Diagnostics
Diagnostic

Anisogramma anomala

Specific scope

This standard describes diagnostic protocols for *Anisogramma anomala*[1].

Specific approval and amendment

Approved in 2009-09.

Introduction

Anisogramma anomala (Peck) E. Müller in E. Müller & Arx (Diaportales) was first described by Peck as a pathogen of the native American filbert, *Corylus americana* Walt. (Gottwald & Cameron, 1979). It is considered as an endemic pest of *Corylus americana*, but on the European (cultivated) hazelnut, *C. avellana* L., it causes eastern filbert blight, a devastating disease with perennial cankers on limbs. Since its first detection in South-West Washington State more than 30 years ago (Davinson & Davinson, 1973), *A. anomala* has slowly spread southward (approximately 2 km/year) into the Willamette Valley of Oregon. This pathogen is distributed in North America namely, Canada (British Columbia and Nova Scotia) and USA (Connecticut, Delaware, Illinois, Iowa, Maine, Maryland, Massachusetts, New Jersey, New York, North Carolina, Oregon, Washington and Wisconsin).

Anisogramma anomala systematically colonizes the phloem, cambium and the outer xylem of branches and produces cankers only after at least a one year incubation period. On American hazelnut, this fungus causes an insignificant canker, measuring 1–10 cm in length. In contrast, on European hazelnut the cankers can expand perennially at rates of up to 1 m per year, girdling branches, causing canopy dieback and death of trees (Johnson *et al.*, 1996).

This pathogen infects actively growing shoots and buds from budbreak to early shoot elongation. It has the typical characteristics of an obligate, biotrophic parasite.

Identity

Name: *Anisogramma anomala* (Peck) E. Müller

Synonyms: *Apioporthe anomala* (Peck) Höhn
Cryptosporella anomala (Peck) Saccardo
Anamorph: None
Taxonomic position: Fungi: *Ascomycota: Diaporthales*
EPPO code: CRSPAN
Phytosanitary categorization: EPPO A1 list no. 201, EU Annex designation II/AI.

Detection

Symptoms of *A. anomala* are very specific. Period for observation of different symptoms given in this section are based on observations of the disease in Oregon state (USA).

Anisogramma anomala infects immature shoots in the spring following budbreak. Initial host invasion occurs by direct penetration of young epidermal cells by germinating ascospores and early establishment of intra-cellular hyphae (Pinkerton *et al.*, 1995). Over the summer the pathogen colonizes cambium, phloem, and secondary xylem without producing visible symptoms. The latency can last about 12–16 months corresponding to the symptomless incubation period and the first visible symptoms consist of bumps on branches (Fig. 1). In spring of the year following initial infection, a perennial canker is formed. Branches are girdled as the cankers expand laterally over a period of 1–5 years. In susceptible cultivars of European hazelnut, *C. avellana*, such as Barcelona, Casina, Daviana, Ennis, Negret, Tonda Romana, and Tonda Gentile delle Langhe (TGDL), canopy dieback is noted in 4–5 years in the absence of treatments (Pinkerton *et al.*, 1993, 1998b; Iriti & Faoro, 2004; McCluskey *et al.*, 2005). This fungus produces multiple perithecia within a compact black stroma and apiosporous ascospores.

The stroma is the main diagnostic character of the fungus. Information on epidemiology is helpful for a better detection of stroma formation. From mid to late spring (May to June)

[1]Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

Fig. 1 Very first symptoms in the late spring or early summer as rows of bumps along a branch (courtesy JW Pscheidt, Oregon State University, Corvallis, OR, USA).

stromata are evident as whitish mycelial mats (Fig. 2). In late summer–autumn (end of August–September) stromata turn black (Fig. 3). They are produced in the cankered area which is sunken due to cambium death (Gottwald & Cameron, 1979) (Fig. 4).

Fig. 2 Immature stromata that have erupted through the bark in early summer (courtesy JW Pscheidt, Oregon State University, Corvallis, OR, USA).

Fig. 3 Mature, black stromata arranged in rows along a branch (courtesy JW Pscheidt, Oregon State University, Corvallis, OR, USA).

On *C. avellana*, cankers contain up to 20 stromata (in single or double rows) in the first year and hundreds of stromata (in three to five rows) from the second year on. Ascospores are the source of inoculum. Spore maturation begins in late summer; by January, over 90% of the spores are morphologically mature. Similarly, both the number of mature ascospores per perithecium and the proportion of ascospores that germinate increase throughout autumn. After January, the number of spores per perithecium decline until May, when few viable spores remain. The ascospores remaining after budbreak are the most important for infection, as this period coincides with the highest susceptibility to infection. Timing and amount of precipitation are the main variables for the ascospore release (Pinkerton *et al.*, 1998b). Ascospores of *A. anomala* are released when stromata on the surface of hazelnut branches are wet due to rainfall, dew does not cause this release to occur. Release of ascospores ceases after branch surfaces dry. The duration of free moisture on branch surfaces regulates the initiation and rate of ascospore release, but no significant effects of temperature, relative humidity, wind or light on ascospore release are apparent. Most (>90%) ascospores are captured during precipitation events that exceed 20 h in duration (Pinkerton *et al.*, 1998a).

Identification

Morphological identification

Identification is based on morphological characters *in vivo*. The pest cannot be cultured from infested tissues without sporulating perithecia. The morphological characters of this fungus are very specific and confusion with other fungi is very unlikely.

Fig. 4 Cankers of *Anisogramma anomala* (courtesy R Cameron, Oregon State University).

Direct examination

If suspicious symptoms of *A. anomala*., e.g. cankers with stromata and fruiting bodies (perithecia) (Fig. 5), are observed on diseased parts of *C. avellana*, a preliminary diagnosis is possible by direct examination of stroma. Spores from fruiting bodies can be removed with the tip of a sterile needle and placed directly in a drop of distilled water on a microscope slide for examination under a compound microscope (×400, ×1000). Perithecial primordia appear in May and develop from the end of spring to the end of August–September.

Fig. 5 Mature erumpent black stromata of *Anisogramma anomala* ejecting ascospores (white globs) on top of the stromata. (copyright JW Pscheidt, for use contact pscheidtj@bcc.orst.edu) source: JW Pscheidt, Oregon State University.

Morphological characters

Gottwald & Cameron (1979) described the fungus on *C. avellana*, European filbert. Mature stromata, which develop within a cankered area, are black and measure 1.5–3 × 2–10 mm and 1–2 mm in height (Figs 6 and 7). Perithecia are 40–60 per stroma, ovate to pyriform (Fig. 8), 250–830 × 1040–2160 µm. Perithecium wall is 40–45 µm wide and the neck is 160–240 µm in diameter, which is often bulbous at the surface, near the ostiole, measuring up to 350 µm in diameter. The size of the neck depends upon the perithecium position in the stroma. Paraphyses and the ostiolar canal are evident in the perithecia at maturity. Asci are deliquescent, broadly clavate, 45–65 × 10–15 µm, with a long, threadlike stipe, 8-spored (Figs 9 and 10). There are about 8400 asci in a mature perithecium. Ascospores are hyaline, two-celled. The smaller cell is completely degenerate and remains as a hemispherical cap cell on one end of the larger cell (Fig. 11),

Fig. 6 Stroma erupting out of the host bark with several perithecia embedded.

Fig. 7 Magnification of a stroma of *Anisogramma anomala.*

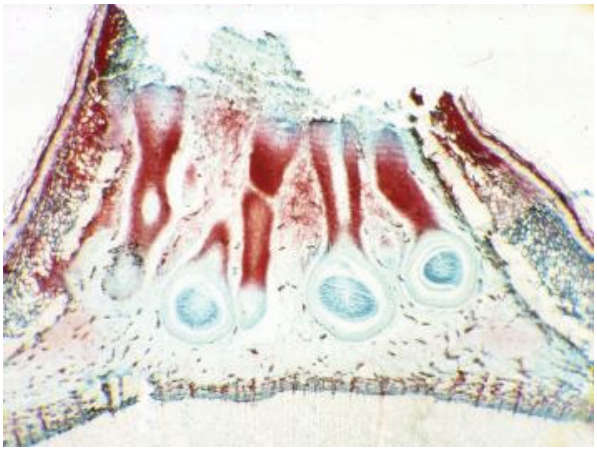

Fig. 8 Cross section through a single stained stroma, showing pyriform perithecia containing blue masses of spores. (copyright, for use contact tgottwald@ushrl.ars.usda.gov) source: TR Gottwald (Pscheidt, 2006) USDA ARS/US Horticultural Research Laboratory).

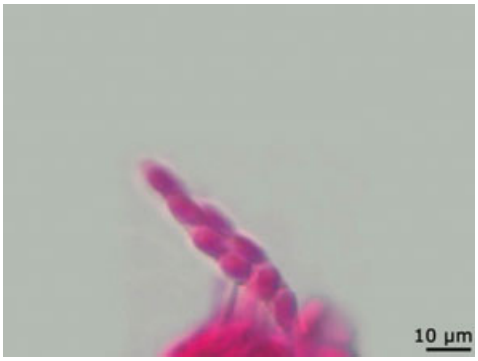

Fig. 9 Stained ascus with 8 ascospores of *Anisogramma anomala.*

measuring 1.1–1.4 × 1.1 µm. The larger cell measures 8–12 × 4–5 µm at the maturity (Gottwald & Cameron, 1979).

This fungus does not grow on standard mycological media. Media for culturing and colonies characteristics are presented in the Appendix.

Biochemical and molecular methods

Enzyme-linked immunosorbent assay (ELISA)

A rapid screening ELISA was developed in Oregon to identify *A. anomala* in host plant tissue and was mainly used in a selection context of breeding programmes. This test proved more effective after a 13–27 month incubation (corresponding to the period that is required to develop cankers). Nevertheless as antisera are no longer available this test is not recommended.

Molecular methods

There is currently no molecular method available for this pest.

Reference material

No reference material available.

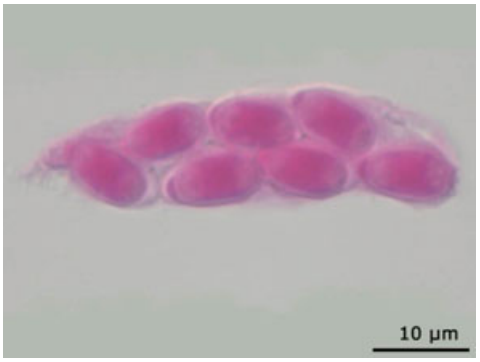

Fig. 10 Magnification of stained ascus of *Anisogramma anomala.*

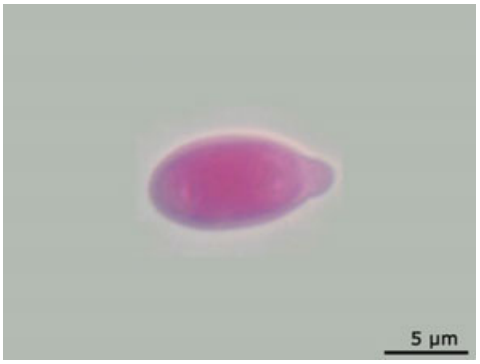

Fig. 11 Stained ascospora of *Anisogramma anomala.*

Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77(1) *Documentation and reporting on a diagnosis* (EPPO, 2006).

Further information

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Acknowledgements

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Appendix

Isolation of *Anisogramma anomala* from sporulating perithecia

Though the fungus does not grow on standard mycological media [PDA (potato-dextrose agar), MA (2% malt agar), MEA (malt extract agar), CMA (2% corn meal agar), OMA (oatmeal agar), etc.], and exhibits characteristics of obligate, biotrophic parasites, Stone *et al.* (1994) described a medium that supports the prolonged growth of *A. anomala*.

This pathogen is strictly host specific to *Corylus* spp. and can infect only live, newly opened buds and young emerging shoots. Ascospores germinate and establish colonies only on media containing a chemical adsorbent such as bovine serum albumin (BSA), β-cyclodextrin (CD), polyvinylpyrrolidone (PVP), amylase (SS), and activated charcoal (AC). Bovine serum albumin at 0.5% and activated charcoal at 0.025% were most effective. Sucrose was the only carbon source of 19 carbohydrates tested that supported prolonged growth in culture. Growth was further enhanced by asparagine and yeast extract at 0.2%. Anagostakis' improved *Cryphonectria* (*Endothia*) *parasitica* medium (EMM, Anagnostakis, 1982) and a modified Murashige and Skoog medium (MMS, see recipe below) Yamaoka & Katsuya (1984) were successful for *A. anomala* growth, and the latter proved the best medium for this fungus to grow at 18°C in the dark (Stone *et al.*, 1994). Ascospores germinate and establish colonies only on media containing a chemical absorbant. The highest ascospore germination is obtained on BSA (0.5–0.05%) and activated charcoal (0.005–0.05%) plates.

Modified Murashige & Skoog medium (1962)

The medium is composed of the following per litre of distilled water: KNO_3, 475 mg; NH_4NO_3, 412.5 mg; $CaCl_2{\cdot}2H_2O$, 110 mg; $MgSO_4{\cdot}7H_2O$, 92.5 mg; KH_2PO_4, 42.5 mg; Na_2 EDTA, 9.325 mg; $FeSO_4{\cdot}7H_2O$, 6.95 mg; $MnSO_4{\cdot}4H_2O$, 5.575 mg; $ZnSO_4{\cdot}7H_2O$, 2.15 mg; H_3BO_3, 1.55 mg; KI, 0.208 mg; $Na_2MoO_4{\cdot}2H_2O$, 0.063 mg; $CuSO_4{\cdot}5H_2O$, 0.006 mg; $CoCl_2{\cdot}6H_2O$, 0.006 mg; myo-inositol, 25 mg; nicotinic acid, 0.125 mg; pyridoxine HCl, 0.125 mg; thiamin-HCl, 0.025 mg; glycine, 0.5 mg; Evans peptone, 2 g; Difco Bacto-soytone, 2 g; glucose, 40 g; 2,4-D, 1 mg; kinetin, 0.1 mg; agar, 8 g. The pH of the medium is adjusted to 5.8–5.9 with 1 N HCl before autoclaving at 121°C for 10 min.

Characteristics of colonies

Colony growth is very slow, producing micro-colonies with an average diameter of only 15–35 mm after 6 months of incubation on sucrose modified MMS medium. Spherical, lobate vesicle (primary vesicle) arising from a short germ tubes develop between 48 and 72 h on MMS plates containing 0.8% BSA. Hyphal branches continue to proliferate from 10 to 14 days and highly-branched, mounded micro-colonies form by 14–21 days (Stone *et al.*, 1994). Ascospore germination only occurs on plates containing intact BSA, and no germination occurs on plates containing only enzymatically hydrolyzed BSA, heat-denaturated BSA, or media without BSA. Cultures of *A. anomala* fail to survive when transferred to any medium lacking adsorbents (Stone *et al.*, 1994).