A COMPENDIUM OF VERTICILLIUM WILTS IN TREE SPECIES

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Edited by

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PREFACE

This compendium is the result of cooperation between eight european research groups, all working on verticillium wilt in tree hosts. During 1997 and 1998 the groups collaborated in a Concerted Action within the FAIR programme of the European Union. One of the aims of this project was to collect all current information on verticillium wilt in trees and make it available to the international community by means of a single publication.

The compendium was compiled as a general reference guide not only for plant pathologists but also for tree growers, crop advisers and extension specialists. The individual parts of the books were written by researchers recognized as experts in that specific area.

The compendium deals with verticillium wilts of fruit, nut and shade trees. Other woody perennial hosts such as grapevine, berry shrubs or ornamentals like rose and lilac are not covered. In the introductory parts of the book some information from non-tree hosts is included in order to provide as complete a picture of verticillium wilts as possible. Moreover, for reasons made clear in the text, the compendium deals almost exclusively with *Verticillium dahliae,* although there are a few references to *V.albo-atrum* and other *Verticillium* species where appropriate.

This publication provides a primary information source for all those involved in the management of trees in which verticillium wilt may be a problem. In addition, we hope the compendium will inspire further research into the disease; and will stimulate development of integrated methods for managing the disease in tree hosts.

Wageningen, 19-12-1998

Jelle A. Hiemstra

Coordinator of the Concerted Action
on verticillium wilts in trees
(EU-FAIR CT96-2015)

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The authors who prepared the chapters of this book are identified under the chapter headings. In chapters 4 and 5 the sections have different authors; they are identified at the start of each section. Illustrations were provided by many individuals. Acknowledgement is given only where they were not provided by the authors of the accompanying text.

We are indebted to our colleagues at HRI-EM and CPRO-DLO for assistance in various stages of the preparation of the book and to the management of the two organisations for allowing time to be spent on compiling the compendium.

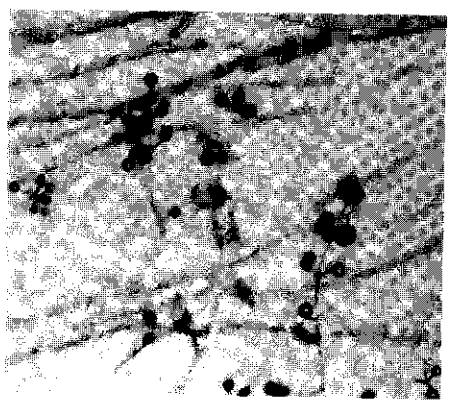
1. AN INTRODUCTION TO VERTICILLIUM WILTS

D.C. Harris

Verticillium wilts affect a wide range of plants in many parts of the world, and in different countries are variously known as verticilliose, flétrissure verticillienne (French), tragueco-verticilosis (Spanish), tracheoverticilliosi (Italian), Verticillium-Welke, Verticillium-Tracheomycosen (German), verwelkingsziekte (Dutch), kransskimmel (Danish), vissnesjuka (Swedish). They are caused by two closely related soil-borne fungi of the genus *Verticillium, V. dahliae* Kleb. and *V. albo-atrum* Reinke & Berth.

From the first reports and descriptions of these diseases, there has been confusion over the identity of the species of *Verticillium* responsible. Thus, in many early reports, authors referred only to *Verticillium*. This problem was compounded until the mid-70s by many investigators, particularly in the USA, who considered that *V. dahliae* was merely a form of *V. albo-atrum*, so that there are many important publications which refer to *V. albo-atrum* where it is now accepted that the pathogen was *V. dahliae*. In reviewing critically the literature of verticillium wilts in tree hosts it is clear that in almost all cases *V. dahliae* is the pathogen responsible, although there are a few well authenticated cases where *V. albo-atrum* is the casual organism. *V. dahliae* therefore forms the main focus of this compendium, although there are references to *V. albo-atrum* where appropriate.

Verticillium is a genus of microfungi in the Order Hyphomycetes whose members produce conidia in moist droplets from the tips of phialides borne in whorls on upright conidiophores, otherwise known as verticils (fig. 1). *V. dahliae* is distinguished morphologically from *V. albo-atrum* and the other closely related but mainly saprophytic soil-borne species *V. nigrescens*, *V. nubilum* and *V. tricorpus* (Isaac, 1949, 1953; Smith, 1965) by a combination of characters: totally hyaline conidiophores; conidiophore and conidia size; and subspherical to elongate, melanised microsclerotia (fig. 2; see also section 4.1). No sexual state is known for any of these fungi.



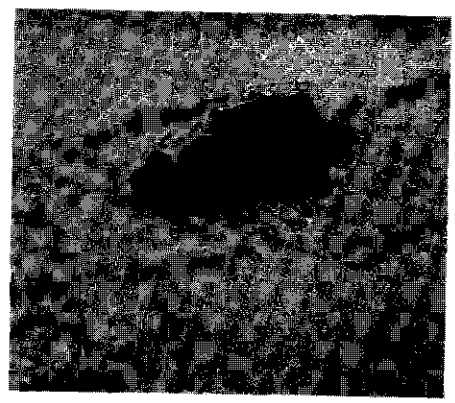


Fig. 1. Conidiophores of *Verticillium dahliae* (× 47). **Fig. 2.** A microsclerotium of *Verticillium dahliae* (× 470).

V. dahliae is widely distributed between latitudes 60°N and 50°S but is generally absent from tropical lowlands (Pegg, 1984). It has been recorded on over fifty crop plants including: soft, pome, stone and other fruits; nuts; vegetables; herbs; root crops; oilseed crops; fibre crops; legumes; on over sixty genera of woody and herbaceous ornamentals; and on some shade and amenity tree species. V.*dahliae* is also known to colonise over twenty-five genera of dicotyledonous weeds (Smith, 1965; Harrison and Isaac, 1969; Skadow, 1969; McKeen and Thorpe, 1973; Thanassoulopoulos *et al.*, 1981b) with or without producing symptoms.

V. dahliae exhibits variabilility in a number of ways, some of which are correlated while others are not. There is quantitative and qualitative variation in pathogenicity. A mint pathotype is known only from the USA and the UK (Okoli *et al.*, 1994). Isolates affecting sweet pepper are also of comparatively limited distribution (Evans and McKeen, 1975). Within populations attacking cotton highly pathogenic defoliating strains have been identified in several widely separated geographic areas (Mathre *et al.*, 1966; Lu *et al.*, 1987; Bell, 1992). Defoliating isolates have also been reported from Spain (Blanco-López *et al.*, 1989), China (Gu *et al.*, 1988) and Iran (Hamdollah-Zadeh, 1993) and the former USSR (Daayf *et al.*, 1995). There appear to be several vegetative compatibility groups (VCGs) within *V. dahliae* (Joaquim and Rowe, 1990; 1991; Strausbaugh *et al.*, 1992). Recent work using molecular methods (Okoli *et al.*, 1993; 1994) has revealed variability which was not suspected hitherto. Variability within *V. dahliae* is considered in more detail in section 4.2.

V. dahliae survives in soil as microsclerotia which are produced in the dying tissues of the host plant. These structures can survive over a range of soil moisture and temperature conditions, but lose viability most rapidly in wet, warm soil (Green, 1980). Once infested with *V. dahliae*, soil remains so for 10 or more years (Wilhelm, 1955) and the longevity of microsclerotia is a major factor in this persistence. The colonisation of dicotyledenous weeds (Skadow, 1969; Evans, 1971; McKeen and Thorpe, 1973; Busch *et al.*, 1978; Vargas-Machuca *et al.*, 1987), legume and cereal rotation crops (Skipp *et al.*, 1986; Mathre, 1986; 1989; Celetti *et al.*, 1990; Krikun and Bernier, 1990) and 'volunteer' potato plants may contribute to bridging the period between susceptible crops. The pathogen can be disseminated with infected planting material of strawberry, hop, olive (Thanassoulopoulos, 1993) and woody ornamentals; with potato seed tubers (Easton *et al.*, 1972); or with true seed of a range of crops (Richardson, 1979). The pathogen may also spread naturally on weed seeds (Evans, 1971).

Microsclerotia germinate in response to root exudates, hyphae penetrate the cortex of young roots and the fungus grows into the stele. In the xylem vessels the pathogen spreads by mycelial growth, but also by producing conidia which are transported in the transpiration stream. In this way the pathogen rapidly becomes systemic in susceptible crops. The major disease effects are believed to be due to occlusion of vessels. A detailed description of the disease cycle (in tree hosts) is provided in chapter 2.

Apart from the intrinsic resistance of the plant attacked, there are a number of factors known to influence the incidence and severity of verticillium wilt outbreaks. Diseases due to *V. dahliae* are favoured by moderate to high temperatures, although temperatures above 28-30°C are inhibitory for most strains of the pathogen (Schnathorst, 1981a). In temperate areas where seasonal climate is variable, diseases

due to *V. dahliae* tend to be more severe in hot, dry summers (Talboys and Bennett, 1961). Drought stress is associated with lower wilt incidence in cotton (Schnathorst, 1981a) but with more severe disease in *Acer* (Caroselli, 1957). Wilt occurs on a wide range of soil types and for any given area is likely to be worse on soils which favour the maintenance of soil temperatures conducive to disease: light soils in cool areas and heavy soils in warmer areas (Schnathorst, 1981a). Verticillium wilt may be exacerbated by plant pathogenic nematodes, particularly of the genus *Pratylenchus* (Martin *et al.*, 1982; Tchatchoua and Sikora, 1983), although cyst nematodes may also have an synergistic effect in potato wilt (Evans, 1987). Vesicular-arbuscular mycorrhiza (VAM) may reduce disease in cotton (Liu, 1995).

Verticillium wilts are generally controlled by a combination of measures and strategies, including: use of resistant or tolerant cultivars or rootstocks, reducing soil inoculum, limiting disease spread and manipulating to advantage factors which influence disease severity.

Tomato cultivars with the Ve resistance gene (Schaible *et al.*, 1951) are widely employed to control verticillium wilt, although types of *V. dahliae* capable of overcoming this resistance have appeared in several countries (see section 4.2). Resistant cultivars have been the mainstay of wilt control in cotton, although the life of some of the earliest resistant types has been curtailed by new strains able to overcome their resistance (Bell, 1992). In several horticultural tree crops resistant rootstocks or cultivars are employed to minimise the effects of wilt (see chapter 3). In high value herbaceous crops like eggplant, grafting to resistant tomato rootstocks has been used for control (Canevascini and Caccia, 1976).

Pre-planting chemical soil disinfestation is used throughout the world to control wilt for high-value crops. Reducing inoculum by solarization has proved successful in several areas (Katan and DeVay, 1991) and, particularly for production under glass or polythene (Tjamos *et al.*, 1989); it can also be used to control wilt by *in situ* treatment of perennial crops (Ashworth and Gaona, 1982; Tjamos *et al.*, 1991). Removal of infested plant residues is also used to reduce soil inoculum. Temporary flooding also reduces *V. dahliae* infestation of soil. In protected cropping, hydroponic culture and artificial growing substrates are commonly used to avoid soil-borne diseases. The reduction of soil populations of nematodes which act synergistically in diseases caused by *V. dahliae* can contribute to disease control (Powelson and Carter, 1973).

There are many reports about the effectiveness of crop rotation in controlling wilt. Rotation with paddy rice has proved particularly effective in eliminating *V. dahliae* from soil (Pullman and DeVay, 1981). Cereals, legumes and cruciferous crops in various rotation systems have been shown to reduce wilt in cotton in the former USSR (Egamov, 1976); and cotton wilt was reduced by ryegrass rotation in the USA (Butterfield *et al.*, 1978) and by a lucerne rotation in Turkey (Sezgin *et al.*, 1982). Wilt in peppermint was controlled in the USA by five years' maize or grass (Green, 1967). However, there are also reports that rotation systems have not been effective in providing control (Huisman and Ashworth, 1976; Busch *et al.*, 1978). These different experiences may reflect differences in the local population of the pathogen, the degree to which weed hosts have been excluded from rotation crops (Minton, 1972) and the levels of soil infestation by *V. dahliae* at the start of the rotation period.

Treatment of soil or seed with preparations containing *Trichoderma* spp. and green manuring, often in combination, have been employed in parts of the former USSR as an aid to controlling wilt in cotton (Muromtsev *et al.*, 1979; Khakimov *et al.*, 1980). Several potential biocontrol agents have been identified but have not yet been developed into commercially viable control methods (Spink and Rowe, 1989; Keinath *et al.*, 1991; Tjamos, 1993; Berg *et al.*, 1994).

Transmission in vegetative planting material for some important crops has been effectively eliminated by making healthy material available through the implementation of certification schemes, whilst for other crops no such schemes are in place. Chemical treatment of seed can reduce transmission in potato (Robinson *et al.*, 1957) and acid delinting of cotton seed effectively removes *V. dahliae* (Shen, 1985).

2. SOME GENERAL FEATURES OF VERTICILLIUM WILTS IN TREES

J.A. Hiemstra

2.1 Woody hosts

Many dicotyledonous trees from a wide range of genera are susceptible to verticillium wilt but woody Monocotyledons (such as palms) and Gymnophytes (Gymnosperms) are not affected (Sinclair *et al.*, 1987). Although many reports refer to *V. albo-atrum* it is clear that in almost all cases wilt in woody hosts is caused by *V. dahliae* (see chapter 1). Thus, Smith and Neely (1979) identified all 51 *Verticillium* isolates from 22 different tree and shrub species as *V. dahliae*.

In Europe (Phillips and Burdekin, 1982; Butin, 1983) and in North America (Sinclair *et al.*, 1987) most reports of verticillium wilt in tree species are of shade, fruit and ornamental trees or young trees in nurseries. Verticillium wilt is rare in forest trees: there is one report for young ash trees in newly planted forest areas in the Netherlands (Hiemstra *et al.*, 1990; Hiemstra, 1995); one for *Ceanothus interregimus* Hook. & Arn. (Harrington and Cobb, 1984) in a coastal Californian forest where the disease was caused by *V. albo-atrum*; and and one for *Liriodendron tulipifera* L. in urban and forest settings in Delaware where both *V. dahliae* and *V. albo-atrum* were responsible (Donohue and Morehart, 1978).

Lists of tree hosts for *Verticillium* have been published for North America by Engelhard (1957), Himelick (1969), Carter (1975) and Sinclair and Hudler (1984). For Great Britain, the woody hosts are reviewed by Piearce and Gibbs (1981). Verticillium wilts of fruit-tree species are listed by Parker (1959). Among the most commonly affected trees are maples, *Catalpa* species, and many fruit and nut plants including almond, apricot, avocado, cacao, cherry, olive, peach, persimmon, pistachio and plum (table 1; see also chapter 3).

Tree genera known not to be affected by verticillium wilt include *Alnus*, *Betula*, *Fagus* (although isolation of *V. dahliae* from a symptomless *F. sylvatica* tree has been reported by Van Dam, 1991), *Platanus*, *Populus*, *Quercus* and *Salix*. Apple (*Malus*) and pear (*Pyrus*) are not affected.

2.2 Symptoms

Symptoms of verticillium wilt on woody hosts are described in detail by Himelick (1968 and 1969), Smith (1979), Piearce and Gibbs (1981), and Sinclair and Hudler (1984). Although the syndrome in different tree species has common features, the combination of symptoms depends on the host species involved and the severity of the attack (see chapter 3 for descriptions of symptoms on individual species). Three main types of effects may be observed: leaf symptoms (wilt, discoloration, defoliation), vascular symptoms (discoloration of the xylem and plugging of vessels) and decline (stunting and dieback).

Table 1. Tree species susceptible to *V. dahliae*. Compiled from Engelhard (1957), Himelick (1969), Piearce and Gibbs (1981) and Sinclair and Hudler (1984).

Genus	Species mentioned
Forest and landscape trees	
Acer	*campestre, ginnala, macrophyllum, mono, negundo, nigrum, palmatum, pennsylvanicum, platanoides, pseudoplatanus, rubrum, saccharinum, saccharum, tataricum*
Aesculus	*hippocastanum* (occasionally)
Ailanthus	*altissima*
Castanea	*sativa* (occasionally)
Catalpa	*bignonioides, speciosa*
Cercis	*canadensis, siliquastrum*
Fraxinus	*americana, excelsior, nigra, pennsylvanica, quadrangulata*
Liriodendron	*tulipifera*
Magnolia	*grandiflora, soulangeana, stellata*
Robinia	*pseudoacacia* (occasionally)
Tilia	*americana, cordata, euchlora, glabra, parvifolia*
Ulmus	*americana, campestris, carpinifolia, fulva, glabra, montana, parvifolia, procera*
Fruit trees and shrubs	
Coffea	*arabica*
Olea	*europaea* (olive)
Persea	*americana* (avocado)
Pistacia	*vera* (pistachio)
Prunus	*amygdalus* (almond), *armeniaca* (apricot), *avium* (sweet cherry), *cerasus, cerasifera, communis* (almond), *domestica* (plum), *laurocerasus, lusitanica, mahaleb, mume,*
Rubus	*pennsylvanica, persica* (peach) *allegheniensis, idaeus, occidentalis, rosaefolius, ursinus*
Theobroma	*cacao*
Ornamental shrubs	
Amelanchier	*canadensis*
Berberis	*thunbergii, vulgaris*
Buxus	*koreana, microphyllum, sempervirens*
Cornus	*alba, florida*
Cotinus	*coggygria*
Daphne	*cneorum, mezereum*
Elaeagnus	*angustifolia*
Erica	*australis, persoluta*
Koelreuteria	*paniculata*
Ligustrum	*amurense, vulgare*
Lonicera	*morrowii*
Rhus	*aromatica, glabra, typhina*
Ribes	*sanguineum*
Rosa	*multiflora* and others
Sambucus	*racemosa* and others
Spiraea	*spp.*
Syringa	*vulgaris*
Viburnum	*burkwoodii, lantana, lentago, tinus, tomentosum*

In most tree species rapid wilting and defoliation are typical symptoms of verticillium wilt. In other species, such as maple, a more chronic type of disease may occur in which symptoms develop more slowly (Piearce and Gibbs, 1981). Symptoms may not affect the whole tree. Leaves may abscise while still green, as in ash (Himelick, 1968), or after yellowing and scorching as in elm, Catalpa and maple (see chapter 3). Occasionally, highly susceptible species, like maple and Catalpa, develop elongated dead areas of bark on stem and branches. These areas are contiguous with a sector of discoloured wood.

Within the sapwood of infected trees *Verticillium* often causes discoloration that appears as streaks or bands in radial and tangential cuts and as spots or partial to complete rings in one or more annual layers of the wood in transverse cuts. The colour of this staining varies with the species. In maples it is greyish green to olive-green; in many other species like Catalpa, elms and cherry trees it is brown; and in the tulip tree it is black. In some other host species, including ash (Himelick, 1968) and lilac (Van der Meer, 1925) only a faint tan streaking may be visible, or discoloration may be absent.

Many tree species show extensive dieback after defoliation. This is often followed by the formation of adventitious shoots, especially from the stem base. Although affected trees may die, recovery in the year after infection is not unusual. In some species, infected trees may even produce a new flush of leaves some weeks after defoliation (Himelick, 1968; Vigouroux and Castelain, 1969; Hiemstra, 1995). Recovery is a particularly common feature of wilt in olive, and has also been reported in other fruit-tree species, such as cherry, apricot and peach; in cacao; and in ornamental and shade trees like Catalpa and ash (see chapter 3).

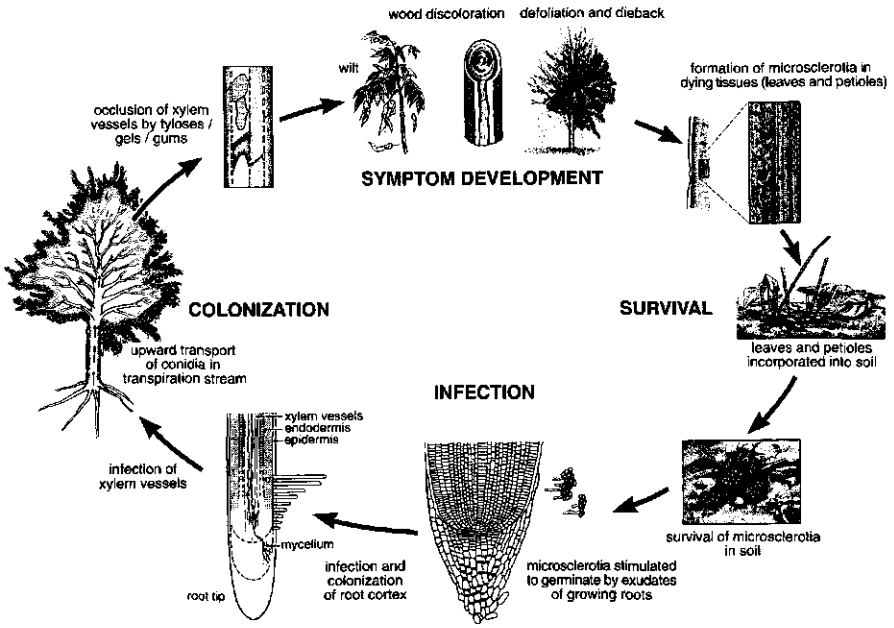


Fig. 3. The disease cycle of *Verticillium dahliae* in tree hosts (courtesy of J.A. Hiemstra and A.J. Termorshuizen; drawing by P.J. Kostense).

2.3 Disease cycle

The literature on the disease cycle of *V. dahliae* in agricultural crops is extensive, but is limited for trees. Therefore, this section has drawn on the results obtained with a wide range of crops and, where appropriate, reference is also made to *V. albo-atrum*.

Several phases can be distinguished in the disease cycle of *V. dahliae* (fig. 3): survival and spread of the fungus, infection of the root, colonization of the vascular system, and symptom development.

Survival and spread of the fungus

V. dahliae survives in soil by means of microsclerotia that are formed in the dead and dying tissues of the host that it has colonised, especially leaves and herbaceous stems. Microsclerotia become incorporated into soil, first localised in host debris but later more widely distributed as a result of decomposition and other activities of soil organisms, and cultural operations. Inoculum of *V. dahliae* in soil may be replenished and increased very effectively in this way. This has been shown for cotton (Blank and Leyendecker, 1951; Benken and Khakimov, 1964; Evans *et al.*, 1966), potato and tomato (Pegg, 1974).

Most microsclerotia are found in the upper layers of the soil (Wilhelm, 1950; Green, 1957) or on the soil surface whence they can be spread by dust storms and excess irrigation or rain water (Easton *et al.*, 1969). They may also be dispersed from one field to another with wind-blown leaves of diseased host plants like cotton. Serious outbreaks of verticillium wilt in olive orchards, not previously infested with *V. dahliae*, have been attributed to the cultivation of cotton in neighbouring fields (Wilhelm and Taylor, 1965).

In perennial species like olive and maple, large quantities of inoculum can be similarly released when leaves containing microsclerotia and hyphae are shed from diseased trees. Moreover, detached leaves may be blown considerable distances thus serving to disperse the pathogen. The colonisation of petioles by *V. dahliae* has been demonstrated for several tree species including *Acer* (Zimm, 1918; Townsend *et al.*, 1990; Hiemstra, 1997a), *Liriodendron tulipifera* (Morehart and Melchior, 1982), ash (Rijkers *et al.*, 1992) and olive (Tjamos and Botseas, 1987).

V. dahliae may also be distributed from one area to another by means of infected planting stock. In tree nurseries, the pathogen may be transmitted by using scions, buds or rootstocks of infected plants (Woolliams, 1967; Sinclair *et al.*, 1987) and probably also during pruning (Dochinger, 1956b; Blodgett, 1964).

Infection of the root

Microsclerotia in soil are stimulated to germinate by root exudates (Schreiber and Green, 1963), or by the addition to or release of nutrients within soil (Green and Papavizas, 1968; Farley *et al.*, 1971) Germination can also be induced by roots of plants immune to vascular infection, including monocotyledonous plants (Lacy and Horner, 1966; Levy and Isaac, 1976; Fitzell *et al.*, 1980).

In cotton, *V. dahliae* colonized all parts of unwounded young roots, from the tip to the hypocotyl (Garber and Houston, 1966). Hyphae penetrated the root cap and epidermal cells and traversed the cortex by inter- and intracellular growth. Some hyphae penetrated the endodermis and, three days after inoculation, had entered the xylem vessels.

In tree hosts, the same pattern of invasion was observed under laboratory conditions in young roots of cacao (*Theobroma cacao*) by Emechebe *et al.* (1972) and in *Fraxinus pennsylvanica*, *Cercis canadensis* (both susceptible), *Gleditsia triacanthos* and *Platanus occidentalis* (both resistant) by Born (1974). In all these species penetration into the xylem vessels was observed within a week.

Penetrating hyphae may be impeded by the inner tangential wall of epidermal cells, by gum-like deposits in the cell walls of cortical cells and by the endodermis (Talboys, 1958a; Garber and Houston, 1966; Emechebe *et al.*, 1972; Born, 1974). Thus, only a small proportion of invasions of the cortex results in systemic infection (Huisman and Gerik, 1989). In wilt-immune plants (plants immune to vascular infection) these processes are apparently highly effective. However, in many other plants, vascular penetration does occur and the ultimate reaction to invasion (susceptible or resistant) depends to a large degree on host plant tissue responses thereafter (Garber and Houston, 1966; Harrison and Beckman, 1982).

Colonization of the vascular system

The rapid upward spread of the pathogen in the vascular tissues is primarily by means of conidia transported with the transpiration stream (Talboys, 1962; Garber and Houston, 1966; Presley *et al.*, 1966; Emechebe *et al.*, 1975). This colonisation strategy enables the pathogen to spread throughout the above-ground parts of its host within one growing season, even in large trees. In elm this mechanism has been clearly demonstrated by Banfield (1941) who showed that in inoculated trees conidia of *V. dahliae* and *Ophiostoma ulmi* may ascend five feet in eight to fifteen seconds, and reach an upward distribution of 20 feet in less than ten minutes. The importance of the transpiration stream in the spread of *V. dahliae* in tree hosts was further demonstrated by Dochinger (1956) and Caroselli (1957).

Within the vessels, conidia are produced by budding or from simple conidiophores (Emechebe *et al.*, 1972; Schnathorst, 1981a). The production of conidia rather than hyphal growth in xylem elements is probably stimulated by the chemical and physical environment peculiar to xylem vessels of trees: low oxygen levels (Ioannou *et al.*, 1977) and a negative pressure potential (Duncan and Himelick, 1988) are both known to stimulate conidial production of *V. dahliae*.

The spread of *V. dahliae* in the vascular tissues may be limited by several resistance mechanisms of the host (Dimond, 1970; Beckman and Talboys, 1981; Bell and Mace, 1981; Beckman, 1987). There are physical (entrapment and vascular occlusion) and biochemical (phytoalexins, for example) components to the defensive response of infected plants (Harrison and Beckman, 1982). Trapping of conidia is achieved by the gridwork of lateral and end-walls of the vessels. In resistant-type interactions, gel plugs and tyloses are formed that completely block off the lumen of the infected vessel above the trapping sites, resulting in immobilization of the pathogen (Beckman, 1987 and 1989). Finally, gel plugs, tyloses and surrounding cell walls become infused with several secondary metabolites like oxidized polyphenols (tannins) and phytoalexins that are exuded from paratracheal parenchyma cells (Mace, 1989). This causes vascular browning, a classic symptom of vascular infection.

Because of their antibiotic nature, polyphenols and phytoalexins contribute to the resistance of the occluding structures. Somers and Harrison (1967) showed that the main tannin fraction extracted from the stained wood of verticillium wilt-affected apricot trees (*Prunus armeniaca*) greatly inhibited spore germination and hyphal

growth of *V. dahliae in vitro* at a concentration well below that present in the wood. In cocoa, elemental sulphur (S_8) and arjunolic acid seem to play a role in resistance, since they are present in stems of infected resistant cultivars at fungitoxic levels (Cooper *et al*, 1995 and 1996; Resende *et al*, 1996a).
The defensive reactions in susceptible hosts are essentially the same as in resistant ones, but the rates, intensities or spatial arrangement of these reactions are inadequate to contain the pathogen (Beckman, 1989; Mace, 1989). Successive attempts to restrict pathogen spread result in widespread local occlusions (Beckman, 1964; Emechebe *et al.*, 1974).

Symptom development
Impedence of water transport is thought to be mainly responsible for the disease syndrome caused by *V. dahliae* and other wilt pathogens (Ayres, 1978; Van Alfen, 1989; DeVay, 1989), although toxicosis may cause effects locally (Beckman, 1987; Bishop, 1988).

The vessels in the stem of a dicotyledonous tree form a complex branching and anastomosing network (Zimmermann, 1983). Hence, limited loss of transport capacity after local occlusion as in resistant plants does not necessarily result in wilt symptoms. In susceptible hosts, however, the many widespread local occlusions eventually clog the vessel network, and symptoms of water stress ensue. If the pathogen reaches the petioles, the effect is even stronger as the vessels here are much smaller, and more easily plugged, and alternative paths for water movement are very limited (Talboys, 1968). The water stress induced leads to wilt, desiccation and defoliation. If the plugged vessels are not replaced by new, healthy xylem, dieback may result.

Fungal metabolites may incite host defence reactions but may counteract them as well by preventing the formation of gel plugs in infected vessels. High molecular weight substances, particularly polysaccharides and glycopeptides, may act by plugging the pores in the pit membranes rather than by being toxic (Bishop, 1988; Sutic and Sinclair, 1991).

The deposition of callose and other materials on cell walls is also an important aspect of the host-pathogen interaction (Bishop and Cooper, 1983 and 1984; Street *et al.*, 1986; Newcombe and Robb, 1989). These deposits help to localize the pathogen by sealing off vessel walls but they also contribute to the development of water stress inside the plant.

Several more host and pathogen factors have been claimed to play a role in the complex interaction between the pathogen and its host, including plant growth hormones (especially IAA, ABA and ethylene), phenolic substances and phytoalexins. These factors are extensively reviewed in contributions to two books on vascular wilt diseases edited by Mace *et al.* (1981) and Tjamos and Beckman (1989).

2.4 Recovery from disease

In species like olive, stone fruits and ash, recovery of trees from verticillium wilt is common even when serious damage is suffered in the season of infection (see chapter 3). The potential for recovery in some tree species results from the highly compartmented structure of the xylem and the presence of a cambium which is able to produce new layers of tissue around the old, diseased xylem. Trees do not overcome injuries and infections by healing damaged or infected tissues, but by isolating the damaged part and replacing it by new functional tissues (Shigo and Marx, 1977).

The cambium may respond to the presence of the pathogen by forming a specific tissue largely consisting of parenchymatous cells, the so-called barrier zone, a tangential layer that serves to isolate or separate the infected wood on the inside from the new, healthy wood that is formed on the outside. This is a general defence mechanism against injury and decay in tree species (Shigo, 1984) but has also been reported to take place after infection by vascular pathogens, including *V. dahliae* (Tippett and Shigo, 1981).

Even without the formation of a barrier zone, the ability to produce secondary xylem greatly contributes to resistance of woody hosts to vascular pathogens (Talboys, 1958b). If new root infection does not take place, affected trees may simply outgrow the disease because of the limited capacity of radial movement of *V. dahliae* (Talboys, 1968; Sinclair *et al.*, 1981). This process of recovery may be strongly enhanced by the fungus being killed by high air temperatures during the summer as was suggested for olive and apricot (Wilhelm and Taylor, 1965; Taylor and Flentje, 1968; Vigouroux and Castelain, 1969). Thus, for most verticillium wilts in trees, the development of disease in one season is generally independent of that in any other season.

Several other anatomical features may contribute to the potential for recovery from infection by vascular pathogens in many tree species. Ash wood has a sheath of marginal parenchyma between successive growth rings (Grosser, 1977; Schweingruber, 1990) that may serve as a built-in barrier zone, provided that the connections between latewood vessels of one growth ring and earlywood vessels of the next growth ring (Braun, 1970) are sealed effectively.

In ring-porous trees like Robinia, oak and ash, water transport is mainly in the current year's wood. Hence, as long as the cambium survives and retains its ability to form a new growth ring, loss of water transport capacity of the current year's xylem poses no problem for survival of the tree. In diffuse-porous trees such as maple (*Acer spp.*) the xylem vessels in each growth ring remain functional for several years, so that the loss of the transport capacity of one (or more) of these rings limits water conduction in following years. This may explain why dieback after an attack of verticillium wilt is much more common in maple than in ash (see chapter 3).

3. VERTICILLIUM WILTS OF MAJOR TREE HOSTS

3.1 Olive

R.M. Jiménez-Díaz, E.C. Tjamos and M. Cirulli

Introduction

Olive (*Olea europaea* L.) is one of the most traditional and important tree crops in the Mediterranean region. The plant originated in an area extending from the southern Caucasus to coastal Syria. From there, cultivation of the olive has spread throughout the Mediterranean Basin and later to Australia, China, Japan and North and South America. Some 750 million trees are grown in approximately 8.5 million hectares, of which about 97% are in Mediterranean countries (COI, 1991).

Verticillium wilt is one of the most important diseases occurring throughout the range of olive cultivation. The disease was first described by Ruggieri (1946) in Italy, and afterwards was reported in California (Snyder *et al.*, 1950), Greece (Sarejanni *et al.*, 1952; Zachos, 1963), Turkey (Saydan and Copcu, 1972), France (Vigouroux, 1975), Spain (Caballero *et al.*, 1980), Syria (Al-Ahmad and Mosli, 1993) and Morocco (Serrhini and Zeroual, 1995).

Verticillium wilt can substantially reduce the production of olive orchards and may cause tree deaths. Thanassoulopoulos *et al.* (1979) concluded that in Greece 2-3% of 14 million trees inspected were affected by verticillium wilt, of which 1% were killed, causing 1.7×10^6 t yield loss. In Andalucía, southern Spain, 38.5% of 122 olive orchards were affected by verticillium wilt with disease incidence ranging from 10 to 90% (Blanco-López et al., 1984). More recently, inspections in newly established olive plantations in Andalucía found that verticillium wilt was present in 27% of orchards seen (Sánchez-Hernández *et al.*, 1996). Studies in Syria reported a 0.85 - 4.5% disease incidence over some 6.5 million trees inspected in nine provinces and annual yield losses of 1 - 2.3% (Al-Ahmad and Mosli, 1993). In Morocco near 60% of 128 olive orchards were affected by verticillium wilt with disease incidence ranging from 10 to 30% (Serrhini and Zeroual, 1995).

Although trees as old as 50 years may be affected by verticillium wilt, disease incidence and severity are usually highest in young orchards. Thus, disease surveys in Greece indicated that olive plantations with 5 to 6 year-old trees were most susceptible, while reports from Morocco (Serrhini and Zeroual, 1995), Spain (Blanco-López *et al.*, 1984), and Syria (Al-Ahmad and Mosli, 1993) indicated that incidence of the disease was highest in plantations up to 10-years-old.

Symptoms

Two forms of verticillium wilt are recognised: 'apoplexy' (or the acute form) and 'slow decline' (or the chronic form) and have been described by several researchers in different countries (Zachos, 1963; Cirulli, 1975; Vigouroux, 1975; Thanassoulopoulos *et al.*, 1979; Blanco-López *et al.*, 1984; Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995; Tosi and Zazzerini, 1998).

Apoplexy develops from late winter to early spring. Leaves first become chlorotic,

then turn light-brown and roll back toward the abaxial side (fig. 4). Apoplexy is characterized by the rapid dieback of shoots and branches, eventually leading to death of the entire tree (fig. 5). The bark of affected shoots and branches often takes on a purple hue (fig. 6). In young trees, partial defoliation of such branches may occur before death. In other cases, in young and adult trees, dried leaves remain attached to affected shoots and branches. Affected trees usually develop a dark-brown discoloration in the inner vascular tissues (fig. 7).

Slow decline is characterized by necrosis of inflorescences; flowers mummify and remain attached to the shoots (fig. 8). Leaves on affected shoots turn dull-green and fall off before withering, except for those at the distal end of the shoots (fig. 9). Usually, symptoms on inflorescences develop before leaf symptoms appear. Both are followed by necrosis of affected shoots. The bark of affected shoots becomes reddish-brown, and the inner vascular tissues show a dark-brown discoloration. Symptoms of slow decline appear in spring, after those of apoplexy, and develop slowly towards early summer. Olive trees affected by slow decline may recover, giving rise to a progressive reduction in disease incidence over several years.

Factors influencing disease

In general, there is cross-pathogenicity between isolates from olive and from other hosts (Snyder *et al.*, 1950; Saydan and Copcu, 1972; Cirulli and Montemurro, 1976). Thus, high incidences of verticillium wilt often occur in olive orchards established in soils with a history of susceptible crops such as cotton, tomato, (Zachos, 1963; Wilhelm and Taylor, 1965; Cirulli and Montemurro, 1976; Thanassoulopoulos *et al.*, 1979; Blanco-López *et al.*, 1984; Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995).

The type of *V. dahliae* as well as the amount of inoculum present in a planting site can affect disease severity in olive. The situation has been made more complex by the appearance of a defoliating strain which is markedly more pathogenic to cotton than other strains (see section 4.2) and which shows the same differential pathogenicity to olive cultivars as to cotton in the USA (Schnathorst and Sibbet, 1971) and in Spain (Rodríguez-Jurado *et al.*, 1993).

Leaves from diseased olive trees harbour the pathogen and can contribute to increasing the number of microsclerotia in soil (Tjamos and Botseas, 1987; Tjamos and Tsougriani, 1990). Dispersal of pathogen propagules over short distances may occur by means of furrow and flood irrigation (Thanassoulopoulos *et al.*, 1981a), by machinery, by wind (Easton *et al.*, 1969) and in infected leaves (Wilhelm and Taylor, 1965). Long distance dispersal of the fungus may occur in infected planting material (Thanassoulopoulos, 1993), and by transport of plant refuse from affected crops to areas free of the pathogen (Schnathorst and Sibbet, 1971).

Al-Ahmad and Mosli (1993) found that incidence of verticillium wilt in olive was correlated with the number of soil discings, and Serrhini and Zeroual (1995) reported that an increase in disease incidence was associated with the use of chisels or discs for soil cultivation. The use of these implements may sever roots and facilitate invasion of the plant by the pathogen (Tjamos, 1993).

Irrigation is another cultural practice that may encourage wilt in olive (Ruggieri; 1948). This effect has been reported for intensive (Cirulli, 1981) as well as extensive olive production (Blanco-López *et al.*, 1984; Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995). Average disease incidences in non-irrigated and irrigated

orchards were respectively: 9% and 21% in Morocco; 17% and 40% in southern Spain; and 4.5% and 13% in Syria.

Soil and air temperatures also influence verticillium wilt in olive trees. Wilhelm and Taylor (1965) pointed out that disease is favoured by 20-25°C air temperature in spring and by slightly higher and fluctuating temperature during the summer, with maximum day temperatures of 30-35°C.

Failure or inconsistency in attempts to recover *V. dahliae* from diseased trees is a common experience of investigators (Blanco-López *et al.*, 1984; Tosi and Zazzerini, 1998), although there are also reports of *V. dahliae* being isolated from highly susceptible cultivars consistently throughout the year (Thanassoulopoulos *et al.*, 1979; Cirulli, 1981; Al-Ahmad and Mosli, 1993). Tosi and Zazzerini (1998) have related consistently successful isolation of *V. dahliae* from stem tissues to the occurrence of a mild winter and summer, and sporadic success to a cold winter (average minimum temperature below 0°C) and warm summer (average maximum temperature above 30°C).

As with other tree species, one of the most striking phenomena in verticillium wilt of olive is the recovery of affected trees and the natural decrease of disease incidence in an orchard over a number of years (Wilhelm and Taylor, 1965; Thanassoulopoulos *et al.*, 1979; Blanco-López *et al.*, 1990) (see chapter 2 for more information on recovery).

Disease management

The best way to manage verticillium wilt in olive trees is through an integrated strategy, involving the application of control measures before and after establishing an orchard (Tjamos, 1993; Blanco-López and Jiménez-Díaz, 1995) (see section 4.6).

Healthy planting material is very important for minimising the worst effects of wilt during the early years of a planting; it is also important for preventing disease spread. The latter takes on particular significance in relation to the defoliating strain of *V. dahliae* which is currently of limited distribution but poses a major threat to olive plantations. However, there are as yet no schemes of production, inspection and certification of planting material to implement this strategy systematically.

Planting sites can be disinfested of *V. dahliae* by soil fumigation with chloropicrin or by soil solarization. Solarization for 6-8 weeks was very effective in eradicating both the defoliating and the nondefoliating pathotypes from a range of soil types infested to various degrees (Melero-Vara *et al.*, 1995).

Once established, there are a number of measures that are available to combat the worst effects of wilt. Wilhelm and Taylor (1965) recommended the encouragement of an antagonistic microflora in the soil around trees by means of a sawdust amendment. Natural recovery of established olive trees may also be encouraged by the use of soil solarization for individual trees (fig. 10). A single season application of this method to adult olive trees increased recovery and symptom remission for at least three successive growing seasons (Tjamos *et al.*, 1991). The benefits of this treatment were apparently due not only to the direct effects of elevated temperature on destroying *V. dahliae* in soil but also to the enhanced effects of naturally occurring antagonists. See section 4.6 for more information.

Several authors have screened olive germplasm for reaction to *V. dahliae* in the quest for resistant cultivars or rootstocks. Inoculation with Italian isolates of *V. dahliae* indicated that cvs Coratina, Fragivento and Frantoio are resistant while

cvs Ascolana, Cellino and Leccino are susceptible (Cirulli and Montemurro, 1976). In California, Wilhelm (1981) screened a large number of olive cultivars, including representatives from the Mediterranean region, under natural conditions in naturally infested soil. Cultivars Chemlali, Manzanillo and Mission proved to be very susceptible, cvs Chitoni, Liguria, Nevadillo and Redding Picholene were moderately susceptible, and Arbequina and Frantoio were considered tolerant to the disease. One clone of "Arbequina" named Allegra was found to be exceptionally resistant to *V. dahliae* and easy to propagate, and it was recommended for use as a rootstock.

In inoculation experiments with Greek table cultivars (Tjamos *et al.*, 1985; Tjamos, 1993), "Kalamon" proved more tolerant of verticillium wilt than the extremely susceptible "Konservolia". Of the oil cultivars tested, "Manaki", "Megantiki" and "Tsounati" were susceptible, whereas "Koroneiki" and "Lianolia of Corfu" were tolerant to moderately resistant.

The importance of pathotype in screening for resistance of olive cultivars was illustrated by López-Escudero *et al.* (1996) in inoculation experiments using the (cotton) defoliating and non-defoliating pathotypes. Cultivars Arbequina, Cornicabra, Hojiblanca, Manzanilla, Picual, and Verdial de Alcaudete were highly susceptible, and "Empeltre" and "Frantoio" were resistant to the defoliating pathotype. Conversely, when the nondefoliating pathotype of *V. dahliae* was used, "Empeltre" and "Frantoio" were resistant and "Manzanilla" and "Verdial de Alcaudete" were moderately resistant, whilst "Arbequina", "Cornicabra" and "Picual"were susceptible.

Partially resistant selections may be useful for replacing severely affected cultivars, but they too may suffer significant damage in areas where soil inoculum is high. Thus, the cv. Kalamon which was planted to replace the highly wilt susceptible " Konservolia" in Greece was badly affected in Fthiotis County.

A highly resistant olive rootstock would allow the use of susceptible cultivars in infested soils. In addition to the resistant "Allegra" rootstock (Wilhelm, 1981), a self-pollinated wilt resistant rootstock named "Oblonga" was identified by Hartman *et al.* (1971). When inoculated with Greek olive isolates of *V. dahliae*, the original "Oblonga" accession was relatively tolerant to susceptible, whilst a self-pollinated "Oblonga" selection (number 113) was resistant (Tjamos *et al.*, 1985). When grafted to selection number 113, susceptible "Konservolia" neither developed symptoms nor harboured the pathogen. In Spain, the defoliating and non-defoliating pathotypes of *V. dahliae* were both able to infect "Oblonga" but only the defoliating type caused symptoms (Rodríguez-Jurado *et al.*, 1993).

All studies carried out to date on chemical management of wilt in olive, indicate that currently available fungicides are unable to control the disease (Tjamos, 1993). There has been no success with xylem-mobile benzimidazoles whether applied to the trunk base (Thanassoulopoulos and Biris, 1980), to the trunk and the plant foliage (Cirulli and Montemurro, 1976), or injected into the trunk (Petsikos-Panayotarou, 1980). Similarly, there has been no success in the control of the disease when artificially infected 1-year-old "Picual" trees were sprayed with carbendazim, quinosol, prochloraz, and the mixtures carbendazim + quinosol and carbendazim + prochloraz (López-Escudero and Blanco-López, 1996).

3.2 Stone fruits

M. Cirulli, M. Amenduni and E.J. Paplomatas

Introduction

The members of the group of cultivated *Prunus* known collectively as 'stone fruits' all suffer from verticillium wilt to varying degrees. The crops affected are apricot *(P. armeniaca* L.), almond (*P. dulcis* (Mill.) D.A.Webb. = *P. amygdalus* Batsch and *P. communis* (L.) Arcang.), sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.), peach and nectarine (*P. persica* (L.) Batsch), european plum (*P. domestica* L.) and Japanese plum (*P. salicina* Lindl. = *P. triflora* Roxb.). *V. dahliae* is mainly responsible but there have been a few authenticated cases of disease due to *V. albo-atrum*: in apricot (Schmidle, 1968), peach (Haenseler, 1923), cherry (Van der Meer, 1925; Van Poeteren, 1928; Schmidle, 1968) and plum (Marchal, 1932 and 1933; Keyworth, 1944). *Prunus* species and hybrids which are used as rootstocks for stone fruits are also susceptible to verticillium wilt. Several accounts of verticillium wilt of stone fruits have been published (Rudolph, 1931; Parker, 1959; Gubler, 1995).

Verticillium wilt on stone fruits has been reported from many areas where these crops are grown (see table 2). In some places it is a significant factor in production, whilst in others disease incidence is sporadic or even rare. In California, the losses due to wilt, as a result of tree deaths, and the costs of replacement trees, supplemental pruning and lost production, in the first five years of two almond plantings exceeded $ 9000 per hectare (Asai and Stapleton, 1995). Currently, verticillium wilt of stone fruits appears to be most significant in North America, eastern Europe, the Mediterranean region and Australasia. In general, verticillium wilt of stone fruits is not an important problem in north-west Europe. Overall, apricot, almond and peach seem to be more severely affected while cherry and plum are less susceptible.

Symptoms

The symptoms of verticillium wilt have much in common on all stone fruits, although there are some differences. Attacks can result in rapid and severe symptom development, from which the tree may or may not recover, known as acute wilt; or a more gradual development of milder symptoms from which the tree usually recovers, though in some suscepts it may be accompanied by a gradual decline. It is common for some branches of a tree to be affected whilst others remain healthy (see figs 11 and 12).

Symptoms usually appear in late spring or early summer. In typical symptom progression, leaves first turn pale green, then yellow, then brown, become flaccid, wither and curl. They may drop off at any stage in this progression (fig. 13) or remain attached (fig. 14). When symptoms develop early, flowers and fruitlets abscise; when symptoms develop later, fruits first shrivel and then fall off or become mummified and remain attached (figs 15 and 16). Symptoms in apricot and almond appear first at the tips of shoots and extend downwards, whilst in cherry, they occur first on spurs on the lower parts of limbs and progress upwards.

Table 2. Reports of verticillium wilt in stone fruits by area and crop.

Country or geographic area	Crop				
	Apricot	**Almond**	**Cherry**	**Peach and Nectarine**	**Plum**
USA and South America	Czarnecki,1923; Parker, 1959; Blodgett, 1964	Czarnecki, 1923; Carter, 1938; Stapleton et al, 1993	Wilhelm *et al.*, 1954; Blodgett,1964	Haenseler*,1923; Rudolf, 1931; Mills,1932; Parker, 1959; Blodgett, 1964; Vergara, 1967	Rudolph,1931; Parker, 1959; Blodgett, 1964
Canada	Berkeley, 1927		McIntosh, 1954	McKeen, 1943; Conners & Saville, 1945;1953	
U.K.			Wormald & Harris, 1932		Keyworth*, 1944
France	Dufrenoy & Dufrenoy, 1927	Dufrenoy & Dufrenoy, 1927	Dufrenoy & Dufrenoy, 1927	Gaudineau, 1949	Dufrenoy & Dufrenoy, 1927
Netherlands			Van der Lek*,1918; Van der Meer*, 1925	Van Koot, 1947	Van Koot, 1947
Germany	Schmidle, 1968		Donandt,1932; Schmidle, 1968		Donandt,1932
Greece	Sarejanni, 1935	Thanassoulopoulos & Kitsos, 1972		Sarejanni, 1935	
Italy	Curzi, 1930; Noviello & Aloj, 1991	Luisi & Sicoli, 1993		Pollacci, 1933; D'Ercole, 1981; Ciccarese *et al.*, 1990; Cirulli *et al.*, 1998	Curzi, 1930
Former Yugoslavia				Arsenijevic, 1977	Arsenijevic & Sevar, 1981
North Africa	Rieuf, 1950				
Hungary	Husz, 1947; Berend, 1958				
Former Czechoslovakia				Anonymous, 1926	
Turkey	Saribay *et al.*, 1973			Saydam *et al.*, 1971	Saribay & Demir, 1984
Bulgaria		Vitanov *et al.*, 1972; Iliev,1974		Atanasoff *et al.*, 1932	Atanasoff *et al.*, 1932
Georgia		Tsakade & Liluashvili, 1975			
Former USSR		Verderevskii & Kropis, 1964	Verderevskii & Kropis, 1964	Azimdzhanov, 1971	Dernovskaya, 1972
Australia	Cheney, 1930; Hutton & Morschel, 1955				Purss,1961
New Zealand	Renouf, 1948			June, 1950	

* *Verticillium albo-atrum*

In almond and peach, thin shoots may flag (fig. 17), the tips curling over to form characteristic hooks and loops (fig. 18). In almond and peach, defoliation may occur suddenly, with few preliminary leaf symptoms (fig. 19). Defoliated trees may produce a new crop of stunted shoots with leaves which are small and pale. Affected branches may die back as the disease progresses through the growing season. Where branch death has occurred, new shoots may eventually develop from below the level of die-back, leading to full recovery of the tree over a 2-4 year period (see chapter 2). The resulting tree may be permanently disfigured by the early attack, though it may be possible by careful pruning and training over several years to restore the shape of affected trees. In the most severe cases the whole tree may die, but this is unusual except in cherry (Wilhelm, 1981).

A brown or dark green to black staining of the wood is a common symptom in verticillium wilt of stone fruits, as it is in other trees. Viewed in cross-section this may be evident throughout one or more annual rings or be confined to patches, mainly in the spring vessels, or to discrete spots (figs 20 and 21). This discoloration occurs mainly in the trunk and main branches (fig. 22) and to a much lesser extent in the roots. Verticillium wilt of apricot is also known as 'black-heart' for this reason (Czarnecki, 1923; Cheney, 1930). Vascular discoloration is least well developed in cherry.

Factors influencing disease

Generally, attacks of wilt are most likely to occur in the early years of a plantation (3-10 years). In peach and plum the disease is uncommon in older trees whilst in apricot, almond and cherry trees of all ages may be attacked. The influence of inoculum level in soils and disease in apricot was reported by Taylor and Flentje (1968), whilst Stapleton *et al.* (1993) showed that less than 5 propagules/g. soil can cause a high incidence of wilt in apricot and almond plantations. As with other verticillium wilts, intercropping with wilt susceptible crops is an important factor affecting disease incidence. In a 4-year-old almond planting in Italy, 50% more trees were affected in the half of the field that had been intercropped with a wilt- susceptible tomato cultivar in the previous year than in the half that was not intercropped (Luisi and Sicoli, 1993). Abundant irrigation and high nitrogen levels in soil predispose apricots to wilt as do nematodes and soil cultivations that lead to root damage (Vigouroux, 1984). In Moldavia, verticillium wilt was a problem on plum orchards only in irrigated areas (Gavrilenko *et al.*, 1977). Ndubizu (1977) found that the effects of *V. dahliae* on cherry seedlings were greater in the presence of nematodes (*Tylenchorynchus claytoni*, *Pratylenchus penetrans* or *Meloidogyne hapla*) than in their absence.

Disease management

The same good practices that apply to managing all wilt diseases apply to stone fruits: avoiding planting sites infested with *V. dahliae*; pre-planting soil disinfestation; not intercropping with wilt susceptible plants; using disease-free planting material; and removal and destruction of diseased plants and plant parts. Resistance is again an important potential control strategy in stone-fruit crops but the extent to which it has been deployed is limited. Because stone fruits are usually grafted to rootstocks, it is in the latter where resistance has been mainly sought. New rootstocks from within *Prunus* species or from interspecific crosses have been selected primarily for innovative agronomic traits such as dwarfing and precocity and for tolerance of

phytophthora root rots but not specifically for resistance to verticillium wilt.

Currently, no resistant rootstock suitable for apricot or apricot cultivars is available for commercial use and there is limited information on rootstock resistance in the several types of apricot, peach and myrobalan (*P. cerasifera* Ehrh.) plum commonly used as rootstocks for apricot. In contrast to an earlier report (Rudolph, 1931) which said that myrobalan seedlings in California were highly susceptible to wilt, Day (1953) found in the USA that this rootstock was more resistant than apricot. Of several species, including *P. ansu* (Maxim.) Komar, *P. armeniaca*, *P. mandschurica* (Maxim.) Koehre, *P. mume* Sieb. et Zucc., *P. persica* and *P. sibirica* L., screened in Australia by Taylor and Flentje (1968) one selection from a wild population of *P. armeniaca* was resistant and considered of potential value as a rootstock. In France, Vigouroux (1984) ranked potential rootstocks in increasing susceptibility: myrobalan, peach, GF 8 seedlings, apricot seedlings, and GF 31 seedlings. More recent work in Australia (Gathercole *et al.*, 1987) has confirmed that plum cultivars are more resistant to *V. dahliae* than apricot.

Although susceptible to wilt in California, the almond cv. Nonpareil has strong recovery powers (Wilhelm, 1981) which enables it to be grown successfully in areas where wilt is common. In Italy, following inoculation of potted trees of three peach x almond hybrid rootstocks and three plum rootstocks, *V. dahliae* was re-isolated after seven months from all except the plum rootstock Pixy (Corazza and Chilosi, 1986), indicating potential for this rootstock as a means of controlling wilt in almond. Possible rootstocks for wilt control in peach, almond and apricot were identified by Cirulli *et al.*, (1998) who tested 30 rootstocks for reaction to *V. dahliae* and found that four, namely, GF 677 (*P. persica* x *P. dulcis*), A5 (*P. persica*), Harroblod (*P. persica*) and Nemaguard (*P. persica*) were resistant and failed to yield the pathogen on isolation. Some differences among almond cultivars to inoculation with a range of isolates of *V. dahliae* were demonstrated by Luisi et al (1994).

Although there have been reports of serious verticillium wilt outbreaks in plum (Pollacci, 1933; Gavrilenko *et al.*, 1977; Saribay and Demir, 1984), this is generally the least affected of the stone fruits (Wilhelm, 1981) and its greater resistance has been generally confirmed in inoculation experiments. Gathercole *et al.* (1987) reported that all plum cultivars tested were resistant to *V. dahliae* whilst all apricot and related selections were susceptible. In tests conducted in Moldavia (Gavrilenko *et al.*, 1977), six out of 22 plum cultivars grafted to myrobalan stock were only slightly affected by wilt. All the experience to date suggests that plum species are likely to offer the best prospects for developing rootstocks for stone fruits which are resistant to verticillium wilt.

The use of clear polythene to raise soil temperature sufficiently to kill *V. dahliae* before planting susceptible crops, otherwise known as solarization, is a well-developed technology (Katan and De Vay, 1991). Soil mulching with black and transparent polythene has also been shown to control verticillium wilt of apricot and almond, but in very warm summer conditions newly planted trees may collapse (Stapleton *et al.*, 1993). This is the only proven control method that can be applied after trees have been planted and has also been shown to be effective for other tree crops (see section 3.1 and 4.6). Judicious use of fertilizers and irrigation can help trees to recover following an attack by verticillium wilt.

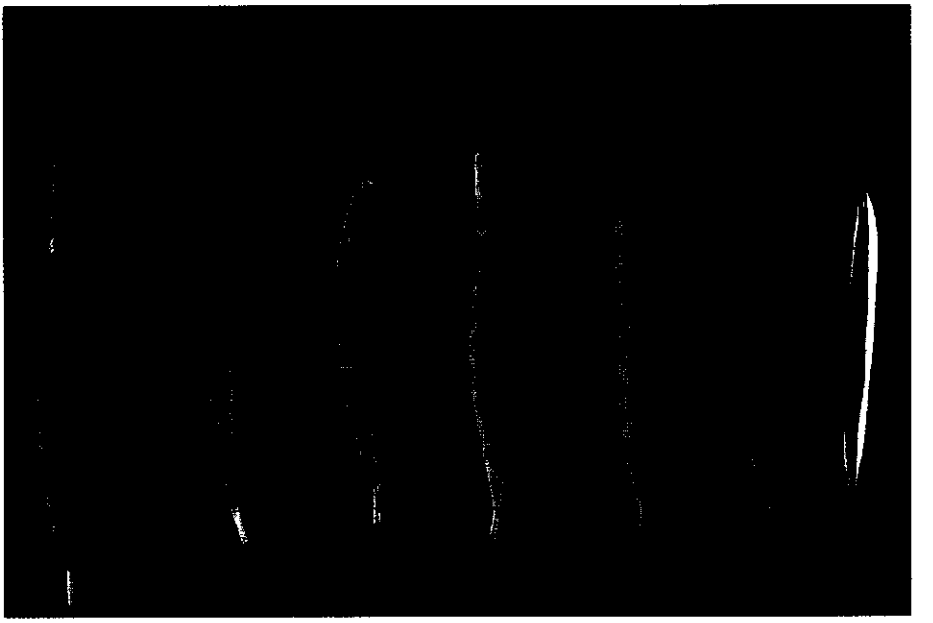


Fig. 4

Fig. 5

Fig. 4. Leaf symptoms on olive: leaves become chlorotic, then light-brown, rolling back towards the abaxial side. **Fig. 5**. Dieback of shoots and branches on an olive tree affected by wilt. (photographs: R.M. Jiménez-Díaz).

Fig. 6

Fig. 7

Fig. 8

Fig. 6. The characteristic purple hue on the bark of an affected olive branch. **Fig. 7.** Dark brown discoloration in the inner vascular tissues of an olive branch. **Fig. 8.** Necrosis of olive inflorescences: mummified flowers remaining attached to the shoots. (photographs: R.M. Jiménez-Díaz).

Fig. 9

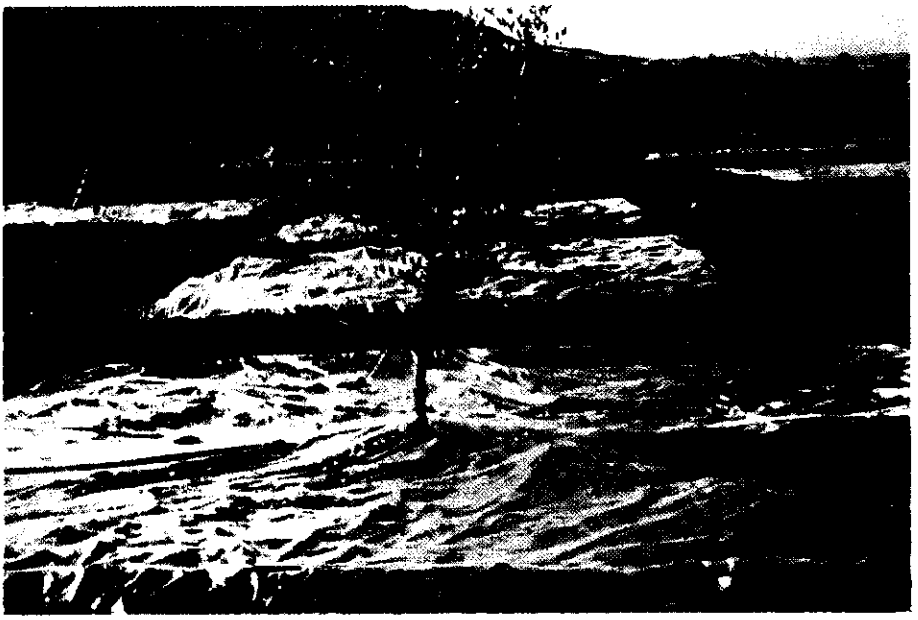


Fig. 10

Fig. 9. Slow decline in olive: dull green leaves that fall off before withering, except for those at the distal end of shoots. **Fig. 10**. Application of soil solarization to individual olive trees. (photographs: R.M. Jiménez-Díaz).

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 11. Typical disease symptoms on one branch in the crown of a three year old peach tree (photograph: M. Cirulli). **Fig. 12**. Scaffold branch of an apricot tree with typical symptoms of verticillium wilt (photograph: S. Frisullo). **Fig. 13**. Sudden and complete defoliation of an almond tree (photograph: N. Luisi). **Fig. 14**. An almond tree with withered leaves remaining attached (photograph: N. Luisi).

Fig. 15

Fig. 16

Fig. 15. Leaf withering and partial shrinking of fruits on apricot (photograph: M. Cirulli). **Fig. 16**. Abortion of fruits and defoliation in almond (photograph: N. Luisi).

Fig. 17

Fig. 18

Fig. 19

Fig. 17. Flagging of thin shoots of peach. **Fig. 18**. Typical hook shaped tips of young peach shoots. **Fig. 19**. Yellowing of veins and veinlets in the leaves of an infected peach tree. (photographs: M. Cirulli).

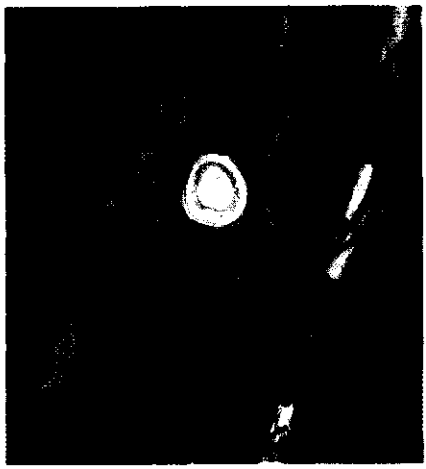


Fig. 20

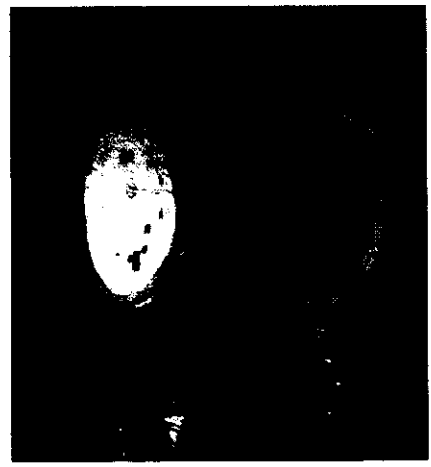


Fig. 21

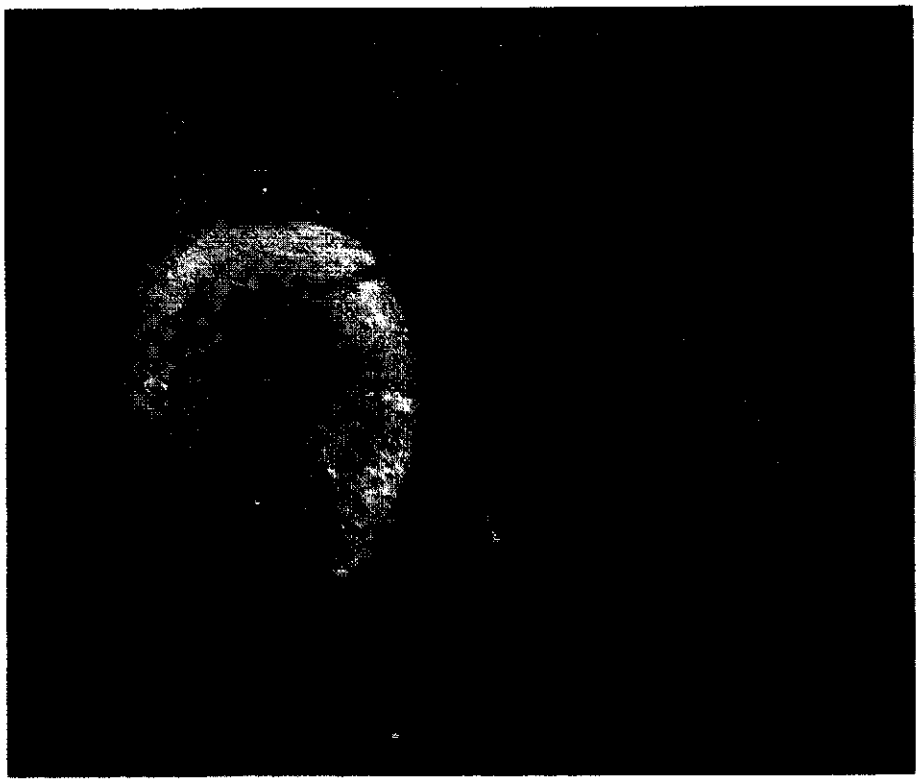


Fig. 22

Fig. 20. Discoloration in one growth ring in the wood of a young peach tree. **Fig. 21**. Two patterns of discoloration in cross sections of apricot shoots. **Fig. 22**. Wood darkening on cross sections of almond scaffold branches ('black-heart'). (photographs: M. Cirulli).

Fig. 24

Fig. 23

Fig. 25

Fig. 23. Leaf symptoms in pistachio; desiccation and necrosis. **Fig. 24**. Partial defoliation and leaf withering in pistachio. **Fig. 25**. A pistachio tree killed by verticillium wilt; withered leaves remaining attached. (photographs: M. Cirulli).

Fig. 26

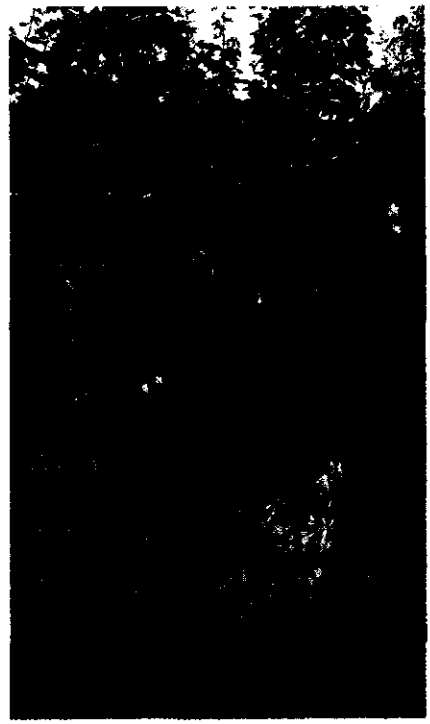


Fig. 29

Fig. 27

Fig. 28

Fig. 26. Wilting and desiccation of leaves on young Norway maple (photograph: J.A. Hiemstra). **Fig. 27**. Discoloration and marginal necrosis of Norway maple leaves (photograph: J.A. Hiemstra). **Fig. 28**. Desiccation and necrosis of Norway maple leaves. **Fig. 29**. Severe dieback of young Norway maple.

Fig. 30

Fig. 31

Fig. 32

Fig. 33

Fig. 30. Wilting, leaf necrosis and defoliation in mature ash trees. **Fig. 31**. Close-up of wilting ash leaves, showing the typical light, greyish green colour. **Fig. 32**. Necrosis of ash leaves and partial defoliation. **Fig. 33**. Reduced new flushes of very small, chlorotic leaves and very short internodes in ash.

3.3 Pistachio

E.J. Paplomatas and K. Elena

Introduction

All pistachio cultivars belong to the species *Pistachia vera* L. This species originates in Asia and Asia Minor and is cultivated in the Mediterranean region, the southern United States and in Australia (Whitehouse, 1957). In the Mediterranean producer countries, *P. vera* is grafted to the rootstock *P. terebinthus* L. However, both rootstock and scion are susceptible to verticillium wilt which is one of the most serious problems of pistachio cultivation (Thanassoulopoulos and Kitsos, 1972; Firuzeh and Ludders, 1978; Chitzanidis, 1995; Michailides *et al.*, 1995). In California, *P. atlantica* Desf. has been used widely as a rootstock but recently, because of its susceptibility to verticillium wilt, it has been replaced in many areas by the more resistant *P. integerrima* Stewart (Pioneer Gold) rootstocks (Wilhelm, 1981; Ashworth, 1984).

Symptoms

In Greece, the earliest symptoms of wilt can be seen on a few trees in spring, but symptoms usually appear in June and become progressively more pronounced into October and November. The leaves of affected shoots turn yellow and assume a light tan colour and gradually dry out, but remain attached to the branches (fig. 23). A brown discoloration of the xylem, extending into the most distal branches, is characteristic of the disease. In young trees the whole foliage is affected (fig. 24). In older trees the disease usually affects one or more branches on one side of the tree.

Some diseased trees die in the year of infection (fig. 25), whilst others recover and remain healthy for several years. Re-infection is necessary for disease development in trees that have naturally recovered (Ashworth and Zimmerman, 1976). Following infection of young trees, the fungus spreads rapidly through the tissues, often resulting in tree death (Papaioanou, 1956; Ashworth and Zimmerman, 1976).

Factors influencing disease

New pistachio orchards that are planted in fields where susceptible hosts like cotton or tomato have been grown, usually develop verticillium wilt problems and, in some cases, up to 30% of the trees die (Ashworth *et al.*, 1982). Moreover, disease incidence is more severe when orchards are intercropped with wilt-susceptible hosts such as cotton, vegetables and ornamentals (Sarejanni, 1935; Papaioanou, 1956).

Disease incidence in young pistachio trees is related to the inoculum density of *V. dahliae* in soil. Over a two-year period, losses in new plantings were relatively small (<2%) where inoculum density ranged from 0.02-0.05 microsclerotia per g. soil, whereas 10-14% of the trees died with 1-2 microsclerotia per g. soil (Ashworth and Zimmerman, 1976). However, within 6 years, 85% of the trees were killed where inoculum density approached 5 microsclerotia per g. soil (Ashworth and Gaona, 1982). Ashworth *et al.* (1985) observed 69% infection of one-year-old trees growing in soil with an inoculum density of about 20 microsclerotia per g. soil.

In California, both potassium deficiency and phosphorus deficiency were correlated with increased susceptibility to infection by *V. dahliae* in soils with low inoculum densities (Ashworth *et al.*, 1985).

Disease management

The principles of planting disease-free trees in pathogen-free soil and avoiding contamination after planting apply as much to pistachio as to other tree crops (see section 4.6). However, because many potential planting sites are already infested with *V. dahliae,* the use of resistant rootstocks and soil disinfestation methods are particularly important elements of current management strategies.

Differences in susceptibility have been demonstrated between and within *Pistacia* species. In mass plantings of young seedlings of *P. atlantica, P. vera, P. terebinthus* and *P. integerrima* in a field with 20 microsclerotia per g. soil, mortality was 21, 8.1, 5.4 and 2.2% respectively (Ashworth, 1984). Screening for resistance by Raabe and Wilhelm (1978) also suggested that selections from *P. terebinthus* could be resistant to the pathogen.

Morgan *et al.* (1992), in greenhouse experiments with seedlings of *P. atlantica, P. integerrima* and interspecific hybrids, also demonstrated intraspecific variation in resistance to *V. dahliae*. They concluded that a currently unused gene pool for crop improvement exists.

Recently, two new hybrid cultivars, Pioneer Gold II (PGII) and University of California, Berkeley I (UCBI), were introduced in California and tested for resistance to verticillium wilt. Hybrid PGII was moderately susceptible, while UCBI was moderately resistant when compared to "Pioneer Gold" (resistant) and "Standard Atlantica" (susceptible) rootstocks. However, more work is required before this rootstock could be recommended for commercial planting in soil infested with *V. dahliae* (Morgan *et al.*, 1992).

Soil fumigation has proved very effective for the control of verticillium wilt of pistachio (Ashworth and Zimmerman, 1976). Fumigation with a mixture of chloropicrin and methyl bromide reduced inoculum densities of *V. dahliae* in soils previously cropped with cotton from five or more microsclerotia per g. to 0.05 or less. This resulted in tree losses no greater than observed in virgin soil groves, two years after planting. In heavily infested orchards, tree removal and spot treatment of soil may be practised. During fumigation, care should be taken to avoid fumigant damage to the neighbouring trees.

Effective control of the pathogen has also been achieved by soil solarization of established trees (Ashworth and Gaona, 1982; Ashworth *et al.*, 1982). See also section 4.6.

3.4 Maple

D.C. Harris

Introduction

Maples (*Acer* spp.) are among the most susceptible tree hosts of *Verticillium dahliae* (Himelick, 1969; Smith and Neely, 1979; Piearce and Gibbs, 1981). Verticillium wilt of maples has been reported extensively from the USA and to a lesser extent from north-west Europe. The disease is most damaging on young trees, particularly in nurseries, where losses of up to 85% have been recorded (Bedwell and Childs, 1938). Verticillium wilt in Europe is comparatively rare on established trees, although damage to urban and landscape maples has occurred on a significant scale in the U.S.A. (Pirone, 1978; Smith, 1979).

Although there is a range of intra-specific susceptibility in *Acer*, which may account for some contradictory reports about the comparative susceptibility of species (Schreiber *and* Mayer, 1992), it is generally agreed that *A. platanoides* (Norway maple) is among the most susceptible (Hoitink *et al.*, 1979; Smith and Neely, 1979). Verticillium wilt is reported to occur naturally in a number of other maples: *A. campestre* L., *A. griseum* (Franchet) Pax, *A. ginnala* Maxim., *A. macrophyllum* Pursh, *A. negundo* L., *A. nigrum* Michaux, *A. palmatum* Thunberg ex Murray, *A. rubrum* L., *A. saccharum* Marshall and *A. saccharinum* L. The sycamore (*A. pseudoplatanus* L.) has been shown to be susceptible to inoculation (Chambers and Harris, 1997) but is effectively field resistant. Because of its susceptibility and pre-eminence in horticulture, Norway maple has been the main subject of scientific investigations on verticillium wilt in *Acer*.

Symptoms

Two forms of verticillium wilt of maple are recognised: acute and chronic (Caroselli, 1957), although they undoubtedly represent a continuum of symptom expression. Acute symptoms are characterised by the rapid wilting of leaves which then desiccate and turn brown, dead leaves usually remaining attached (fig. 26). This form of disease usually results in death of the tree. Symptom development is much slower in the chronic form of wilt: leaves become progressively discoloured and desiccated, usually from the margins inwards (figs 27 and 28); affected leaves become epinastic and fall off. In chronic wilt, the outward effects of disease may be limited to leaf discoloration, slightly premature leaf fall or stunting. Shoots affected by wilt usually dieback (fig. 29). This dying back may begin during the growing season, especially in acute wilt, and continue through the winter, leading to the death of branches, or possibly the whole tree. Overwinter die-back may also occur following chronic disease in which few or no obvious symptoms have occurred during the growing season. The wood of affected shoots usually exhibits a greenish-black discoloration extending longitudinally upwards from the tree base. It is common for symptoms to be restricted to a sector or one side of a tree or branch. Trees that have suffered severely in one year may recover in the following season by producing new shoots below the level of die-back; such shoots may remain healthy or may also be affected by wilt subsequently.

Factors influencing disease

Most disease has its origins in the nursery. If the soil is infested with *V. dahliae* as a result of its previous cropping history, young trees or seedling rootstocks become infected when lined out. This can lead to various degrees of disease severity, including death or severe mutilation. Some trees may become infected but show few or no obvious symptoms of disease until they are transplanted to another location where the symptoms may appear in the first growing season or be delayed for some time; levels of disease may fluctuate from year to year. It is believed that the use of former agricultural land infested with *V. dahliae* for nursery production caused the prevalence of the wilt problem in the USA in the 1960s and 70s (Sinclair *et al.*,1981) (compare with wilt in ash, section 3.5).

Maples, as in other tree suscepts of *V. dahliae*, are able to restrict the spread of the pathogen from one year's growth of xylem to the next. The ability to do this is correlated with the resistance of the species (Sinclair *et al.*, 1981). This would explain the fluctuation in disease from year to year and how trees are able to recover following an attack. Thus, disease levels in each year are determined mainly, though not exclusively, by the incidence and development of new root infections in the same year.

Root wounds are thought to be an important contributory factor in disease and there is some evidence (Santamour, 1992) that wilt of maple is exacerbated by the presence of root knot nematodes (*Meloidogyne* spp.). Drought stress is also thought to be an important factor in determining the severity of wilt (Caroselli, 1957; Manion, 1981).

The pathogen colonises leaf petioles which become detached and may contribute to the accumulation of inoculum in soil (Hiemstra, 1997a).

Some authors have found that isolates of *V. dahliae* from *Acer* are more pathogenic to this host than are isolates from other hosts (Adams and Tattar, 1975; Hiemstra, 1995).

Disease management

Wilt can be largely avoided by producing trees on nursery sites that are free of *V. dahliae* or are infested below a disease risk threshold. Soils can be tested in advance of planting to determine the level of risk (see Harris and Yang, 1996). An unacceptable risk may be reduced by treating soil with a suitable disinfestant or, preferably, a site with an acceptable risk should be found.

Irrigation (Smith, 1979) and the supply of additional potassium (Smith, 1983) have been advocated as a means of reducing the impact of the disease on established trees.

Resistance to (or tolerance of) *V. dahliae* has been identified in seedlings of *A. rubrum* (Townsend and Hock, 1973) and *A. platanoides* (Valentine *et al.*, 1981), and some efforts have been made to exploit resistance as a strategy for control. Two scion cultivars of *A. platanoides*, Jade Glen and Parkway were found to be comparatively resistant in the USA (Townsend *et al.*, 1990) though it is not known how well these will perform if budded to susceptible rootstocks. In the Netherlands, resistant individuals of *A. platanoides* have been selected as possible clonal rootstocks (Hiemstra, 1997b). The exploitation of such rootstocks depends on developing economic methods of vegetatively propagating what is a 'difficult' subject.

3.5 Ash

J.A. Hiemstra

Introduction

Verticillium wilt is not generally an important problem of ash (*Fraxinus spp.*) but it may be locally serious in nursery and landscape trees. In the USA, *F. americana* L. and *F. pennsylvanica* Marsh. are the most commonly affected species (Forer and Longenecker, 1973) and an increasing incidence of the disease has been reported recently (Worf *et al.*, 1994; Heffer and Regan, 1996). Other species like *F. nigra* Marsh., *F. excelsior* L. and *F. quadrangulata* Michaux are also known to be affected (Himelick, 1969). In Western Europe verticillium wilt of the only native species, *F. excelsior,* has been known for many years (Buisman, 1933). Recently, severe attacks of established ash trees in young forest stands and landscape plantations has been reported in the Netherlands (Hiemstra, 1995). Since the end of the 1970's, "ash wilt disease" has become common in young plantings all over the Netherlands. Almost all reports on ash wilt in forest trees (Hiemstra *et al.*, 1990) come from recently afforested areas. Hence, most affected trees are less than 30 years old. Occurrence of the disease is not related to site conditions or management practices. Affected trees have been found on clay, loam, sand and peat soils, in all kinds of plantings: roadside, amenity and recreational plantations as well as forest stands, both pure and mixed.

Symptoms

The most striking early symptoms are wilting and defoliation (fig. 30). Wilting is usually accompanied by a subtle change of the colour of leaves towards a lighter, greyish green (fig. 31). In extreme cases, withering or complete necrosis of leaves occurs (fig. 32). All these early symptoms may affect the entire crown, or only part of it. Secondary symptoms of the disease may include: reduced new flushes of very small, pale green to chlorotic leaves and very short internodes (fig. 33); scorch and marginal necrosis of the remaining leaves; elongated dead areas of bark on the diseased stem or branches; dying back of part of the tree; epicormic shoots on surviving parts of the tree, sometimes followed by dying back of these shoots.

Some affected trees show a discoloration in the cambial zone, the wood or the pith of stems or branches. However, in many cases there is little or no discoloration. Experienced growers may recognize wilt in young trees by a greyish hue of the wood. Under the microscope, obstruction of vessels by tyloses and granular deposits may be clearly seen in the wood of affected trees (Miller and Hiemstra, 1987).

Leaf symptoms usually become conspicuous in August or September, after a period of normal growth. The processes of wilting and defoliation can occur very suddenly, particularly in trees up to 15 or 20 years old. Death of affected trees may take place within a few weeks after initial symptoms or may occur during the following winter; however, most of the affected trees recover (Hiemstra, 1995 and 1995b). In trees of 20 years and older, disease progress may be more gradual. These trees do not show the sudden, complete wilting and leaf fall as described above but decline over a period of months or even years. Such cases are characterized by late flushing, sparse and pale-coloured foliage, gradual wilting and premature leaf fall, and a partial dying back of the topmost shoots.

Factors influencing disease

Much of the recent ash wilt in the Netherlands may have arisen from infection in the nursery (Sinclair *et al.*, 1981).The Dutch nursery industry has recently undergone considerable expansion, and almost all the soils available for this expansion had a history of agricultural use, including potato growing, and may therefore have been infested with *V. dahliae*. On the other hand, many of the new ash plantations were also established on former agricultural land, and the inoculum may already have been present in the planting site.

The number of diseased trees in forest plots gradually increases during the growing season, usually peaking strongly in August and September. During a 5-year-period, the average yearly disease incidence was about 10% of the trees in each of two plots, yet the annual mortality rate was less than 1% of the dominant trees in a plot. Complete recovery of *Verticillium*-affected ash trees was a common occurrence: out of 212 trees with distinct symptoms 73% were free of any symptoms in the following year (Hiemstra, 1995).

Microsclerotia of *V. dahliae* produced in dying host tissues can contribute to soil inoculum and can be a means of distributing the pathogen (see chapter 2). Rijkers *et al.* (1992) demonstrated that petioles from *Verticillium*-affected ash trees may contain large numbers of microsclerotia. In forest plots, where no soil cultivation is practised, these petioles are rapidly incorporated into the soil by earthworm activity (fig. 34) where they contribute to soil inoculum. The limited lateral movement of comparatively large ash petioles helps to explain the patchy disease distribution which develops in affected forest stands (Hiemstra, 1995).

Fig. 34. Incorporation of ash petioles into soil by the activity of earthworms.

Disease management

So far, ash wilt disease is not a serious problem in forest management. Because of the low mortality rate and the diffuse distribution of dead trees in affected forest stands the disease is effectively a slowly acting 'natural thinning agent' rather than a threat to the stand (Hiemstra, 1995). But in other types of plantation, death or dieback caused by ash wilt disease may be more serious and justify control measures. In line plantings and landscape plantations and in tree nurseries, each dead tree means a loss of either production or value, or both. However, in roadside plantings, and particularly in gardens and parks, dead trees are unacceptable. Soil fumigation may be appropriate in some nursery situations. Not enough is known about the reaction of *Fraxinus* species to *V. dahliae* to be able to exploit resistance for control.

3.6 Other tree species

J.C. Goud and J.A. Hiemstra

Many other tree species are affected by verticillium wilt (see chapter 2). In this section wilts of some of the more important other tree hosts are described.

Cocoa

Verticillium wilt has long been the most serious soil-borne disease of cocoa (*Theobroma cacao* L.) in Uganda (Emechebe *et al.*, 1971; Matovu, 1973) and is now an increasingly serious problem in Brazil (Resende *et al.*, 1994). Verticillium wilt in cocoa has also been observed in Colombia (Granada, 1989 in Cooper *et al.*, 1995).

Disease symptoms on cocoa include flaccidity, followed by chlorosis and necrosis of leaves and fine branches (Resende *et al.*, 1994; 1996b). Leaves and soft shoots on affected trees wilt rapidly, although flaccid leaves are usually retained and later turn brown. Often the wilt starts with one branch at the jorquettee (branching node) and progresses to the next in a clockwise or anticlockwise direction until all five branches are wilted. In some cases the wilted tree may produce new suckers, but in most cases it dies (Matovu, 1973). In disease caused by 'defoliating' strains, wilting and defoliation starts with the uppermost leaves (Resende *et al.*, 1996b). Presently, it is not clear whether the defoliating and non-defoliating strains of cocoa are comparable to those occurring in cotton and olive in Spain and California (see sections 3.1 and 4.2). Vascular discoloration is often, but not always present in plants affected by verticillium wilt (Emechebe *et al.*, 1975).

Wilt in cocoa is favoured by soil moisture levels favouring plant growth (Emechebe, 1975b). Though most diseased trees do not recover (Matovu, 1973), spontaneous recovery has been reported for cocoa (Emechebe *et al.*, 1974), especially in more resistant varieties (Resende *et al.*, 1995, 1996a). In inoculation trials older seedlings were found to be more susceptible to verticillium wilt than younger ones (Emechebe, 1975a; Resende *et al.*, 1995).

It has been reported that some reduction of disease may result from the shading effect of growing the crop under thinned forest (Matovu, 1973). However, the main emphasis for control has been on the development of resistant varieties (Resende *et al.*, 1995 and 1996a).

Different levels of resistance have been reported to exist in cultivars and selections of cocoa (Braga and Silva, 1989; Resende *et al.*, 1995). Braga and Silva (1989) detected the involvement of an allele pair which was responsible for resistance, susceptibility being the dominant character. The cultivar Pound 7, reported as being one of the most resistant (Braga and Silva, 1989; Resende *et al.*, 1996a) was shown to possess both recessive alleles.

Avocado

The culture of avocado (*Persea americana* Mill.) is generally based on varieties that are budded or grafted to seedling rootstocks. Verticillium wilt of avocado has been observed in Argentina (De Ramallo, 1972), Chile (Zentmyer, 1959; Latorre and Allende, 1983), Ecuador (Zentmyer, 1959), Israel (Barchilon *et al.*, 1987; Tjamos,

1991), Mexico (Garcia and Téliz-Ortiz, 1984), Morocco (Vanderweyen, 1978), Spain (Galan-Sauco, 1976) and the U.S.A. (Zentmyer, 1949; Marlatt and Goldweber, 1969). Zentmyer *et al.* (1955) reported an average disease incidence of 10.5% in trees on Guatemalan rootstocks (*P. americana*) and of 1.3% in trees on Mexicola rootstocks (*P. americana var. drymifolia* (Schlechtendal and Chamisso) Blake). In Mexico, *Verticillium* was detected in 10-15% of cultivar Hass and native trees (García and Téliz-Ortiz, 1984). In Chile, disease incidence in seven groves varied from 1.3 to 10.4%, with an overall incidence of 2.9% (Latorre and Allende, 1983).

Symptoms on avocado are typical of those seen in tree hosts, as described in chapter 2. As in peach (section 3.2), fruits borne on diseased branches do not usually reach maturity but become desiccated, turn brown and remain on the tree (Zentmyer, 1949; Marlatt and Goldweber, 1969; Vanderweyen, 1978; Latorre and Allende, 1983). Trees from 2.5 to 20 years of age can be affected by wilt (Zentmyer, 1949). Infected seedlings may remain symptomless for some time (Pinkas and Kariv, 1984).

As with other crops, the disease occurs mainly in new avocado groves that have been planted after susceptible crops, but rarely after non-susceptible crops (Latorre and Allende, 1983). Disease incidence depends both on the cropping history of the field and on the resistance of the cultivars and rootstocks grown. After the initial attack trees usually recover, showing vigorous regrowth from buds on the tree trunk. However, the crop is usually lost and much of the tree crown may also be lost (Zentmyer, 1949; Marlatt and Goldweber, 1969; Vanderweyen, 1978; Latorre and Allende, 1983). Recovery can either be complete and long-lasting or may be followed by a recurrence of symptoms in the next growing season (Zentmyer *et al.*, 1955). Although effects may be extensive and severe, death of mature trees is unusual (Latorre and Allende, 1983).

In managing the disease the usual careful choice of site, planting material and the avoidance of intercropping are important (see section 4.6). Planting after tomato, potato, aubergine, cotton, melon, squash, cauliflower, cabbage, carthamus, artichoke, chrysanthemum (Latorre and Allende, 1983) should be avoided. It is usually safe to plant after Gramineae, beans and bulb crops (Vanderweyen, 1978; Latorre and Allende, 1983). *In situ* solarization is effective, but shading by the tree canopy may be a limiting factor for complete eradication of *V. dahliae* in established avocado orchards (Tjamos, 1991).

Rootstocks should be screened for *V. dahliae* because it may be present in the tissues of symptomless plants (Pinkas and Kariv, 1984). There is progress in the development of resistant rootstocks (Zentmyer *et al.*, 1955; Ben Ya'acov and Frenkel, 1974; Pinkas and Kariv, 1984). Some resistance has been demonstrated in the rootstocks 'Stewart' (Barchilon *et al.*, 1987), and 'Degania 234' and 'Ashdod 5' (Ben Ya'acov and Frenkel, 1974). Guatemalan rootstocks are more susceptible than Mexican rootstocks (Zentmyer, 1949; Zentmyer *et al.*, 1955). Of the cultivars, 'Fuerte' is considered more resistant than 'Hass' (García and Téliz-Ortiz, 1984).

Elm

Kelsheimer and May (1940) reported that verticillium wilt was an important elm disease which was widespread throughout the U.S.A. Verticillium wilt of elm has also been reported from the Netherlands (Van der Meer, 1926). *V. dahliae* has been isolated from American or white elm (*Ulmus americana* L.), English, common or small-leaved elm (*U. procera* Salisb.), Dutch elm (*U. hollandica* Mill.), smooth-

leaved, feathered or European field elm (*U. carpinifolia* Gled. Suckow), Chinese elm (*U. parvifolia* Jacq.), slippery, red or gray elm (*U. rubra* Muhl.) and winged elm (*U. alata* Michx.) (Van der Meer, 1926; Kelsheimer and May, 1940; Holmes, 1964; Carter, 1975; Affeltranger *et al.*, 1976). Sometimes verticillium and cephalosporium wilts and Dutch elm disease occur together, causing tree mortality (Affeltranger *et al.*, 1976).

Other reports mention the use of preventive inoculation of elm trees with *V. dahliae* to protect against Dutch elm disease. In this case inoculation with *Verticillium* seems to elicit a resistance response of the tree (Scheffer, 1990). The action of tyloses and gel plugs, which are formed after *Verticillium* infection, may be to restrict (Smith, 1970) the growth of *Ophiostoma ulmi* and *O. novo-ulmi*, the causal agents of Dutch elm disease.

In elm, suppression of growth is often the main symptom of verticillium wilt (Lester, 1975; Rauscher *et al.*, 1974). However, the disease can also cause true and severe wilt symptoms, such as the sudden collapse of foliage on a single limb or branch, accompanied by a loss of turgor in the shoot (Kelsheimer and May, 1940). Leaves can show yellow blotches and plants can be stunted and partly defoliated (Van der Meer, 1926). Verticillium wilt cannot be identified by symptoms alone, because of confusion with Dutch elm disease. Both diseases cause similar vascular staining (Kelsheimer and May, 1940). Banfield (1941) described the vascular discoloration of both diseases as usually starting reddish brown, sometimes of low intensity, or faint steel-blue, becoming a conspicuous reddish to intense dark brown. Symptoms discernible with the aid of a microscope include yellowed vascular walls and gum-like substances, tyloses and mycelium in the vessels (Van der Meer, 1926).

The disease has been found mostly on young trees, although large trees are also affected (Kelsheimer and May, 1940). Different levels of resistance have been reported in elm species (Smalley and Lester, 1973; 1983). Holmes (1964) reported that *U. hollandica* clone 'Belgica' and *U. carpinifolia* 'Schuurhoek' were the most susceptible, whereas *U. hollandica* 'Groeneveld' was the most resistant to the disease. *U. hollandica* 'Commelin' layers, 'Commelin' grafts on 'Belgica' and clone '148' showed intermediate susceptibility.

4. TECHNICAL ASPECTS

4.1 Disease diagnosis and identification of the pathogen

A.J. Termorshuizen

The symptoms of verticillium wilt are described fully in chapters 2 and 3 and may suffice for disease diagnosis. However, it is often necessary to use additional criteria for confirmation.

Microsclerotia in dead or dying tissues are a good indication of *V. dahliae*, and can best be observed by teasing tissues apart or by clearing (Rijkers *et al.*, 1992). They are subglobose, black, and mostly 50-80 µm in diameter. Microsclerotia of *V. dahliae* are distinct from those of most other fungi. It is possible to extract microsclerotia from dead tissue and confirm *V. dahliae* by culturing them on suitable media (see section 4.4)

The most commonly used method of diagnosis is by isolation of the pathogen from diseased tissues which are still living. General methods for isolating fungi from plant tissue (Dhingra and Sinclair, 1995) may be applied equally well to *V. dahliae* and *V. albo-atrum*. In this, a shoot or leaf is surface sterilised, and small tissue pieces are excised aseptically from within and transferred to a culture medium such as potato dextrose or malt agar including 50 mg l^{-1} oxytetracyclin (streptomycin is not recommended) to suppress bacterial growth. The isolation of *V. dahliae* is usually much easier from woody material than from soft tissues and it may suffice only to wash the material in water, remove the bark aseptically and plate out small pieces of wood. Identification is usually possible after incubation at 20-23 °C for 7 days.

In pure culture *V. dahliae* can be identified by its white mycelium, verticillate conidiophores and microsclerotia (see chapter 1, figs 1 and 2). Microsclerotia produced in culture tend to be more elongate than those formed in plant tissues. Conidiophores of *V. albo-atrum* are larger than those of *V. dahliae* and have a darkly pigmented base; moreover *V. albo-atrum* produces dark resting mycelium and not microsclerotia.

The related fungus *V. tricorpus* might under certain circumstance be confused with *V. dahliae* and *V. albo-atrum* (see also section 4.3). It produces conidia on verticillate conidiophores and microsclerotia, but it also produces chlamydospores and a yellow pigment that diffuses into the culture medium. However, this species is only weakly or non-pathogenic and has not been reported from tree species.

It is important to base an identification on fresh isolates, since plant pathogenic *Verticillium* species may lose some of their distinctive characters in culture (see section 4.4).

4.2 Variability in *V. dahliae*

D.J. Barbara, E.J. Paplomatas and R.M. Jiménez-Díaz

Introduction

Apart from the existence of defoliating and non-defoliating strains in olive (see below), there is little information about variability in *V. dahliae* causing wilt diseases in trees. However, when considering *V. dahliae*-like isolates from the full range of hosts, it is evident that the species exhibits variability at several levels and that isolates can be resolved into groups on the basis of a number of criteria.

Variation in pathogenicity

There is much evidence for variation in pathogenicity in *V. dahliae* which may be quantitative or qualitative. Many isolates have broad host ranges but may vary in their ability to cause disease in specific hosts (Tjamos, 1981), isolates often being found more pathogenic to the host from which they were isolated than to other hosts (Sinclair *et al.*, 1987). The same was found to be true of isolates from cotton cultivars of different resistance (Ashworth, 1983). Japanese isolates were divisible into sweet pepper and tomato pathogenicity groups but these overlapped with a low pathogenicity group, able to infect only eggplant Horiuchi *et al.*, (1990). There is further evidence for host-adapted pathotypes within *V. dahliae*. For example, isolates from mint are strongly pathogenic to that host but, at most, only weakly pathogenic to other hosts (Green, 1951). This mint pathotype has a limited geographic distribution and probably a common origin (Okoli *et al.*, 1994). Isolates causing wilt in sweet pepper are also strongly host-adapted and of comparatively limited distribution (Kendrick and Middleton, 1959; Evans and McKeen, 1975). Such isolates may also be distinctive in other characters. There are reports of isolates from *Acer* being more pathogenic to this host than isolates from other hosts (Adams and Tattar, 1975; Hiemstra, 1995).

Within *V. dahliae* only one example of race structure based on a specific gene-for-gene interaction is known. The *Ve* gene (Schaible *et al.*, 1951) in tomato, which has been widely exploited in breeding wilt resistant cultivars, has been overcome by a so-called Race 2 which has appeared in North America (Grogan *et al.*, 1979), Australia (O' Brien and Hutton, 1981), South Africa (Ferreira *et al.*, 1990) and several european countries (Tjamos, 1980; Montorsi, 1986; Ligoxigakis and Vakalounakis, 1992). However, molecular evidence suggests that Canadian Race 2 isolates are heterogenous and that the ability to overcome the *Ve* gene has arisen independently several times (Dobinson *et al.*, 1998). Isolates attacking cotton occur world-wide, and in Russia have been divided into a number of races and biotypes (Kasyanenko, 1980; Gu *et al.*, 1988).

A defoliating pathotype, first recognised in cotton (Schnathorst and Mathre, 1966) has received particular attention because it represents a quantum increment in pathogenicity on this host. Such isolates have been recorded in Peru (Mathre *et al.*, 1966), China (Lu *et al.*, 1987), Spain (Blanco-López *et al.*, 1989), and Iran (Hamdollah-Zadeh, 1993) and the former USSR (Daayf *et al.*, 1995); but molecular studies indicate that Spanish isolates are clonal and distinct from those in at least some other countries (Pérez-Artés *et al.*, 1997), again indicating the independent evolution

of pathogenicity phenotypes. Moreover, the differential pathogenicity to cotton of cotton-defoliating isolates is exhibited in olive (Schnathorst and Sibbet, 1971; Rodriguez-Jurado *et al.*, 1993). Defoliating types may also be distinguishable from non-defoliating types by several morphological and physiological traits: the shape of microsclerotia, optimum temperature for growth, growth rate and fluorescence on sanguinarine-amended media (Presley, 1969; Bejarano-Alcázar *et al.*, 1996)

Most isolates of *V. dahliae* are unable to cause disease in cruciferous hosts but two important new wilt diseases of *Brassica* crops, on oil seed rape in Europe (Karapapa *et al.*, 1997) and on cauliflower in the USA (Subbarao *et al.*, 1995), are associated with strains with higher than normal nuclear DNA content. Similar isolates occur in a wider range of cruciferous hosts in Japan (Horiuchi *et al.*., 1990; Karapapa *et al.*, 1997).

Although *V. dahliae* is quite variable in culture, only rarely are morphological characters correlated with pathogenicity. For example, cotton-defoliating isolates from Spain have elongate microsclerotia and some crucifer infecting isolates have longer than normal conidia.

Vegetative compatibility

Vegetative compatibility has been widely used to examine populations of *V. dahliae*. Potentially, gene flow within the species is possible through parasexual interactions (Heale, 1988) and vegetative incompatibility may to serve to limit this and help maintain population differentiation and clonal lineages. Studies with nitrate non-utilising (*nit*) mutants have identified four main vegetative compatibility groups (VCGs) with some sub-groups (Joaquim and Rowe, 1990 and 1991; Strausbaugh *et al.*, 1992; Strausbaugh, 1993; Chen, 1994; Elena and Paplomatas, 1998).

Some VCGs are more widely distributed than others and some have been linked to other biological differences. Thus cotton-defoliating isolates (though not always isolated from cotton) belong to VCG 1 (Puhalla and Hummel, 1983; Joaquim and Rowe, 1990; Daayf *et al.*, 1995); and most isolates from solanaceous hosts belong to VCG 4 (Jeger *et al.*, 1996), whilst American isolates from woody hosts are mainly VCG 1 (Chen, 1994).

Cotton isolates from southern Spain and Israel fell into four groups (1, 2A, 2B and 4B). Isolates in 2A and 4B were all non-defoliating types from both countries, whereas isolates in VCG 1 were defoliating types and those in 2B were of a type that induced severe wilting and partial defoliation (Korolev *et al.*, 1998). Although the VCG groupings corresponded with the pathogenicity classification of isolates in this study, Bell (1994) found a wide range of virulence among isolates within each group with the exception of VCG 1 and cautioned about assuming virulence category from VCG.

Variation at molecular level

Molecular markers are also being used to examine relationships within *V. dahliae*. In general, these molecular markers are not of themselves biologically important, but may indicate discrete populations or clonal lineages within the species. Several different types of molecular markers have been used in studies of *V. dahliae*: RFLPs (Carder and Barbara, 1994; Okoli *et al.*, 1993 and 1994), RAPDs (Koike *et al.*, 1996, Karapapa *et al.*, 1997; Paplomatas and Lampropoulos, 1997) and ITS sequencing (Robb *et al.*, 1993; Morton *et al.*, 1995). Overall, these studies have shown that

molecular variation is readily detectable in *V. dahliae* and may resolve into more or less discrete groups; and that, in all the countries where studies have been conducted, only a limited range of the total molecular variability known is present. Molecular groups may be correlated with other characters, such as the tomato/sweet pepper pathogenicity groups in Japan (Horiuchi *et al.*, 1990), although in molecular terms these groups overlap with the low pathogenicity eggplant group. The presence of only a limited sub-set of the total molecular variation in each of the countries so far studied implies that *V. dahliae* has spread to many parts of the world from a centre of diversity of *V. dahliae* which has not yet been identified.

At the molecular/genetic level the clearest differentiation within *V. dahliae* is between those isolates mentioned above which are generally (but not exclusively) pathogens of cruciferous hosts and the majority of other isolates. Most of these crucifer isolates have a nuclear DNA content roughly twice that of normal (haploid) isolates but may have either normal sized or long conidia (Subbarao *et al.*, 1995; Karapapa *et al.*, 1997). Although they produce microsclerotia and are thus identified as *V. dahliae,* at least some of these isolates seem to be hybrids between *V. dahliae* and *V. albo-atrum* (Karapapa *et al.*, 1997). High DNA content isolates do not appear to have been isolated from trees as yet but this may only reflect the fact that the DNA content of most isolates is not measured.

4.3 Quantification of *Verticillium dahliae* in soil

A.J. Termorshuizen

Determining the amount of viable *V. dahliae* (in the form of microsclerotia) in soil is important in disease risk assessment of possible planting sites and for evaluating the performance of soil disinfestation procedures (see section 4.6).

Most of the methods of inoculum quantification available depend on applying soil evenly to a semi-selective solid medium and counting colonies which develop in the agar after a period of incubation. Soil is applied as a suspension in water: 'wet plating' or WP (Nadakavukaren and Horner, 1959; Menzies and Griebel, 1967); or dry: 'dry plating' or DP (Harrison and Livingston, 1966; Butterfield and DeVay, 1977). WP may include wet-sieving as a preliminary stage to remove particles smaller (e.g. <20 μm) and larger (e.g. >100 μm) than microsclerotia. DP involves the use of the Andersen sampler (Andersen, 1958; Buxton and Kendrick, 1962) to distribute the soil particles evenly over the agar medium (Butterfield and DeVay, 1977). For both methods the soil is dried prior to analysis to kill conidia and mycelium of *V. dahliae*, to reduce inoculum of other fungi, and to facilitate grinding and mixing of the soil sample. On the basis of a recent comparison of different methods by 13 research groups (Termorshuizen *et al.*, 1998) it was concluded that dry plating soil samples leads to a higher recovery of *V. dahliae* than wet plating. However, the considerable variation observed among laboratories carrying out DP methods indicates that further standardisation is necessary.

Identification of *V. dahliae* in soil plates requires some skill. Colonies can be recognized because new microsclerotia are formed in the agar. However, the appearance of microsclerotia in soil plates is different from that in pure culture and depends on the medium used (Goud and Termorshuizen, 1997). On modified soil extract agar (Harris *et al.*, 1993), colonies of *V. dahliae* can be identified by the presence of subglobose to elongate, black microsclerotia (18-41 x 9-17 μm), which are produced on single, hyaline hyphae. The main source of possible confusion is the related species *V. tricorpus* which may be common in some soils and which produces dark microsclerotia superficially similar to those of *V. dahliae*. However, in comparison with *V. dahliae*, the microsclerotia of *V. tricorpus* are larger, more irregularly shaped, and several hyphae, pigmented at the point of attachment, radiate outwards into the agar (Davis and McDole, 1979). Typically, the new microsclerotia of both species are formed in the agar and not on its surface.

V. dahliae may not be uniformly distributed within fields (Smith and Rowe, 1984; Wheeler *et al.*, 1994; Bejarano-Alcázar and Jiménez-Díaz, 1997) and the optimal sampling method should take any unevenness of distribution into account. In the absence of prior knowledge of the spatial pattern, the mostly widely adopted sampling method is to take multiple subsamples in a w- or double-w shaped pattern from the uppermost 20 cm of soil. These are then bulked to make up one sample. However, it is probably wise not to bulk subsamples from an area in excess of two hectares (Harris *et al.*, 1991). It is also important to take account of cropping history. For example, if one part of the field has a history of potatoes and another part has a history of grain, the two areas should be sampled separately (Harris *et al.*, 1991).

4.4 Manipulation of *V. dahliae*

D.C. Harris

A general account of methods for *V. dahliae* and *V. albo-atrum* is given by Melouk (1992).

Culturing

V. dahliae will grow on a range of standard fungal media such as potato dextrose and malt extract and on mineral media such as Czapek's Dox. Talboys (1960) recommended a prune extract medium for culturing which was particularly useful for distinguishing *V. dahliae* and *V. albo-atrum*. The optimum incubation temperature is about 23°C.

V. dahliae is subject to spontaneous degradation in artificial culture. The most obvious manifestation of this is a reduction or complete loss of the ability to produce microsclerotia and is first seen as the formation of hyaline or semi-hyaline sectors in growing cultures. In prolonged culture isolates may become completely hyaline. The ability to produce conidia may also be reduced or lost. Cultural degradation may also result in the reduction or loss of pathogenicity, which may or may not be correlated with morphological changes in the fungus. All these changes are irreversible. For these reasons care is needed in maintaining isolates of the pathogen.

Maintaining isolates

It is important not to keep growing cultures for more than two weeks before subculturing because there is an increasing incidence of sectoring when using cultures of greater age (Heale and Isaac, 1965). Several storage methods are recommended for maintaining isolates unchanged in culture (see Smith and Onions, 1983). Cultures may be refrigerated for several months without obvious changes but this should only be used for short-term storage. It is also possible to store dried agar cultures for several years as long as microsclerotia are present. Other techniques used for long-term storage include: maintenance of small cubes of young agar culture in sterile distilled water at room temperature in airtight containers (Boeswinkel, 1954); refrigerated storage of 2-3 weeks' growth from conidia cultured on sterilised soil (Joaquim and Rowe, 1990); and cryostorage of conidia suspended in 10% glycerol solution either in glass vials at about – 80 °C or in liquid nitrogen using the straw method of Elliott (1976).

Preparation of inocula for experimentation

Conidial suspensions are commonly used in inoculation experiments and for other purposes. To produce standardised and consistent inoculum it is important to use young conidia, that is, from cultures up to 7 days old. Conidia may be produced in liquid (Hiemstra, 1995; Resende *et al.*, 1995) or on solid media (Chambers and Harris,1997). In liquid culture, conidia are separated from mycelium by filtration and then may be washed and resuspended by centrifugation or the suspension may be used directly. Conidia are harvested from plate cultures after flooding with water by using a glass rod; mycelial fragments and other material are removed from the suspension by filtration. Conidia of similar age may be produced in large quantity by these methods.

Numbers of conidia in suspensions may be determined by means of a bacterial counter slide. Conidia of *V. dahliae* are small, weakly refractile and are difficult to see on a conventional slide with a 0.2mm deep counting surface. Rapid standardisation when working with numerous suspensions can be achieved by using a prepared calibration curve of conidial concentration and light absorbance in an absorptiometer.

Microsclerotia may be required for use as inoculum or for studies in their own right. They can be produced in culture (Francl *et al.*, 1988; Hawke and Lazarovits, 1994) or, for some purposes, it may be better to obtain those naturally produced in diseased plants. Microsclerotia are readily produced on plant material (such as straw) in culture and should be allowed four or more weeks to mature. They can be detached from the material by gentle blending or shaking, and separated and concentrated by repeated washing and sedimentation. Microsclerotia can be separated into the desired size range or ranges by using sieves of appropriate pore size. Survival and germination rates are related to size (Hawke and Lazarovits, 1994). It is generally more difficult to separate microsclerotia embedded in culture medium or plant material. However, microsclerotia produced in 0.25% agar cultures are relatively easy to separate and purify (Hawke and Lazarovits, 1994). Similar methods can be used to obtain microsclerotia from diseased plant material such as potato haulms or linseed stems. For pure culture studies it may be necessary to surface-disinfect microsclerotia. Very dilute solutions of mercuric chloride are effective and cause no loss of viability; sodium hypochlorite (or domestic bleach) is a safer material to handle but its use may result in some loss of viable microsclerotia. Microsclerotia can be maintained as a homogeneous suspension more effectively if suspended in dilute agar solution (*c.* 0.1%) than in water. They may be stored in the refrigerator using sterilised soil or sand as a storage medium and 'carrier' (Francl *et al.*, 1988). Dry microsclerotia can be stored for a year or more with no loss of viability at a constant temperature of 24, 4, -25 or -70°C (Hawke and Lazarovits, 1994).

Several investigators have used blended agar cultures as inoculum to add to growing medium for challenging plants with *V. dahliae* (see section 4.5).

4.5 Selection and screening for host resistance

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Introduction

The use of host resistance is the most effective and ecologically sound method for managing plant disease (see section 4.6). The main techniques used for detection and evaluation of host resistance are summarized here. When screening for resistance it is important to distinguish between resistance and tolerance (Schafer, 1971). If a plant is resistant, infection and colonization by the pathogen are restricted. It is not an absolute condition, but there is usually a range of responses from complete resistance or immunity, where no infection takes place, to high susceptibility where the host is readily colonised and the colonisation results in serious damage or death. Tolerance is the capacity of a plant to become colonised by the pathogen without sustaining severe losses in yield or quality. In the absence of resistance, tolerance may provide a useful level of disease control and may be an important target in breeding. For developing new rootstocks, however, only high levels of resistance are acceptable because the pathogen may grow through the rootstock, ultimately causing serious disease on a susceptible scion cultivar.

Methods

The objective may be to select the most resistant individuals from a population or to compare the incidence of resistant individuals in one or more populations. In these cases each plant tested is unique. Alternatively, the object may be to evaluate the resistance of a particular genotype by comparison with other genotypes of known resistance. Replication is required for the latter. In both cases the methods are essentially the same: plants are challenged with an appropriate inoculum and the degree of symptom development is used to assess resistance. Plants can be challenged by planting in soil which is naturally or artificially infested by the fungus, allowing infection to occur naturally. However, in order to minimize the number of escapes, plants are usually inoculated.

Inoculation can be done in several ways. The most common methods are of two types: dipping the roots into the inoculum (root dip: RD) or inserting the inoculum into the vascular system of the stem (stem inoculation: SI). Both methods have many variants such as spraying the inoculum over the root ball before planting (RB) or making holes in the stem (SI) and applying the inoculum to it (table 3). The response of plants is usually determined against a range of disease criteria: degree of stunting, leaf symptoms, dieback, death of plants, and recovery after initial symptom development are all aspects that need to be considered. Resistance can also be evaluated in other ways such as the amount of pathogen in the plant and the extent of invasion and colonization (by determining the percentage of tracheary elements colonised, the numbers of conidia in the sap or the extent of fungus distribution in the plant), but such methods are very labour intensive.

Table 3. Techniques used for screening for resistance to *Verticillium dahliae* in trees.

Host	Inoculation method[a]	Inoculum type[b]	Inoculum density[c]	Plant age	Soil/ compost[d]	First symptoms	Reference
Acer platanoides	RD	-	-	4 yr	-	-	Wittberg, 1983
	RD	C	10^6	4-6 wk	-	3 wk	Hiemstra,1997
	SC	C	10^6	2 yr		3-4 wk	Hiemstra,1997
	SD, RD	C, C+M+MS	10^4 -10^6	38 d	I, NI	1 mo.	Chambers & Harris, 1997
Acer spp.	SI, RB	C+MS	-	2, 4-7 yr	NI	-	Sinclair, *et al.*, 1981
Avocado	RD, SI	C, C+M	-	-	C	12 d	Zentmyer, 1949
	RD	C	-	-	I	1 mo.	Zentmeyer *et al.*, 1955
Cocoa	SD, SI	C	10^4-10^8	135 d	NI	2 wk	Resende *et al.*, 1995
	RD	C	10^6, $5x10^6$	2-25 wk	NI	-	Emechebe, 1975a
Fraxinus excelsior	SC	C, M+MS	10^6, -	3	-	3, 5 wk	Hiemstra, 1995
Olive	RD	C+M+MS	$6x10^4$	8 mo.	C	25 d	Cirulli & Montemurro, 1976
	RD	C	10^7	9 mo.	C	24 d	Rodriguez-Jurado *et al.*, 1993
	RB	C	10^7	1 yr	-	1 mo.	Schnathorst & Sibbet, 1971
	RB, SC	C	-	1 yr	I	-	Wilhelm and Taylor, 1965
Pistachio	RD	C	-	seedling	-	3-4 wk	Raabe & Wilhelm, 1978

[a] RB: Root ball spray; RD: Root dip; SC: Stem cut; SD: Soil drench; SI: Stem injection
[b] C: Conidial suspension; M: Mycelium; MS: Microsclerotia
[c] conidia/ml
[d] (Soil or Substrate) I: Infested; NI: non infested; S: Sterilised
- information not available

Young plants (one to four years old) are usually used for screening material in the field. Soils may be naturally infested through prior cultivation of crops susceptible to *V. dahliae*, such as potato, tomato or linseed. Sometimes the plants are intercropped with herbaceous hosts to maintain or increase the inoculum potential of soil (Wilhelm *et al.*, 1965). Plants may be given a double challenge by inoculation before or after planting in infested fields (Wilhelm and Thomas, 1950; Wilhelm and Taylor. 1965; Sinclair *et al.*, 1981). In field experiments, the sources of variability are considerable, including uneven distribution of inoculum in soil and variation of inoculum potential in soil as a result of mixtures of isolates differing in pathogenicity.

Plants in pots may be tested under controlled conditions. When comparing the resistance of cultivars it is convenient to include in each experiment cultivars with known resistance or susceptibility. The conditions must be standardised in order to give reproducible results and should simulate natural conditions favouring disease development as far as is possible (see below).

Factors influencing the response of plants challenged with *V. dahliae*

Seedling inoculation has often been used to screen for host resistance in tree hosts since large plant populations can be handled in a minimum amount of space and time. However, plant age may play an important role in many wilt diseases. In cocoa and olive for example, the use of very young plants can hinder successful and consistent infection and disease development of *V. dahliae* (Emechebe, 1975a; Rodríguez-Jurado, 1993; Resende *et al.*, 1995).

Inoculum potential is another factor that needs to be taken into account. In elm the effect of inoculum density of *V. dahliae* on disease reaction was significant and different dose-response curves were found for different elm species (Rauscher *et al.*, 1974). Many authors use a high spore concentration in inoculum (up to 10^7 conidia/ml) to prevent disease escape. Types of *V. dahliae* differentially pathogenic to different hosts and genotypes are known to exist (Alexander, 1962; Schnathorst, 1969; Tjamos, 1981; Blanco-López, *et al.*, 1989) (see section 4.2). It is therefore important to include isolates representing an appropriate range of virulence in all programmes for evaluating host resistance to *V. dahliae*. In olive for example, evaluation for resistance should include defoliating and non-defoliating pathotypes of *V. dahliae* (Rodríguez-Jurado *et al.*, 1993) (see section 3.1).

When plants are screened for resistance, the environment should simulate conditions which are conducive to wilt because verticillium wilts are strongly influenced by environmental conditions. In fact, the disease may develop faster under controlled conditions than under natural conditions because ideal conditions for wilt development can be maintained continuously.

Verticillium wilt is especially influenced by temperature. In cotton, high temperatures caused plant recovery of diseased plants and curtailed infection of healthy plants (Bell and Presley, 1969). Moreover, sub-optimal conditions may delay symptom development. Thus, Ruggieri (1948) found that olives artificially inoculated with *V. dahliae* in autumn and incubated under natural conditions developed dark-brown vascular discoloration two and a half months after inoculation, indicating that infection had taken place, but did not show external symptoms until the following spring and summer. Similarly, Vigouroux (1975) found that it took two years for olive trees transplanted into artificially infested field soil to develop symptoms of verticillium wilt.

Soil nutrients can also influence wilt and may modify the results of screening for resistance. In many hosts, resistance to verticillium wilt is positively related to K availability and negatively related to N availability. The effect of P usually depends on the N and K levels (Dwinell and Sinclair, 1965). In American elm (*Ulmus americana*) and sugar maple (*Acer sacharum*), the addition of P did not directly affect susceptibility, but K alone or added to any other treatment reduced susceptibility of both host species (Dwinell and Sinclair, 1965). These nutrient effects may explain the influence of compost in screening *Acer platanoides* seedlings for resistance to *V. dahliae* (Chambers and Harris, 1997).

It is clearly of the utmost importance that genotypes are thoroughly tested under natural disease conditions in the field before their resistance can be fully evaluated.

4.6 Management of disease

E.C. Tjamos and R.M. Jiménez-Díaz

Verticillium wilts are among the most difficult plant diseases to manage. Factors contributing to this difficulty include: the ability of the pathogen to survive for a long period in soil; the wide host range of the pathogen; and the inaccessibility of the pathogen to fungicides during its parasitic phase in the xylem. The prolonged period of exposure to infection because of the host's long life is an additional problem for controlling the disease in trees. Consequently, the best way of dealing with verticillium wilt in trees is through implementation of an integrated disease management strategy, involving the application of control measures before and after planting. This section provides general information on possible elements of such a strategy. More details for individual tree species may be found in chapter 3.

CONTROL MEASURES BEFORE PLANTING

Choice of proper planting site.
This aims to avoid using soils with inoculum levels of *V. dahliae* in excess of potentially damaging thresholds. Thresholds will vary with the susceptibility of the crop and with the pathogenicity of the prevailing pathotype(s), a point of particular importance in relation to the defoliating strain (see sections 3.1 and 4. 2). Thus, only 6-8 microsclerotia/g soil of the defoliating strain can cause nearly 100% incidence of wilt in the susceptible cotton cv Coker 310, whilst approximately 35 microsclerotia/g of the non-defoliating pathotype were required to cause similar levels of disease in this cultivar (Bejarano-Alcazar *et al.*, 1995). Information about previous crop rotations at planting sites available may give some indication of wilt risk but the only certain method of site selection is by quantification of the pathogen in soil samples (see section 4.3) as it practised for strawberry growing in the U.K. (Locke and Harris, 1993) and for tree nursery crops in the Netherlands (Van Zaayen, 1995).

Disinfestation of soil.
Infested soils can be restored for establishing healthy tree plantings if inoculum is eradicated or significantly reduced. This can be achieved by physical methods (soil solarization, steaming) or chemical treatments (chloropicrin, formalin, metham-Na, methyl bromide). Both types of methods can be applied over large areas or in strips or at individual planting sites. However, these chemicals are not permitted in all countries and their use is generally under threat from environmental and consumer pressures.

Soil solarization is a hydrothermic process that develops in moist soil tightly covered with transparent polyethylene sheets (usually 50-100 μm thick) during periods of high solar radiation. Soil has to be moist for maximal effect. As a result of this treatment the numbers of viable microsclerotia in soil are greatly reduced. Solarization also has a beneficial effect on heat tolerant antagonists of *V. dahliae* such

as *Talaromyces flavus* and *Aspergillus terreus* (Tjamos, 1993). Clearly, this treatment is only possible in areas with a suitable climate.

Both inoculum of *V. dahliae* in soil and its potential to cause disease can also be significantly reduced by means of green amendments like brassica residues (Xiao *et al.*, 1998) or sudangrass (Davis *et al.*, 1990 and 1997). The effect of brassicas is thought to be mainly due to fungicidal volatiles released from the hydrolysis of glucosinolates in the tissues, a process which has been called 'biofumigation' (Angus *et al.*, 1994). The disease suppressiveness induced by sudangrass was associated with significant increases in populations of *Fusarium* spp. (Davis *et al.*, 1997). However, the consistency of this effect is influenced by soil type and microflora (Parks and Powelson, 1997). An alternative approach is the enforced anaerobic decomposition of fresh plant residues which has destructive effects on a range of soilborne pathogens including *V. dahliae* (Termorshuizen *et al.*, 1997).

Use of *V. dahliae*-free planting material

Because of the possible presence of *V. dahliae* in symptomless planting material (see chapter 3), great care should be exercised to use only healthy material for propagation and to use disease-free plants for new plantations. This is doubly important when plantings are being established in areas free of *V. dahliae*.

Use of resistant cultivars or rootstocks

In the long run, the exploitation of host resistance must be considered as the most practical and economical measure for controlling verticillium wilt in tree species. For crops in which cultivars are grafted onto a rootstock the use of resistant rootstocks may be sufficient to prevent verticillium wilt. However, the rootstock in such cases should be completely resistant because, otherwise, *V. dahliae* may grow through it and cause wilt in the scion. If resistance is not available, tolerant cultivars (see section 4.5) may be used to limit production losses. However, the use of tolerant cultivars may lead to an increase in the amount of the pathogen in soil. Possible sources of resistance in the main tree species are described in chapter 3. Methods for detecting and evaluating host resistance are described in section 4.5.

CONTROL MEASURES AFTER PLANTING

Cultural practices

The importance of several cultural practices in the development of verticillium wilt in trees has been discussed in chapter 3. This leads to the following recommendations for disease management: avoid intercropping with plants susceptible to *V. dahliae*; use chemical weed control and avoid or minimise cultivation practices that damage roots; avoid using vehicles or machinery that have been used in *V. dahliae* infested fields; use drip irrigation instead of furrow or flooding irrigation to reduce dissemination the pathogen; remove infected shoots and branches as soon as possible, preferably before defoliation occurs, and destroy diseased material to prevent newly produced microsclerotia being incorporated into the soil.

Apply soil solarization to affected trees in irrigated orchards

The benefit of soil solarization in aiding recovery from and preventing new infections by wilt has been demonstrated for several tree crops (see chapter 3). Soil solarization is employed when solar radiation is maximal. Prior to solarization, weeds should be destroyed, trees should be pruned to remove diseased branches and to allow better irradiation of soil. Soil around trees should be moistened to saturation level (300-400 liter water/tree). Then, a soil area equal to or slightly larger than the dimensions of the tree canopy is covered by polyethylene sheets. Tight anchoring of the plastic is important.

In spite of considerable effort by agrochemical companies and many scientific investigations, no fungicide has been identified which gives satisfactory economic control of verticillium wilt in tree species, either as a protectant or as a cure. Because of the systemic nature of the pathogen, a similarly systemic fungicide is essential for effective control (Talboys, 1984). The closest that has been attained is carbendazim and the carbendazim-generating bezimidazoles and thiophanates. Some success has been achieved against wilt in strawberry (Talboys *et al.*, 1976) and other herbaceous crops (Talboys, 1984) but repeated doses in large volumes of water are necessary for control. Studies with woody species have shown that they are generally ineffective regardless of the method of application (see section 3.1).

5. PROSPECTS AND FUTURE RESEARCH NEEDS

J.A. Hiemstra and D.C. Harris

Verticillium wilt has long been a serious problem in the cultivation of a wide range of economically important herbaceous and woody plants in many parts of the world. An unfortunate consequence of some recent developments within european agriculture has been an increase in wilt of tree species. A policy of afforestation of areas with a history of agricultural or horticultural crops has exposed susceptible tree hosts to wilt on an unprecedented scale (section 3.5). A great expansion in the nursery stock industry, also largely into former agricultural land, has had similar effects. Moreover, the increased movement of planting stock between countries as a result of greater market integration within Europe has heightened the risks of disseminating *V.dahliae*. This represents a significant hazard to the health of all crops susceptible to wilt, but is of particular importance in relation to the possible spread of highly virulent strains of the pathogen, such as the cotton-defoliating strain to which olive is very susceptible (section 3.1 and 4.2). The pressures driving these changes will undoubtedly continue so that an increase of verticillium wilt problems in trees is to be expected.

As there is no single effective method of control, dealing with verticillium wilts requires an integrated approach, involving the application of measures before and after planting (section 4.6). In order to be able to develop effective management strategies, several underpinning areas of research must be developed.

The appearance of a strain of *V.dahliae* in Spain and of similar strains elsewhere with enhanced pathogenicity to cotton and olive illustrates the inherent variability within the species and its apparent ability to produce new and more dangerous variants. When viewed globally, it is evident that there is considerable variability in *V.dahliae* and there is a risk of introducing important pathogenicity genes to countries where they are not yet present. The information currently available on variability within *V.dahliae* and its significance in terms of pathogen populations is at best fragmentary and a major research effort is required in this area.

The application of molecular methods to understanding variability in *V.dahliae* has already brought some important insights and demonstrates the power of these tools for shedding light on relationships (section 4.2). Vegetative compatibility analysis is another important approach to understanding relationships and evolution in *V.dahliae*. Little is known about the range and distribution of VCGs in Europe and only preliminary information is available on their relation to American VCGs. A full understanding of relationships in *V.dahliae* is fundamental to all aspects of verticillium wilts: the potential for new, more pathogenic strains to arise; the risks of disseminating potentially dangerous strains; the range of pathogenicity genes required to challenge plant genotypes when breeding and screening for resistance; and the assessment of risk when planting susceptible crops in *V.dahliae* infested land.

Infected planting material is clearly a major source of disease for many tree crops and yet there are few systems of control in place. The implementation of healthy planting material schemes for valuable tree species would bring considerable benefits, and the development of improved methods of screening plant material for *V.dahliae* is important to the success of such schemes. DNA-based techniques undoubtedly have much to offer as means of developing such methods. This technology could also be

applied to developing rapid and sensitive methods for detecting and quantifying *V.dahliae* in soil to assess the wilt risk of possible planting sites. However, basic studies to develop suitable probes and primers are essential in order to exploit this approach. Meanwhile, much can and should be done to improve the sensitivity and reliability of existing methods which have already proved useful management tools for verticillium wilt in herbaceous and woody crops (section 4.3).

The reduction of inoculum in soil prior to planting has been a main, and often the only means of controlling wilt in some crops. Although trees are exposed over a long period and reinfestation of land is a common hazard, pre-planting soil disinfestation remains important for high value crops. However, the standard means of achieving this through the use of biocidal chemicals like methyl bromide is becoming unacceptable, and more environmentally sound methods need to be devised. In areas with sufficient sunshine, solarization is employed very successfully for this purpose. In other areas other approaches will have to be adopted. Biological disinfestation methods like 'biofumigation' with brassicas and through anoxic decomposition of fresh plant residues evidently have considerable potential but need to be thoroughly investigated.

The single most important and valuable control method is to use resistant (or tolerant) cultivars or rootstocks, and this is already being exploited in a number of crops (see chapter 3). It is apparent that for many tree crops there are as yet untapped resistance resources available for exploitation. Currently, the exploitation of resistance is restricted to that already available in existing genotypes: for all too few woody species are there programmes of breeding for wilt resistance. A general problem in tree breeding is the long generation time, but there are a number of new technologies available to help bypass this difficulty. The development of genetic markers for resistance followed by marker-assisted selection would greatly speed the development of wilt-resistant genotypes. Genetic transformation is another possible approach to developing resistant cultivars, using either homologous genes for resistance if they have been identified, or heterologous genes capable of conferring resistance, potentially from a variety of sources. Understanding the genetic control of resistance is inextricably linked to understanding the mechanisms of resistance, which is therefore another important area for study.

One of the most intriguing features of verticillium wilt in trees is recovery. This is another aspect of resistance and is poorly understood. A greater understanding of this phenomenon could lead to developing techniques for stimulating recovery as a component of better management procedures.

Because of the perennial nature of the host, wilt in trees may need to be controlled over a long period. Apart from employing resistance, a number of ways have been and are being developed to provide long-term protection. *In situ* solarization has been successful in reducing wilt, and is now being developed in conjunction with local application of biocontrol agents. The use of biocontrol agents offers considerable promise in the management of chronic wilt problems in high value tree crops such as olive and stone fruits. Various organisms including, fluorescent pseudomonads, *Bacillus spp,* mycorrhizal and other fungi such as *Talaromyces flavus* have shown potential as biocontrol agents in laboratory or greenhouse experiments. The need now is to provide a knowledge base for developing effective ways of applying these organisms in the field and management practices that maximise their effectiveness. The possible exploitation of endophytic bacteria as biocontrol agents is

also an important area for investigation. The potential of antagonistic compost material developed from disposal of organic waste, alone or in conjunction with biocontrol agents is another important opportunity which needs to be researched.

The challenge therefore is to design integrated systems for managing verticillium wilt under different circumstances and in different host plants. There are clearly a number of areas in which greater understanding of *V. dahliae* and of the interactions between the pathogen and its tree hosts is necessary in order to develop the most effective, most economic and most environmentally acceptable systems of disease management.

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