

## SOIL-BORNE DISEASES

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There are three soil-borne diseases known to afflict pistachio trees in California. All are caused by fungi and occur in most pistachio-growing regions of the state. Of these, *Verticillium* wilt has killed many trees and as a result growers have suffered severe economic losses in early planted orchards with susceptible rootstocks. Pistachios are relatively resistant to crown gall, a serious bacterial disease of other perennial crops; crown gall has not been reported on pistachio. The relative importance of soil-borne diseases varies with changes in choice of rootstocks. As new rootstocks are developed and introduced into pistachio culture, the array of root diseases may differ.

### VERTICILLIUM WILT

Historically, the most destructive disease of pistachio in California is *Verticillium* wilt, caused by the fungus *Verticillium dahliae* Kleb. Trees of any age are subject to attack and hundreds die each year (Plate 26A). *Verticillium* also attacks cotton, tomato, melon, stone fruit, and olive in addition to pistachio in the southern San Joaquin Valley. Non-cultivated plants, both indigenous and weed species, also serve as hosts, and these enable the pathogen to become established in non-cultivated land. *Verticillium* wilt of pistachio is more common in the southern half of the San Joaquin Valley than in other areas of the state, and the disease has been most destructive where pistachio trees were grown in fields previously planted to other susceptible crops. The disease occasionally is found in the Sacramento Valley.

### Symptoms

The first symptoms of *Verticillium* wilt are interveinal patches of yellowing or scorching of the leaves on affected branches. Collapse of

the branch or tree ensues with the first warm weather because the pathogen causes blockage of vital water-conducting tissues, and the tree demands more water than the root system can provide. The classic symptom of *Verticillium* wilt is rapid desiccation and death of one or more scaffolds or the entire tree, usually in late spring or early summer.

In another manifestation of *Verticillium* wilt, called "thin leaf decline," symptoms develop slowly over several years before the tree becomes economically unproductive or succumbs to the disease. Thin leaf decline is characterized by slow loss of vigor and reduction in growth and yield. A gradual thinning of the canopy until most of the remaining leaves are clustered in tufts at the ends of branches and shoots may also be seen (Plate 26B). Why *Verticillium* causes some trees to collapse suddenly and others to decline slowly has not been explained. An overall lack of vigor may be the most common symptom of *Verticillium* wilt.

*V. dahliae* invades and colonizes the plant's vascular system. The water-conducting tissue, the xylem, is composed of small vertical tubes that together form a cylinder just inside the cambium. Only the young xylem, that which is formed in the current year, is able to transport water. Xylem elements infected by *Verticillium* become clogged by substances elaborated by the fungus and the tree. These substances are darkly pigmented, and a cross-section of an infected root, trunk, or branch reveals the pattern of *Verticillium* infection in the new xylem: scattered black speckles or a more or less discontinuous black ring, depending upon how many vessels are infected (Plate 26C). The uniform discoloration of the innermost wood is a consequence of the natural aging process. The discolored xylem elements are non-functional and cannot transport water to

the upper portions of the tree. The branches that die are those that are connected to the infected root and vascular system and no longer receive adequate water. The history of *Verticillium* infection in older trees is recorded in the necrosis of each year's ring of xylem (Plate 26D). *Verticillium* wilt does not cause rot of bark or wood and does not invade through the above-ground parts of the plant.

### Disease cycle

*Verticillium* has only limited saprophytic activity in soil, so it does not readily grow and reproduce there. Instead, it either lives on the roots of alternate hosts or it survives in soil in the form of resting structures called microsclerotia. Each microsclerotium is a tiny cluster of approximately 30 dark-colored cells that are extremely hardy. A percentage of the microsclerotia can remain viable in soil for many years. In a soil that was previously planted with an alternate host of *Verticillium* such as cotton or a solanaceous crop, several hundred microsclerotia can be present in a cubic inch of soil. Most microsclerotia are found in the upper six inches of soil where there is the most biomass from a previous host.

The microsclerotium is also the infective unit of the fungus. It remains dormant in soil until a plant root grows nearby releasing substances (nutrients) which stimulate it to germinate. These substances can be produced by the roots of both host and non-host plants, but only young or wounded roots produce sufficient quantities of nutrients to cause the microsclerotium to germinate. Many infections remain in the outer, cortical tissues of roots and never invade the xylem. However, entry occurs just behind the root tip where the young cells are undifferentiated, and the fungus may grow into the new xylem. Inside the xylem element, the fungus produces mycelium and conidia (spores). The conidia are carried upward with water. As conidia become lodged in xylem cells, they germinate, more mycelium is formed which produces more conidia that again move upward with the water flow. In this manner the fungus spreads from root to shoot, plugging the xylem vessel along the way. Neighboring xylem vessels apparently are not easily infected. Thus, many independent

infections may be needed to involve a large portion of the water-conducting system.

Infections located away from the root tip usually follow another course of events. The fungus invades a few cortical cells, outside the vascular tissues, and there forms a new microsclerotium which later is returned to the soil. This 'refreshing' of the microsclerotial population occurs with non-host as well as host plants and helps the fungus to persist, especially in the absence of susceptible species. A few such cortical colonies later establish xylem infections which then proceed as described above.

### Inoculum production

Production of microsclerotia in infected plant parts has been studied in annual crops such as cotton and tomatoes but not in tree crops. In annuals, many thousands of microsclerotia are formed in leaves, stems and roots as the plant withers and dies. When the dead plant is incorporated into the soil, the tissues decay, freeing the microsclerotia. It is not known whether the incorporation of diseased pistachio leaves and branches into the soil contribute to the inoculum in the soil. Microsclerotia are formed in infected pistachio roots, which may contribute to the inoculum level in the soil. However, in a pistachio field trial in California in *Verticillium*-infested soil, and a relatively weed-free planting, the number of microsclerotia per gram of soil dropped from 40 in the first year to four by the fifth year and stayed at two from the eighth to tenth years. Thus, although a pistachio planting may maintain a relatively low population of microsclerotia, pistachios in a weed-free field do not support a high inoculum concentration.

### Conditions that affect disease

*Verticillium* wilt is favored by cool temperatures. Extended spring weather and mild summers often are accompanied by severe losses to this disease. The fungus apparently is eliminated from above-ground portions of trees during hot summer weather. Survival in root tissue and reinvasion of the upper stock is possible but has not been demonstrated. Instead, repeated attacks of wilt apparently represent new infections each year.

The disease is more prevalent in wet soils, presumably partly because they are cooler than dry soils, and irrigation influences the incidence of *Verticillium* wilt in some annual crops. In pistachio, the disease increased more down than across rows in an experiment in a drip irrigated orchard. Roots were more concentrated down rows and between trees than in the aisle between rows. This suggests that the denser root system down rows improved the chances for infection. Also, inoculum build-up would increase where more infected roots are present. An irrigation system that would discourage root growth in this area might alleviate damage from the disease. In a recent experiment, drip irrigation systems buried at different depths fostered changes in root density. More roots developed deeper in the soil, but no decrease in the microsclerotial population or incidence of *Verticillium* was measured.

Plant vigor affects susceptibility to *Verticillium* wilt; stressed trees are more vulnerable than non-stressed trees. Inasmuch as the nutritional status contributes to the overall health of the tree, nutrition may affect susceptibility. However, there is no evidence that nutrient imbalance enhances disease development or that improvement in nutritional status of trees prevents or alleviates infection by *Verticillium*.

The population of *V. dahliae* is genetically diverse. There are many differences in the pathogenicity and virulence of isolates of the fungus, and several strains and races have been identified. To date, isolates cultured from pistachios are also pathogenic to cotton and can be further separated according to their effects on tomato. The significance of this grouping is unclear, but no strong pattern of 'wilt' and 'decline' isolates has emerged. Nonetheless, variability within the fungus population is an important factor in breeding and screening plant material for resistance.

The nature of resistance to *Verticillium* wilt by pistachio is not well understood. The fungus is able to invade roots of both susceptible and resistant plants, but once inside the xylem, movement of the fungus up the vascular tissue is slower in resistant than in susceptible plants.

## Control

The best defense against *Verticillium* wilt is the use of the resistant or tolerant rootstocks, Pioneer Gold (*P. integerrima*) PG I or UCB I (a *P. atlantica* x *P. integerrima* hybrid). UCB I is a relatively new rootstock that may exhibit slightly less tolerance to *Verticillium* wilt when compared to PG I, but showed more frost and salt tolerance. *P. atlantica* and *P. terebinthus* rootstocks are very susceptible and should be avoided where *Verticillium* is present. Another pistachio hybrid, PG II (*P. integerrima* x *P. atlantica*), is extremely susceptible to *Verticillium*.

Criteria for site selection for an orchard should include a crop history and an assessment of the inoculum level in the soil. The microsclerotial population of the soil can be determined, and this service is available through private laboratories. Pre-plant or planting site fumigation can reduce soil inoculum levels but will not eradicate the fungus or prevent its re-establishment. Plastic mulches (soil solarization) on fallow soil for several weeks in summer can lower inoculum levels. Application of plastic mulches to planted pistachio orchards is limited in effectiveness and does not work well in shade. Chemical treatments to soil or by tree injection have not protected trees from *Verticillium* wilt.

## ARMILLARIA ROOT ROT

Armillaria root rot, also called oak root fungus, is caused by the soil-borne fungus, *Armillaria mellea* (Vahl.) Quel. *A. mellea* is a pathogen of many cultivated and wild plant species, including some species of oak. Orchards planted where oak trees once grew often are severely damaged by the disease, hence the name oak root fungus. Plants growing in the soils of flood plains and along rivers and streams generally are more at risk than are those in other soils on the valley floor. Thus, the disease is most frequently encountered in orchards planted in the Sacramento Valley and along the eastern side of the San Joaquin Valley. *Armillaria* root rot limits cultivation of several tree crops in California but only occasionally affects pistachios.

## Symptoms

The first symptoms, which usually begin on one side of the tree, are reduced growth, early yellowing and defoliation. Within a few years the entire tree becomes affected and eventually dies. The disease progresses to adjacent trees leaving areas of dead trees in its wake. Internal tissues of bark and outer wood of the upper roots and crown are discolored, and distinctive white, fan-shaped sheets of fungal mycelium, called plaques, are located between the bark and wood (Plate 26E). With the onset of winter rains, clumps of honey-colored mushrooms may develop at the base of infected trees (Plate 26F). Rhizomorphs, which are flat, root-like structures composed of white mycelium covered by a dark, protective coating, grow along the surface of infected roots. Rhizomorphs are not always present or may be difficult to find and can be confused with small roots. The presence of mycelial plaques within, or rhizomorphs on infected roots, is sufficient evidence to confirm a diagnosis of Armillaria root rot.

## Disease cycle

*A. mellea* does not live freely in the soil. It is always associated with infected or dead woody tissues, with which it can survive for decades if not subjected to desiccation. Deep-rooted plants, such as oak, when infected by Armillaria, leave a legacy of many potential sources of inoculum distributed through the soil.

Infection of healthy plants occurs when roots grow near dead or diseased roots which harbor the fungus. Close proximity between infected and healthy roots is necessary for infection because rhizomorphs are infective only for short distances away from their food source. Rhizomorphs present on such material penetrate the nearby root and establish an infection. Both the formation of rhizomorphs and infection are favored by moist soil. The fungus also moves from infected to healthy individuals through root grafts. The pattern of disease spread in the orchard -- enlarging circles of affected trees -- reflects these methods of transmission.

*A. mellea* is spread when infected roots are moved. This is accomplished by floods or

human activity. Soil or water that does not contain infected plant parts does not carry the fungus. The spores produced by the mushrooms have not been implicated in natural spread of the disease. Often, Armillaria root rot appears to be naturally restricted to certain areas of an orchard and in such cases does not threaten an entire planting.

## Control

Control is extremely difficult and usually not successful. Removal of infected roots followed by fumigation as pre-plant treatment can slow progress of the disease, but eradication is not likely. Nothing is available for use in established plantings. Other soil microorganisms, chiefly *Trichoderma* spp., are antagonistic to *A. mellea* and provide some measure of biological control. This biocontrol occurs only when the fungus has been weakened by sublethal doses of fumigant or by heat or drying. When healthy, *Armillaria* is not harmed by *Trichoderma*.

Resistant rootstocks would offer the best protection against oak root fungus disease. Recent field trials indicate that *P. terebinthus* and UCBI (a *P. atlantica* x *P. integerrima* hybrid) are tolerant, whereas *P. atlantica* and *P. integerrima* are susceptible. However, pathogenicity and virulence in the natural population of *A. mellea* ranges from weak to severe. Thus, disease response may vary with different combinations of host and pathogen. Breeding programs may incorporate some resistance in future stock.

## PHYTOPHTHORA ROOT AND CROWN ROT AND TRUNK CANKER

Root and crown rot can devastate stone fruit, almond and walnut orchards but is not common on pistachio trees in California. Trunk cankers occur occasionally in sprinkler-irrigated orchards. These diseases caused by soil-borne fungi belong to the genus *Phytophthora*. Most of our knowledge of *Phytophthora* disease on pistachio is borrowed from research on other crops.

## Symptoms

All parts of the root system may be attacked by *Phytophthora*. The term 'root rot' refers to infection of small roots, including the fine

feeder roots, and results in reduced uptake of water and nutrients. This leads to a weakening and decline of the tree. Above-ground symptoms of root rot usually develop slowly, and diseased trees show reduced growth, thinned canopy and early defoliation for several years. By contrast, crown rot girdles the tree by destroying the crown and large roots that anchor the tree in the soil. Trees with crown rot typically die within a year or two after infection; they leaf out in spring then collapse with the first hot weather. The bark and outer wood of infected roots and crowns are discolored, but the mycelium of *Phytophthora* in the infected tissues cannot be seen by the naked eye.

*Phytophthora* infections on the trunk or scaffolds of trees form cankers in the bark, and the tree gums profusely. If the trunk or scaffold is girdled by the infection, the portion of the tree above the site also dies.

### Disease cycle

*Phytophthora* species live independently in the soil and survive long dry periods as oospores, the sexual fruiting bodies of the fungus, or chlamydospores, which are resistant asexual spores. The infective units of *Phytophthora* are called zoospores. These are one-celled structures that are capable of swimming through water present in the soil. In response to certain conditions of moisture and temperature, the fungus mycelium in the soil produces reproductive structures called sporangia. As many as 100 zoospores develop inside each sporangium, and when the soil is flooded, the zoospores are released. They are attracted to root exudates and swim to the root surface. There they attach themselves and invade the tissues to establish an infection. Free moisture, i.e. liquid water, is required for both the production and dissemination of the zoospores. Consequently, root and crown rots are associated with heavy soils and prolonged periods of high soil moisture. Alternating cycles of wet and dry soil are known to exacerbate *Phytophthora* diseases in some crops. Stressed plants often are more susceptible to infection.

Many *Phytophthora* spp. are present in canal and river water. The fungi are introduced

into fields and orchards irrigated with water from these sources. To date, *Phytophthora* has not been found in well water.

*P. parasitica* has been cultured from rotted roots and crowns and usually is found in trunk and canker infections of pistachio trees. *P. cactorum* and *P. cryptogea* also have been isolated from trunk and scaffold cankers. In other crops, certain species of *Phytophthora* are associated with root rot, others with crown rot, and some may cause both. Species also differ in their temperature, moisture, and nutritional requirements. Much is known about the interaction of different *Phytophthora* species with various rootstocks and the environment for other crops but not for pistachio.

### Control

Because prolonged periods of high soil moisture promote disease, water management is the basis for control of *Phytophthora* root and crown rot. Cultural practices such as planting on berms, shortening irrigation time, and improving water penetration, all contribute to lessened root and crown rot. Where root or crown rot are of concern, soil should not be wet longer than 24 hours at a time. The relative susceptibility of pistachio rootstocks to *Phytophthora* species is unknown. No chemicals are registered for control of this disease on pistachios. Sprinklers should be set to avoid water hitting the tree or causing puddling around the crown. This is especially important where irrigation water is taken from canals or rivers that may be contaminated with *Phytophthora*.

### SEEDLING BLIGHT

Seedling blight was first reported from California in 1995. In one nursery, over 10,000 seedlings, consisting of *Pistacia atlantica*, *P. integerrima*, and *P. atlantica* × *P. integerrima*, were lost. The disease has not been observed in orchard trees.

**Symptoms.** Leaves turn brown, wither, and cling to the shoots. Brown cortical lesions develop on roots, which become discolored. Seedlings are stunted, blighted, and die (Plate 26G). The rotting tissues decompose and dry, forming a sunken area filled with dried plant

parts mixed with fungus mycelium and sclerotia.

**Causal Organism.** *Rhizoctonia solani* Kühn is a collective species of several strains that are distinguished from each other by anastomosis between isolates of the same anastomosis group. Isolates causing seedling blight belong to anastomosis group AG-4. *R. solani* exists primarily as a sterile mycelium that is hyaline when young but turns yellowish or light brown with age. Branches grow at approximate right angles to the main hypha and have a cross wall near the slightly constricted junction. Under certain conditions the fungus produces hyphal tufts that form brown to black sclerotia or short, barrel-shaped monilioid cells that function as chlamydospores. The teleomorph, *Thanatephorus cucumeris* (A. B. Frank) Donk has not been produced by pistachio isolates.

**Disease Cycle and Epidemiology.** *R. solani* overwinters in soil as mycelium, sclerotia or chlamydospores. The fungus is present in most

soils and once established remains indefinitely. Disease is usually more severe in soils that are moderately wet and when plant growth is slow due to adverse environmental conditions. *R. solani* spreads with rain, irrigation or flood water, equipment, and anything carrying contaminated soil or infected propagative materials. In greenhouses where pistachio rootstocks are germinated and initially planted, infested benches can be a source of inoculum. Optimum temperature for infection is 15-18° C (59-65°F) but the disease is more common in warm areas (75-95°F). Seed transmission has not been demonstrated and is unlikely.

**Control.** Wet, poorly drained areas should be avoided and seeds should be planted in clean soil on raised beds under conditions that encourage rapid growth. Greenhouse benches should be cleaned, especially if a susceptible crop was grown previously. Drenching soil or planting mix with pentachloronitrobenzene helps reduce damping off in seed beds and greenhouses.

## SUMMARY

DISEASE	SYMPTOMS	WHEN SYMPTOMS APPEAR	CONTROL
<b>Verticillium wilt</b>	Sudden collapse of branches  Poor vigor which may be accompanied by slow thinning of canopy	Spring, early summer  Growing season over several years	Plant resistant rootstocks Plant resistant rootstocks
<b>Phytophthora root rot</b>	Slow thinning of canopy  Reduced growth, early senescence	Growing season over several years Growing season over several years	Plant resistant rootstocks Irrigation management
<b>Crown rot</b>	Sudden collapse of entire tree	Spring or early summer	Irrigation management
<b>Trunk canker</b>	Branch death, profuse gumming	Growing season	Keep sprinkler water off trunk
<b>Armillaria root rot</b>	Reduced growth, early senescence, rhizomorphs on infected roots	Growing season	Preplant fumigation; Plant resistant rootstocks
<b>Seedling Blight</b>	Leaves turn brown, wither, and cling to shoots. Seedlings are stunted, blighted, and die	Seedling stage in the nursery greenhouse	Sterile potting soil and clean nursery benches

## References

1. Epstein, L., R. Beede, S. Kaur and L. Ferguson. 2004. Rootstock effects on pistachio trees grown in *Verticillium dahliae*-infested soil. *Phytopathology* 94: 388-395.
2. Holtz, B.A., T.J. Michailides, L. Ferguson, J.D. Hancock and A.R. Weinhold. 1996. First Report of *Rhizoctonia solani* (AG-4) on Pistachio Rootstock Seedlings in California. *Plant Dis.* 80(11):1303.
3. Holtz, B.A. 2002. Pistachio Seedling blight. Pages 70-71 In. *Compendium of Nut Crop Diseases in Temperate Zones*. B.L. Teviotdale, T.J. Michailides and J.W. Pscheidt (eds.). American Phytopathological Society (APS) Press, St. Paul, MN.