruits inoculated with *Alternaria* spp. (E), *Fusarium* spp. (F) and *X. a. pv. juglandis* (G) isolated from “apical necrosis”-affected fruits.

quently associated with the disease (Moragrega *et al.*, 2008). In fact, this bacterium was isolated alone from more than 70% of the fruits showing apical necrosis and was found in association with *Fusarium* and *Alternaria* species in 20-30% of the samples (Table 1).

Recent investigations from Turkey (Özaktan *et al.*, 2009), show that *Xaj* is the most frequently isolated microorganism from apical necrosis-affected walnut fruits (Table 1). In this country, *Xaj* was consistently present throughout the growing season in tissues of fruits either laying on the ground or still attached to the tree. Fungi were present with low frequencies in apical infections of fruits on the tree, but were much more common in dropped fruits.

Fusarium species isolated from apical necrosis-affected fruits in Spanish orchards were identified as *F. chlamydosporum*, *F. lateritium*, *F. semitectum* and *F. solani*. *Alternaria* spp. were not present in seed lesions and only occasionally in endocarp lesions, but they were present in necrotic mesocarp. In fruits dropped to the ground, *Alternaria* spp. were present in all tissues including the seed, especially in mature fruits with old apical infections. Pathogenicity tests on young walnut fruits showed that only field isolates of *Xaj* were able to induce apical infections consisting of brown necrotic spots at the end of the nuts. External necrosis progressed from the stigmatic end of the nuts to the surrounding area and in some cases reached the equatorial zone of the fruits (Fig. 1G). Internally, fruits developed

progressive infection of all tissues including the seed. Some *Fusarium* spp. isolates were able to develop external black necrosis which, in some cases, affected internal tissues (Fig. 1F) Only *Alternaria* spp. were involved in some external necrosis at the end of the incubation period, as saprophytes growing on dead fruit tissues, and some lesions were covered by fungal mycelium (Fig. 1E) (Moragrega *et al.*, 2008).

According to these results, *Xaj* can be considered as the main causal organism of apical necrosis of walnut whereas *Fusarium* spp. can occasionally be involved in the disease, interacting with bacterial infections. The presence of *Alternaria* spp. on necrotic tissues may be related more to the opportunistic colonization of dead tissues previously infected by the bacteria. Studies from Garcin and Duchesne (2001) also consider *Xaj* as the microorganism involved in the development of apical necrosis of walnut. Further studies, including characterization of *Xaj* populations from apical necrosis and comparison with populations causing walnut blight, could contribute to elucidate the role of this bacterial species in both walnut diseases.

Several studies have pointed out that apical necrosis could be partially related to crop nutritional problems in addition to microbial infection (Bouvet, 2005), and that soil characteristics and phenolic contents of walnut fruits could predispose trees to infection and enhance apical necrosis severity (Garcin and Duchesne, 2001).

EPIDEMIOLOGY

Although apical necrosis can be observed from fruit set to harvest, infections are more frequent and severe in the early developmental stages. Initial infections occur after fruit set and are localized at the stigmatic area of the hulls. If climatic conditions are favourable, infection spreads externally and internally through the inner walnut fruit tissues. In young nuts the infection progresses internally from epicarp and mesocarp and can reach the seed 10-20 days after initial infections whereas, after shell hardening, infections remain restricted to the pericarp. The highest levels of apical necrosis incidence are observed from fruit set to Gf+30, in June and early July, causing the most extensive fruit dropping. From Gf+30 to complete shell sclerification incidence is slow and less intensive, and after Gf+45 fruit stage only few new infections develop (Aletà and Ninot, 2002; Moragrega *et al.*, 2008).

Infection progress from external to internal fruit tissues suggests that epiphytic populations of *Xaj* may act as the primary inoculum source, rather than bacterial populations overwintering in the internal tissues of walnut buds or twigs. *Fusarium* spp. may also be involved in apical necrosis of walnut and their involvement could cause secondary infections or colonization on tissues infected already by bacteria, enhancing disease symptoms and severity. In fact, some *Fusarium* species have been reported to be pathogenic to walnut and could attack the fruits which, combined with *Xaj* tissue colonization, may result in development of more severe apical necrosis. In dropped fruits, secondary colonizers such as *Alternaria* spp. contribute to increase the disease severity and the necrosis progress into inner fruit tissues.

Yield reduction due to premature fruit drop caused by apical necrosis depends on environmental factors, such as rain or temperature. Although climatic conditions favourable to apical necrosis are not yet clearly determined, it was observed that walnut orchards located in the coastal zone are more affected by apical necrosis than those of inland areas and that a relative humidity above 70% and mean temperatures higher than 24°C may trigger apical necrosis development (H. Özaktan, unpublished information).

CULTIVAR SUSCEPTIBILITY

Most commercial walnut cultivars are susceptible to apical necrosis. For instance cvs Hartley, Chandler, Serr, Lara and Vina rank as very susceptible, together with the Turkish cvs Bilecik and Rendede. Nuts of these cultivars are usually damaged when young and drop at the beginning of July. 'Hartley' and 'Mayette', however, can also be affected at mature nut stage, disease progress continues until harvest and often black stains on the shells reduce

the fruit value (Aletà and Ninot, 2002). Under Spanish climatic conditions cv. Franquette is less affected by apical necrosis, probably due to its late fruit set and delayed fruit growing period (Moragrega *et al.*, 2008). 'Pedro' has also been reported as low susceptible to the disease in some walnut-growing areas. Local Turkish cvs Yalova 1 and Sebin are slightly susceptible (Özaktan *et al.*, 2009).

DISEASE MANAGEMENT

Most epidemiological aspects of the disease are still unknown, thus management strategies cannot readily be defined and disease in affected areas is usually not well controlled. However, field observations indicate that preventive sprays with copper derivatives, consisting of 3-4 applications from bud break to fruit set as proposed for walnut blight control (Ninot *et al.*, 2002), contribute to a containment of apical necrosis (Aletà and Ninot, 2002). Removal of mummified nuts from trees reduces inoculum pressure and may prevent further yield losses. Some soil conditions as light texture, acidic soils, low manganese and magnesium contents and nutrient deficiencies (mainly phosphorous and calcium) seem to predispose trees to apical necrosis and measures to counteract these deficiencies appear to be appropriate for reduction of damage (Garcin and Duchesne, 2001).

ACKNOWLEDGEMENTS

Part of information presented in this review was financed by public funds from the Spanish Government (research project INIA RTA2005-00104-00-00). We thank the COST-873 Action for supporting this mini review.

REFERENCES

- Aletà N., Ninot A., 2002. La bactériose et la nécrose apicale du noyer en Espagne. *InfoNOIX* 19: 8.
- Arquero O., Lovera M., Rodriguez R., Salguero A., Trapero A., 2005. Characterization and development of necrotic lesions of walnut tree fruits in southern Spain. *Acta Horticulturae* 705: 457-461.
- Bandyopadhyay R., Mwangi M., Aigbe S.O., Leslie J.F., 2006. *Fusarium* species from the cassava root rot complex in West Africa. *Phytopathology* 96: 673-676.
- Belisario A., Forti E., Santori A., Balmas V., Valier A., Corazza L., 2001. *Fusarium* necrosis on Persian (English) walnut fruit. *Acta Horticulturae* 544: 389-393.
- Belisario A., Maccaroni M., Corazza L., Balmas V., Valier A., 2002. Occurrence and etiology of brown apical necrosis on Persian (English) walnut fruit. *Plant Disease* 86: 599-602.
- Belisario A., Maccaroni M., Coramusi A., Corazza L., Figuli P., Pryor B.M., 2004. First report of *Alternaria* species

- groups involved in disease complexes of hazelnut and walnut fruit. *Plant Disease* **88**: 404.
- Bottalico A., 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles in Europe. *Journal of Plant Pathology* **80**: 85-103.
- Bouvet G., 2005. Walnut blight-apical necrosis: test of agronomic control. *Acta Horticulturae* **705**: 447-449.
- Garcin A., Duchesne D., 2001. Walnut blight and apical necrosis. *Acta Horticulturae* **544**: 379-387.
- Hong S.G., Maccaroni M., Figuli P., Belisario A., Pryor B.M., 2006. Polyphasic classification of *Alternaria* isolated from hazelnut and walnut fruit in Europe. *Mycological Research* **104**: 1312-1321.
- Miller P.W., Bollen W.B., 1946. Walnut bacteriosis and its control. *Oregon Agricultural Experimental Station Technical Bulletin No. 9*.
- Moragrega C., Aletà N., Rovira M., Matias J., 2008. Disease progress and microorganisms associated to brown apical necrosis of walnut. Files of the *COST 873 WG1-4 Meeting: Active research to combat bacterial diseases of stone fruits and nuts*. Athens 2008. Available from: http://www.cost873.ch/_uploads/_files/Moragrega_BANSpain.pdf
- Ninot A., Aletà N., Moragrega C., Montesinos E., 2002. Evaluation of a reduced copper program to control bacterial blight of walnut. *Plant Disease* **86**: 583-587.
- Nitschke E., Nihlgard M., Varrelmann M., 2009. Differentiation of eleven *Fusarium* spp. isolated from sugar beet, using restriction fragment analysis of a polymerase chain reaction-amplified translation elongation factor 1 α gene fragment. *Phytopathology* **99**: 921-929.
- Özaktan H., Akat S., Akköprü A., Yavas M., 2009. Etiological approach to brown apical necrosis on walnut fruits in Turkey. Files of the *COST 873 WG1-4 Meeting: Active research to combat bacterial diseases of stone fruits and nuts resistance and Control strategies against bacterial diseases of stone fruits and nuts*. Cetara 2009. Available from: http://www.cost873.ch/_uploads/_files/Hozaktan_BAN-Turkey_Italy_1.pdf
- Serdani M., Kang J.C., Andersen B., Crous P.W., 2002. Characterisation of *Alternaria* species-groups associated with core rot of apples in South Africa. *Mycological Research* **106**: 561-569.
- Simmons E.G., 1990. *Alternaria* themes and variations (27-53). *Mycotaxon* **37**: 79-119.
- Simmons E.G., Roberts R.G., 1993. *Alternaria* themes and variations (73). *Mycotaxon* **48**: 109-140.
- Stewart J.E., Kim M.S., James R.L., Dumroese R.K., Klopfenstein N.B., 2006. Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. *Phytopathology* **96**: 1124-1133.
- Teviotdale B.L., Viveros M., Pryor B.M., Adaskaveg J.E., 2001. First report of *Alternaria* leaf spot of almond caused by species in the *Alternaria alternata* complex in California. *Plant Disease* **85**: 558.

