United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

New Pest Response Guidelines

Monilinia fructigena (Aderhold & Ruhland) Honey ex Whetzel

Brown Rot/Apple Brown Rot

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Cover Image

Monilinia fructigena on fruit exhibiting diagnostic concentric rings of sporodochia (image courtesy of Radek Sotolář)

Chapter

1

Introduction

Four *Monilinia* species—*M. fructicola* (G. Winter) Honey, *M. fructigena* (Aderhold & Ruhland) Honey ex Whetzel, *M. laxa* (Aderhold & Ruhland) Honey and *Monilia polystroma* (G. van Leeuwen) (van Leeuwen *et al.*, 2002a)—are of concern as significant causes of disease in cultivated fruit trees, particularly apples, pears, stone fruits and ornamentals. These species can cause serious blossom and twig blight and brown rot in fruit. *Monilinia fructicola* and *M. laxa* have already proven detrimental to stone fruit in the United States (Zehr, 1982). *Monilinia fructigena* (Aderhold & Ruhland) Honey ex Whetzel primarily causes fruit rot before and after harvest with less frequent infection of shoots and twigs (Daff, 2008).

For information regarding the use of this document, refer to *Appendix A: How to Use the Guidelines.*

Chapter

2

Taxonomy

The classification and nomenclature of *M. fructigena* and related fungi have undergone many changes. Persoon (1796) described the anamorphic (conidial) stage of a fungus found on the decayed fruit of *Prunus domestica* L., *Amygdalus persica* L. and *Pyrus* spp. and named it *Torula fructigena* (Matheny, 1913). However, Harrison (1933) concluded that "it is impossible with certainty to allocate Persoon's description to any of the present day species of *Brown Rot Fungi*." Persoon (1801) further described the conidial fungus, changing its name to *Monilia fructigena* (Matheny, 1913). Later Kunze and Schmidt (1817) described the same fungus as *Oidium fructigenum* (Persoon) Fries 1832 based on buff-colored pustules. In 1819, Schmidt included this fungus in a descriptive collection of German specimens (Byrde and Willetts, 1977). *Oidium fructigenum* was recognized and used by leading British mycologists including Duby, Fries and von Thümen until the late 19th century (Byrde and Willetts, 1977).

Assuming the fungus was generically different from the one he originally described, Persoon (1822) gave this specimen another name, *Acrosporium fructigenum*. However, the new name was never accepted by other authors. Wallroth (1833) first distinguished between two species of the "brown rot fungi" using coloration and named the anamorphic stage *Oospora fructigena* (Persoon: Fries) Wallr. 1833 then changed the name to *O. candida* Wallroth. Von Thümen (1875) classified it as *Oidium wallrothii* Thümen and, in 1879, changed the name to *O. fructigenum*. Schröter (1893) categorized the fungus as an ascomycete and transferred *M. fructigena* to *Sclerotinia fructigena* (Persoon: Fries) J. Schröter 1893 based on an analogy with other *Monilia* species known to have a perfect stage (Wormald, 1954). Therefore, the name was considered invalid. Aderhold and Ruhland (1905) were first to describe the apothecia (teleomorph) of this brown rot fungus and described the conidial stage obtained from ascospores isolated from the ascocarps. Aderhold and Ruhland's (1905) description of the anamorphic stage was comparable to that provided by Kunze and Schmidt (1817) for *O. fructigenum*. Aderhold and Ruhland provided the name designation *S. fructigena* for the anamorphic stage, which is also valid for this fungus. Eventually, the fungus became known as *M. fructigena* (Aderhold and Ruhland) Honey following Honey's (1928) creation of the genus *Monilinia*. Whetzel (1945)

validated Honey's 1936 placement of *fructigena* in the genus *Monilinia* (USDA–ARS–SMML, 2014).

The taxonomic classification of *M. fructigena* is presented in Table 2-1.

Table 2-1 Classification of *Monilinia fructigena* (Aderhold & Ruhland) Honey, causal organism of apple brown rot disease

Rank	Taxon
Kingdom	Fungi
Phylum	Ascomycota
Class	Leotiomycetes
Order	Helotiales
Family	Sclerotiniaceae
Genus	*Monilinia*
Species	*Monilinia fructigena* (Aderhold and Ruhland) Honey ex Whetzel

Synonyms

Monilinia fructigena is synonymous with the previously accepted name *Sclerotinia fructigena* (Aderhold and Ruhland). The following is an exhaustive list of other names synonymous with *Monilinia fructigena*:

- *Acrosporium fructigenum* (Persoon) Persoon 1822
- *Monilia fructigena* (Persoon ex Persoon) Eaton: Anamorph
- *Monilinia fructigena* (Aderhold and Ruhland) Honey
- *Monilinia fructigena* Honey ex Whetzel
- *Oidium fructigenum* Kunze and Schmidt
- *Oidium wallrothii* von Thümen 1875
- *Oospora candida* Wallroth 1833
- *Oospora fructigena* (Persoon: Fries) Wallroth 1833
- *Sclerotinia fructigena* (Persoon) Aderhold and Ruhland
- *Stromatinia fructigena* (J. Schröter) Boudier
- *Torula fructigena* (Persoon)

Common Names

The preferred common name for *M. fructigena* is brown rot; however, several other common names are associated with this pathogen (Table 2-2).

Table 2-2 Common names associated with *Monilinia spp.*

Common name	Language
apple brown rot	
appelrotkelkje	Dutch
Asian/European brown rot of Rosaceae	
blight	
blossom blight of fruit trees	
blossom wilt	
bluetenduerre: kern- und steinobst	German
braunfaeule: kern- und steinobst	German
brown fruit rot	
brulure sclerotique du cerisier	French
brulure des rameaux du cerisier	French
fruchtfaeule: kern- und steinobst	German
fruit brown rot	
fruit canker	
fruit rot	
momificado	Spanish
momificado de las frutas	Spanish
monilia des arbres fruitiers	French
moniliás gyümölcsrothadás	Hungarian
monilinia brown rot	
moniliose des arbres fruitiers	
mycose des arbres fruitiers à noyau	French
podredumbre de las frutas	Spanish
polsterschimmel: kern- und steinobst	German
pourriture brune des arbres fruitiers	French
pudricion café de la manzana	Spanish
pudricion café de la pera	Spanish
pudricion café del chabecano	Spanish
pudricion café del durazno	Spanish
rot-brun des arbres fruitiers	French
spur blight	
spur canker	
twig blight	
twig canker	
zweigduerre: kern- und steinobst	German
zweigduerre: obst	German

Chapter

3

Identification

Species Description/Morphology

Although a plausible assessment of the brown rot species can be made based on its host and country of origin, an accurate identification can only be obtained in the laboratory (CABI, 2014). Identifying species within *Monilinia* is often problematic. The criteria required to determine cultural and morphological differences are time consuming and may yield inconclusive results as they rely on variable characteristics. However, identification becomes possible when cultural characteristics such as growth rates, growth pattern and colony color are combined with morphological data such as conidial dimensions and germ tube length. Because these characteristics are quantitative and may overlap, any identification must be conducted under standardized conditions and initiated from pure cultures (Daff, 2008).

Morphology

Entostroma

The entostroma is hollow sphere that surrounds the core or seed of the fruit (Byrde and Willetts, 1977) and consists of a 2- to 3-cell-thick outer rind. The outer rind contains cells that measure 5–6 µm in width with a dark-brown cell wall (Batra, 1991). The medulla contains the stromatal region primarily consisting of interwoven hyphae with infrequent branching (the basketwork effect) *in vitro* (Batra, 1991). The loosely packed hyphae are surrounded by a 4- to 6-µm-wide lumen with interhyphal spaces filled with gelatinous material (Batra, 1991).

Microcondium

The microcondium is typically enteroblastic, existing as chains that merge to form whitish to cream or greenish-yellow microscopic mucilaginous masses (Byrde and Willetts, 1977). The mature spore is round to slightly pear shaped and approximately 2.0 µm in diameter with a wall approximately 0.15 µm thick (Byrde and Willetts, 1977).

Macroconidia

The conidia are elongate ellipsoid, limoniform or ovoid and hyaline when observed under light microscopy. *En masse*, they appear buff (Rayner) or yellow brown. The lateral walls of the spore are approximately 0.2 µm thick. Under transmission electron microscopy (TEM), the conidia exhibit a thin electron-dense outer layer and an inner electron-transparent layer (Byrde and Willetts, 1977). A flattened papilla and pore, which is plugged just before fragmentation of the conidial chain, exist at each pole (Byrde and Willetts, 1977).

Monilinia fructigena conidia are typically larger than those of *M. fructicola* ((10–) 25 × 8 to 20 µm) and *M. laxa* (12 to 18 (–24) × 9 to 10 (–12) µm) (Byrde and Willetts, 1977), ranging in size from 12 to 25 × 12 to 16 µm; however, the mode for the size is 21 × 14 µm (Batra, 1991). Batra (1991) found arthric conidia measuring 12 to 34 × 6 to 15 µm, averaging 21 × 13 µm. The conidia form in 1-mm branched chains (Figure 3-1).

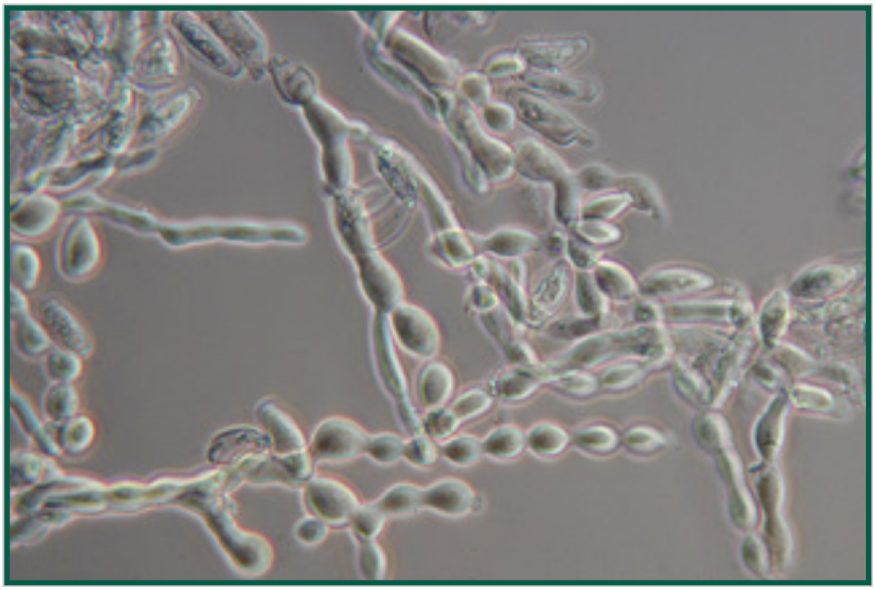


Figure 3-1 Conidiophores and conidia of *Monilinia fructigena* (image courtesy of Malcolm Storey © 2011-2012 /www.discover.org)

Apothecia

The form of the apothecium is similar in the three *Monilinia* species (Wormald, 1954). For *M. fructigena* specifically, the apothecium, which occurs either singly or in groups of twos and threes, is cupulate with discs measuring 3–5 mm in width (Batra, 1991). The receptacle exterior is vinaceous with a hymenium that is slightly darker than the receptacle exterior (Batra, 1991). The stipe is stout, typically light brown and smooth toward its

tip, while dark brown near the base, sometimes with numerous slender, septate, rhizoid-like hyphae approximately 1 mm long (Byrde and Willetts, 1977).

Apothecial Anatomy

In section, the hymenium is 184 to 200 µm thick. The asci are cylindrical with 8 spores ranging in size from 160 to 180 (–200) × 9 to 11.5 µm (Batra, 1991). The apex of the asci is rounded with J+ channel walls and no croziers. The ascospores are arranged uniseriately or are irregularly biseriate. The ascospores are narrow ellipsoids with one or both ends tapering, but not pointed, hyaline, eguttate and smooth, measuring 9 to 12 (–13) × (4.3) 5–6.6 µm (Batra, 1991).

While not enlarged above, the numerous paraphyses can be branched or unbranched, subhyaline and either equal to or slightly above the asci in places (Batra, 1991). The subhymenium is 40 to 64 µm thick, easily distinguished from the medullary excipulum and consists of densely packed, closely septate hyphae that are 5 to 6 µm wide (Batra, 1991). The medullary excipulum is 64 to 80 µm thick consisting of textura intricata to textura porrecta with 5- to 7-µm-wide pale brown hyphae. The hyphae occasionally exhibit encrusted walls. The ectal excipulum is differentiated into an inner outwardly radiating plectenchyma or textura angularis, an outer textura porrecta and possesses cells 3–5 cells thick (Batra, 1991). The entire ectal excipulum is 100 µm thick with narrow brown hyphae banded together in certain regions by an amorphous, granular material (Batra, 1991).

Cultural Characteristics

At 25 °C, 8.5-cm colonies appeared on yeast extract malt extract agar at 7 days under a 12-hour light/dark cycle. These colonies were leathery with an even margin and aerial mycelium that was initially sparse, effuse and white. In many isolates, a circular cushion-like fluffy band—5–6 mm wide, 3–4 mm high and approximately 5 cm from the colony center—formed underneath on the 8th–11th day, appearing flat or loaf-shaped, nearly superficial with initially discrete, but later (between the 12th and 21st day) confluent stromata (Batra, 1991). Two to four similar additional concentric circles subsequently formed outside the first circle with moniloid chains of conidia abundant throughout the colony (Batra, 1979a). Some isolates formed intramatrical stromata, whereas no stromata are formed in others; only a dark-brown pigmentation appears in the intramatrical hyphae and in the agar medium with age. *M. fructigena* sporulates during light or light/dark cycles (Batra, 1991).

Qualitative Colony Characteristics

The colony characteristics of *M. fructigena* were assessed on potato dextrose agar (PDA) at 22 °C under 12-hour light/12-hour dark cycle (Figures 3-2 and 3-3). *Monilinia fructigena*'s growth was radial and continuous with a fairly even colony margin. However, under darkness, several *M. fructigena* isolates produced a rosette-like colony pattern (van Leeuwen and van Kesteren, 1998). The *M. fructigena* formed distinct concentric rings of aerial mycelium on which sporulation occurred. The sporogenous tissue of the *M. fructigena* exhibited a distinct buff/luteous color (van Leeuwen and van Kesteren, 1998).

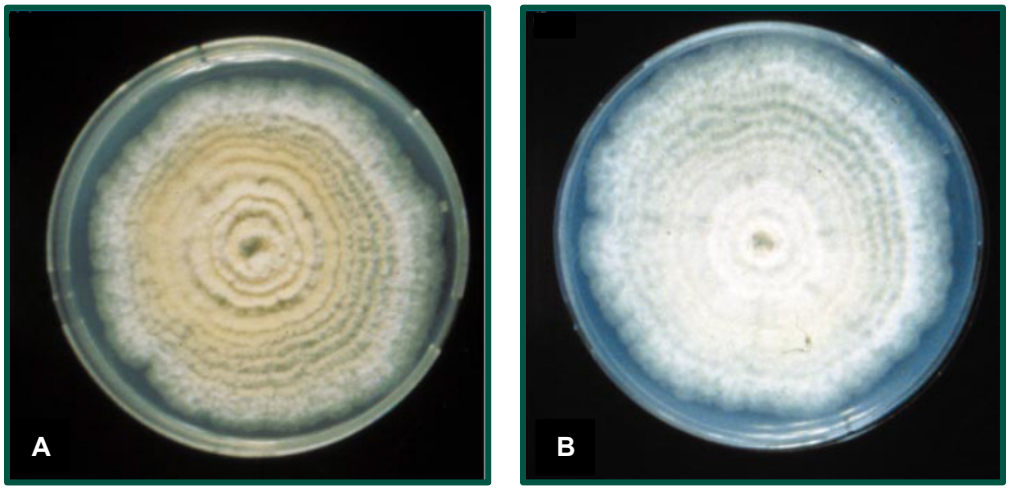


Figure 3-2 *Monilinia fructigena* colonies grown on 4% PDA (10 days, 12 hour light/12 hour dark at 22 °C: (A) upper surface, (B) lower surface (Lane, 2002)

Quantitative Colony Characteristics

Van Leeuwen and van Kesteren (1998) established a framework for a standardized assay based on quantitative characteristics to delineate the three species of brown rot fungi and concluded that germ tube morphology (length of the germ tube and the number of germ tubes per conidium) provides the most robust method for distinguishing the *Monilinia* species. The addition of other parameters (colony diameter and sporulation intensity) decreased the incidence of isolate misclassification. Therefore, the mean colony diameter and germ tube features were used for identification. The mean colony diameter for *M. fructigena* was similar to that of *M. laxa*: After 3 days the mean colony diameter for *M. laxa* was 21.5 µm and that for *M. fructigena* was 21.0 µm with a light/dark regime (van Leeuwen and van Kesteren, 1998). After 5 days, the mean colony diameter was 30.8 µm for *M. laxa* and 33.6 µm for *M. fructigena*. The *M. fructigena* germ tubes were long and straight before branching (Wormald, 1920). The mean germ tube length for *M. fructigena* conidia ranged from 806 to 307 µm, which represents an intermediate range.

The occurrence of more than one germ tube per conidium was most prominent in *M. fructigena* (van Leeuwen and van Kesteren, 1998). The *M. fructigena* isolates displayed branching of the germ tube close to the conidium below 100 µm. In contrast, other reports indicate that *M. fructigena* germ tubes grow out as a single hypha for 400–1200 µm before branching (Wormald, 1920). Van Leeuwen and van Kesteren (1998) defined branching as a result of a leading germ tube that exceeds 20 µm in length.

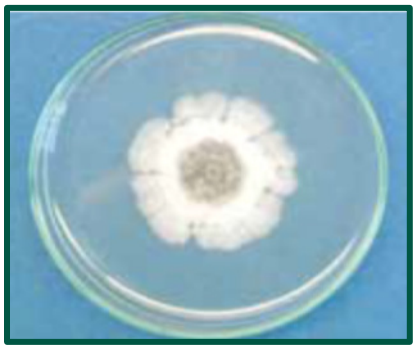


Figure 3-3 *Monilinia fructigena*: compact light yellow mycelium with lobate margins on PDA medium seven days after inoculation (Hrustic *et al.*, 2012)

Diagnostics

Much effort has been expended to develop molecular based methods to detect and differentiate *Monilinia* species. *M. fructigena*, *M. fructicola*, *M. laxa*, and *Monilia polystroma* differ in regulatory status. A timely identification method is required to monitor imported and exported fruit for the presence of quarantined *Monilinia* species. Polymerase chain reaction (PCR) primers for the *M. fructicola* small subunit (SSU) rDNA intron and some SSU sequences were developed for species identification (Fulton and Brown, 1997). However, the method proved unreliable due to the inconsistent amplification of the intron-containing PCR product in some *M. fructicola* isolates, which suggests the lack of an intron in these isolates (Côté *et al.*, 2004; Förster and Adaskaveg, 2000; Fulton *et al.*, 1999; Hughes *et al.*, 2000). The sequences of the ribosomal internal transcribed spacer (ITS) regions of the three *Monilinia* species have been reported (Fulton *et al.*, 1999; Holst-Jensen *et al.*, 1997), and several molecular methods based on ITS sequence variations or other genetic markers were developed (Förster and Adaskaveg, 2000; Ioos and Frey, 2000; Snyder, 1997). Snyder and Jones (1999) proposed a diagnostic method based on restriction fragment length polymorphism (RFLP) of the PCR-amplified ITS1, digested with the endonuclease MseI, which allowed differentiation between *M. laxa* and *M. fructicola*. This method could also differentiate *M. fructigena* from the other two species according to the published ITS1 sequences. In addition, Snyder and Jones (1999) assessed a microsatellite-

primed PCR assay for differentiating *M. laxa* from *M. fructicola*. Microsatellite sequences, however, could not directly determine species from infected fruits possibly because plant DNA was amplified simultaneously. Other molecular identification methods were also developed based on unique species-specific repetitive sequences and microsatellite regions (Boehm *et al.*, 2001; Ma *et al.*, 2003a). Szodi *et al*. (2012) utilized the microsatellite-primed assay to characterize *M. laxa* and *M. fructigena* isolates; however, these assays required the isolation and extraction of the DNA prior to the microsatellite and PCR amplification.

Côte *et al.* (2004) developed an identification method using multiplex PCR (MP-PCR) to differentiate the three most common *Monilinia* species and *M. polystroma*. Random amplified polymorphic DNA (RAPD) analysis was used to generate an *M. fructigena*-specific band, which was subsequently sequenced. Using the as-obtained sequence, primers were designed to amplify bands in the same genomic region for *M. fructicola* and M. *laxa*. These bands were also characterized via sequencing. From all three sequences, an MP-PCR method based on a common reverse primer (MO368-5) and three species-specific forward primers (MO368-8R, MO368-10R and Laxa-R2) was established to differentiate the three *Monilinia* species (Table 3-1) (Côté *et al.*, 2004). The MP-PCR method was tested using additional isolates and consistently produced a 402-base pair (bp) PCR product for *M. fructigena*, a 535-bp product for *M. fructicola* and a 351-bp product for *M. laxa*. The method was also used with isolates of the recently characterized *M. polystroma*, all of which amplified a 425-bp PCR product (Côté *et al.*, 2004). The identification method demonstrates amplification of a PCR product directly from inoculated apples, and the as-produced PCR band is specific to the inoculated *Monilinia* or *Monilia* species.

To evaluate direct PCR from plant material, Gell *et al.* (2007) developed a PCR protocol utilizing sequence-characterized amplified region (SCAR) markers for diagnosing brown rot. The protocol was able to discriminate three *Monilinia* species (*fructicola*, *fructigena* and *laxa*). Hily *et al.* (2011) assessed potential QoI resistance development in the most prevalent *Monilinia* species and designed PCR primers to discriminate these species based on the cytochrome b gene. In (2011), Hu *et al.* analyzed Chinese *Monilinia* isolates and found *M. fructicola*, *M. mumecola* and *M. yunnanensis* occurring in China. However, the aforementioned methods were unable to adequately distinguish these Chinese isolates. As a result, Hu *et al.* (2011) established a MP-PCR protocol that could differentiate *fructicola*, *mumecola* and *yunnanensis* using primers based on G3PDH gene (Table 3-2).

Hrustic *et al.* (2012) utilized the Côte's PCR protocol to identify *M. fructigena* as a causal agent on quince. These primers are listed in Table 3-1.

Table 3-1 Primers used for the molecular detection of *Monilinia* species using PCR (Hrustic *et al.*, 2012)

Species	Primer Name	Sequence (5′–3′)
common reverse primer	MO368-5	GCA AGG TGT CAA AAC TTC CA
M. fructigena and *M. polystroma*	MO368-8R	AGA TCA AAC ATC GTC CAT CT
M. fructicola	MO368-10R	AAG ATT GTC ACC ATG GTT GA
M. laxa	Laxa-R2	TGC ACA TCA TAT CCC TCG AC

Table 3-2 Primers used for the molecular detection of *Monilinia* species using PCR in China (Hu *et al.*, 2011)

Species	Primer Name	Sequence (5′–3′)
common reverse primer	Mon-R	ATC TCC AAG ATC CCG TGA GGA G
	PRCmon-F	ATC TCC AAC GCT TCT TGC AC
all *Monilinia* species	PRCmon-R	CTT CTT GAC GAC AGC CTT GA
M. yunnanensis	*Cola*-F	CTG TAT GAT GAC CGA GAA GG
M. fructicola	*Ensis*-F	GGA AAC CAA GTG GTT GAG AT
M. mumecola	*Mume*-F	AAA GGT AGA AGA CAT CTT AAG G

A protocol for real-time (TaqMan) PCR has been established to differentiate *M. fructicola* from other brown rot fungi that affect fruit crops. Two differentially labelled TaqMan probes were used: a 6-carboxyfluorescein (FAM)-MGB probe P_fc to detect *M. fructicola* and a VIC-MGB probe P2_fgn/1×/ps to detect the group consisting of *M. fructigena*, *M. laxa* and *M. polystroma*. One generic specific primer pair was used to amplify the section of the ITS region of the nuclear ribosomal RNA gene repeat for all four brown rot fungi. The Mon139F and Mon139R primers amplified the 140-bp fragment (Table 3-3) (van Brouwershaven *et al.*, 2010). This methodology increased the rate of detection to 97% as compared with 65% for conventional PCR (van Brouwershaven *et al.*, 2010).

Real-time PCR Reaction

TaqMan Universal PCR Master Mix
200 nM of the Mon139F and Mon139R primers each
200 nM of probe P_fc and 200 nM of probe P2_fgn/l×/ps
5 µL of DNA
Reaction final volume 25 µL

Cycle Conditions

95 °C for 10 min
Followed by 40 to 55 cycles of the following:
  95 °C for 15 s
  60 °C for 1 min

The emission was measured at the annealing/extension step. The threshold was set at a fluorescence (DRn) of 0Æ1. A cycle threshold (Ct) below 40 was scored as a positive result. To assess the analytical sensitivity and specificity, 55 PCR cycles were performed to monitor signals above the cut-off Ct of 40 (van Brouwershaven *et al.*, 2010).

Table 3-3 Oligonucleotides used as primers and probes for the real-time PCR protocol (van Brouwershaven *et al.*, 2010)

Oligonucleotide	Sequence (5′–3′)	Target
Mon139F	CAC CCT TGT GTA TYA TTA CTT TGT TGC TT[a]	*Monilinia* spp.
Mon139R	CAA GAG ATC CGT TGT TGA AAG TTT TAA	*Monilinia* spp.
P_fc	FAM-TAT GCT CGC CAG AGG ATA ATT-MGBNFQ	*M. fructicola*
P2_fgn/1x/ps	VIC-AGT TTG RTT ATT CTC TGG CGA[b]-MGBNFQ	*M. fructigena*, *M. laxa*, *Monilia polystroma*

[a] Y = C or T
[b] R = A or G

Similar Species

Other *Monilinias* that primarily infect rosaceous plants and have a *Monilia* as the conidial stage are similar to *M. fructigena* (Wormald, 1954) and are categorized as belonging to the *M. fructicola* group. The species within this group (*M. fructicola*, *M. laxa* and *M. mume* (Hara) Yamamoto) are junctors: the entostromata are in the ripe fruit, and the infection develops from the outside in. Other species belonging to the *M. padi* group are similar to *M. fructigena* and are parasitic on *Prunus* species with racemose inflorescences. These fungi (*M. seaveri* (Rehm) Honey, *Sclerotinia chaenomelis* E. Fischer, *M. padi* (Woronin) Honey syn. *S. padi* Woronin) possess disjunctors (narrow barren separating cells between conidia), the entostromata occur in the young fruit, and the infection develops in the ovary chamber (Batra, 1991). Another group of fungi similar to *M. fructigena* belong to the *M. mali* group and are parasitic on the Rosaceae family. These organisms have well-developed entostromata with two rinds of fungal cells. However, the species in this group most like *M. fructigena* are *M. aucupariae* (F. Ludwig) Whetzel, *M. amelanchieris* Honey, *M. mali* (Takahashi) Whetzel syn. *S. malicora* Mirua and *S. mali* Takahashi, *M. linhartiana* (Prillieux and Delacroix) N.F. Buchwald syn. *S. cydoniae* Schellenberg, *M. mespili* Whetzel syn. *S. mespili* Schellenberg and *M. johnsonii* (Ellis and Everhart) Honey syn. *S. crataegi* Magnus (Batra, 1991). The *M. urnula* group is largely restricted to parasitizing plants in the *Vaccinium* genus. The structure of the entostromata is similar to that of the *M. mali* group. The species in this group that are similar to *M. fructigena* are *M. oxycocci* (Woronin) Honey syn. *S. oxycocci* Woronin and *M. vaccinii-corymbosi* (J.M. Reade) Honey syn. *S. vaccinii-corymbosi* J.M. Reade (Batra, 1991).

In Japan, the brown rot fungus previously considered to be *M. fructigena* was identified as a separate anamorphic species, *M. polystroma.* Marked differences were noted in its cultural characteristics (van Leeuwen *et al.*, 2002a) and the sequence of the ITS region of the rDNA gene (Fulton *et al.*, 1999). The Japanese isolates produced more stromata on cherry agar, had a significantly higher mean growth rates on PDA and produced smaller conidia on both cherry agar and inoculated pear fruit (van Leeuwen *et al.*, 2002a). *Monilia mumecola* Y. Harada, Y. Sasaki & T. Sano originally isolated from *Prunus mume* Siebold & Zucc. in 1982 is more closely related to *M. laxa* based on symptomology of host part affected (mainly flower and twig) on species of *Prunus*, cultural characteristics on PDA and sequence data in the ITS region of ribosomal DNA (Harada *et al.*, 2004).

In China, a new species, *Monilinia yunnanensis,* was reported for the first time in 2011. Based upon comparison of sequence data for the ITS, glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and β-tubulin genes and cultural characters (conidia size and stromatic tissue production) *M. yunnanensis* is closely related to *M. fructigena* (Hu *et al.*, 2011).

Chapter 4

Biology

Life Cycle

Monilinia fructigena overwinters as fruit mummies either in tree canopies or on the ground and as mycelium in cankers on twigs and branches (Batra, 1991; Byrde and Willetts, 1977). In early spring, the apothecia develop from the overwintered entostromata, which form only in the infected fruits. Ascospores are produced from the apothecia providing the primary inoculum to initiate the disease cycle. However, ascospores are rarely observed in *M. fructigena*. Therefore, the major source of primary inoculum for this fungus is the conidia from overwintered sporodochia on mummified fruits or that formed in the spring from the mycelium retained in cankers from the previous summer (Figure 4-1) (Batra, 1991). Van Leeuwen *et al*. (2002e) demonstrated that fruits infected by *M. fructigena* early and late in the season also contribute to the production of primary inoculum in spring. The sporodochia consist of conidiophores supported by a pseudoparenchymatous cushion-like tissue. The unspecialized, short conidiophores elevate the branched conidial chains above the infected tissues (Byrde and Willetts, 1977). The conidia are established by budding of the terminal portion of the conidiophore (Hashmi *et al.*, 1972) and are dispersed via wind, rain, insects or birds (Byrde and Willetts, 1977; Lack, 1989). Whether the spore is asexual or sexual in nature, it lands on the fruit, penetrating it through wounds caused by either abiotic or biotic factors. Although the direct infection of fruits by *M. fructigena* conidia through lenticels has been observed in the laboratory (Sharma and Kaul, 1990), naturally occurring brown rot is believed to result primarily from wound parasitism (Byrde and Willetts, 1977), as confirmed through detailed orchard studies of brown rot in apple and pear (Xu and Robinson, 2000). Secondary inoculum can arise from any infected tissue with sufficient moisture content for conidial sporulation (Landgraf and Zehr, 1982). Depending on the climatic conditions, several generations may occur during the growing season. These conidia infect fruit and may either cause brown rot under favorable climatic conditions or remain latent under unfavorable climatic conditions. When conditions become favorable for disease expression, brown rot develops (Byrde and Willetts, 1977; Emery, 2000). Conidia are produced throughout the growing season and can infect fruit in any stage of development.

Stem and branch cankers and fruit mummies assure the survival of the fungus from season to season. Decay during storage results from infections immediately preceding harvest.

Figure 4-1 Tufts of sporodochia (image courtesy of Malcolm Storey © 2011–2112 /www.discoverlife.org)

Hosts

Hosts include most pome and stone fruits in the Rosaceae family and a few members of the Ericaceae and Myrtaceae (CABI, 2014). *Monilinia fructigena* was identified on tea-rose hybrid pseudo fruits, the first occurrence of this pathogen on this host (van Leeuwen *et al.*, 2002a). The known hosts of *M. fructigena* can be found in Table 4-1.

Table 4-1 Known hosts of *Monilinia fructigena*

Scientific name	Family name	References
Amelanchier canadensis (L). Medik.	Rosaceae	Lovisolo (1955)
Berberis vulgaris L.	Berberidaceae	Negru *et al.* (1957)
Chaenomeles japonica (Thunb.) Lindl. ex Spach		Batra (1991)
Chaenomeles speciosa (Sweet) Nakai	Rosaceae	Petro´czy et al. (2012)
Cornus mas L.	Cornaceae	Sorauer (1897)
Corylus avellana L.	Betulaceae	Buchwald (1943), Tzavella-Klonari (1985)

Cotoneaster divaricatus	Rosaceae	Petro´czy *et al.* (2012)
Cotoneaster obtusa Wall.	Rosaceae	Negru *et al.* (1957)
Cotoneaster spp.	Rosaceae	Mordue (1979)
Crataegus oxyacantha L.	Rosaceae	Lovisolo (1955)
Crataegus spp.	Rosaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Crataegus subvillosa Schrad. ex Torr. & A. Gray	Rosaceae	Mordue (1979)
Cydonia oblonga Mill.	Rosaceae	Batra (1991), Byrde and Willetts (1977)
Cydonia spp.	Rosaceae	Wormald (1954)
Diospyros spp.	Ebenaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Elaeagnus macrophylla Thunb.	Elaeagnaceae	Takahashi (1911)
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Feijoa sellowiana (questionable host)	Myrtaceae	Kechakmadze and Kikvadze (1976)
Ficus carica L.	Moraceae	Walker and McLeod (1970)
Fragaria ananassa Duchesne ex Rozier	Rosaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Fragaria spp.	Rosaceae	Schellenberg (1923)
Solanum lycopersicum L. var. *lycopersicum*	Solanaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Malus baccata (L.) Borkh.	Rosaceae	Wormald (1954)
Malus domestica Borkh.	Rosaceae	Wormald (1954)
Malus pumila Mill.	Rosaceae	Byrde and Willetts (1977)
Malus prunifolia (Willd.) Bork	Rosaceae	Negru et al. (1957)
Malus × purpurea	Rosaceae	Petro´czy et al. (2012)
Malus × scheideckeri (L.H. Bailey) Späth ex Zabel	Rosaceae	Petro´czy et al. (2012)
Malus sieboldii (Regel) Rehder	Rosaceae	Lovisolo (1955)
Malus sylvestris (L.) Mill.	Rosaceae	Wormald (1954)
Mespilus germanica L.	Rosaceae	Wormald (1939)
Prunus armeniaca Marshall	Rosaceae	Byrde and Willetts (1977)
Prunus avium (L.)	Rosaceae	Batra (1991)
Prunus cerasifera Ehrh.	Rosaceae	Wormald (1940)
Prunus cerasus L.	Rosaceae	Batra (1991)
Prunus domestica L.	Rosaceae	Wormald (1940)
Prunus dulcis (Mill.) D.A. Webb	Rosaceae	Wormald (1940)
Prunus domestica subsp. *insititia* (L.) C. K. Schneid.	Rosaceae	Wormald (1940)
Prunus hortulana L. H. Bailey	Rosaceae	Christoff (1938)
Prunus laurocerasus L.	Rosaceae	Mordue (1979)
Prunus mume Siebold and Zucc.	Rosaceae	Takahashi (1911)
Prunus persica var. *nucipersica* (Suckow) C. K. Schneid.	Rosaceae	Byrde and Willetts (1977)
Prunus persica var. *persica*	Rosaceae	Byrde and Willetts (1977),

		Wormald (1954)
Prunus pseudocerasus Lindl.	Rosaceae	Takahashi (1911)
Prunus salicina Lindl.	Rosaceae	Protection (1972)
Prunus sieboldii (Carriere) Koidz	Rosaceae	Mordue (1979)
Prunus spinosa L.	Rosaceae	Christoff (1938)
Prunus triloba Lindl.	Rosaceae	
Pyrus communis L.	Rosaceae	Byrde and Willetts (1977), Wormald (1954)
Pyrus purpurea (name unresolved)	Rosaceae	Wormald (1940)
Pyrus elaeagrifolia Pall.	Rosaceae	Petro´czy *et al.* (2012)
Pyrus pyrifolia (Burm. f.) Nakai	Rosaceae	Protection (1972)
Pyrus pyraster L. subsp. pyraster (L.) Ehrh.	Rosaceae	Petro´czy *et al.* (2012)
Pyrus serotina var. *culta* (Makino) Rehder	Rosaceae	Harada (1977)
Psidium guava L.	Myrtaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Rhododendron spp.	Ericaceae	Boerema (1971), Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Roberts and Dunegan (1932), Wormald (1954)
Rosa spp.	Rosaceae	
Rubus spp.	Rosaceae	Moore and Talboys (1953)
Rubus occidentalis L.	Rosaceae	Harrison (1928), Lovisolo (1955), Negru *et al.* (1957), Reade (1908), Roberts and Dunegan (1932), Wormald (1954)
Sorbus aucuparia L.	Rosaceae	Mordue (1979)
Sorbus commixta Hedl.	Rosaceae	Harada (1977)
Sorbus dacica Borbás	Rosaceae	Mordue (1979)
Vaccinium spp.	Ericaceae	Schellenberg (1923)
Vitis vinifera L.	Vitaceae	Takahashi (1911)

[1] Susceptible host

Natural Dispersal

Water is important in spreading conidia within the tree canopy; thus, short-range transport of conidia occurs via splash dispersal (Byrde and Willetts, 1977). Long-range dispersal likely depends on vector-borne or airborne mechanisms (Byrde and Willetts, 1977). *Monilinia fructigena* relies on wounds for infection. Insects are the major catalysts of brown rot infection, causing injuries or transporting spores to susceptible tissue. As early as 1932, hornets and wasps were known as potential *Monilinia* spp. vectors (Joessel, 1932). In Europe, *Rhynchites bacchus* Linnaeus appeared to be among the chief insects causing fruit injuries that permit brown rot (Wormald, 1954). In addition, codling moths (*Cydia pomonella* Linnaeus) acted as wound agents and precursors of apple brown rot. Croxall *et al*. (1951) indicated that earwigs (*Forficula auricularia* Linnaeus) can produce

injuries that promote the infection of apples by *M. fructigena*. Other researchers pointed to wasps (*Vespula* spp.), beetles—particularly the nitidulid beetles (*Carpophilus* spp. and *Haptonchus luteolus* (Erichson))—and the nut weevil (*Balaninus nucum* Billberg) as significant wounding agents, and thus vectors of *M. fructigena* (Byrde and Willetts, 1977). Lack (1989) described species of Diptera and Hymenoptera as important to the spread of *M. fructigena*. Birds, specifically blackbirds (*Turdus merula* Linnaeus), starlings (*Sturnus vulgaris* Linnaeus), crows (*Corvus* spp.) can peck holes in fruit increasing the likelihood of brown rot infection. One incident of birds transporting infected mummified fruit from one place to another has been documented (Wormald, 1954). Primary infection of fruit by other fungi occasionally creates ruptures in the skin that serve as ports of entry for the brown rot fungi. For example, apple scab (*Venturia inaequalis* (Cooke) G. Winter) lesions allow the entry of *M. fructigena* (Wormald, 1954).

Kable (1965) demonstrated that airborne conidia ensure a wide dispersal within an orchard. The aerial density of *M. fructigena* conidia increases continuously from the appearance of the first infected fruit to harvest (Holb, 2008). The density of airborne spores is related to the incidence of brown rot infection in fruit (Holb, 2008; Luo *et al.*, 2005; van Leeuwen, 2000). A diurnal fluctuation in spore concentration was observed with the highest spore concentrations during the afternoon (Bannon *et al.*, 2009). Xu *et al*. (2001a) demonstrated that airborne *M. fructigena* conidia deposited on apple surfaces can remain viable on the fruit surface for up to 20 days and infect the fruit under relatively low temperatures and high relative humidity. The number of *M. laxa* and *M. fructigena* conidia on peach surfaces also correlates with the incidence of latent infections in stone fruit (Gell *et al.*, 2008).

Geographic Distribution

Ecological Distribution

A combination of humid weather and temperatures of 15–20 °C in spring induces the formation of a fresh conidia crop on mummified fruit surfaces (Byrde, 1954). Conidia can germinate over a wide temperature range, from at least 3–30 °C with an optimum range of 23–25 °C (Xu *et al.*, 2001a). The conidia can germinate and infect wounded fruit within 2 hours (Xu *et al.*, 2001a). No additional free moisture may be necessary for infection if sufficient moisture is available from the fruit tissues on the wound surfaces. Once the fruits are infected, the subsequent colonization and sporulation are not limited by temperature or relative humidity (RH) within the range typically experienced during summer in the United Kingdom (Xu *et al.*, 2001a). Temperature and duration of wetness are also important climatic variables affecting

the incidence of latent *M. fructigena* infection (Gell *et al.*, 2008).

The main regions of *M. fructigena* distribution are Europe, Japan, Manchuria, Turkey and Uzbekistan (Wormald, 1954). Table 4-2 lists the geographic distribution of *M. fructigena* by continent.

Table 4-2 Geographic distribution of *M. fructigena,* causal agent of apple brown rot

Location		Notes	References
Continent	Country		
Europe	Austria	affecting *Pyrus spinosa*	Japp (1908)
	Belarus		Lesik (2013)
	Belgium	included in a list of new species in the region of Gembloux	Marchal and Marchal (1921)
	Bulgaria	recorded on apple, pear and plum; evidence on five species of *Prunus* and *C. japonica*	Christoff (1934, 1938), Malkoff (1908)
	Croatia	low percentage of total infestations	Ivić *et al.* (2013)
	Czechoslovakia	stone fruits severely attacked by *S. fructigena*	Vielwerth (1938)
	Denmark	serious losses in Denmark due to *S. fructigena* attacking apples, pears and hazel nuts with potential spoilage of 60% of ripening pears	Weber (1926, 1938)
	Estonia	*S. fructigena* caused serious infection of certain apple varieties	Kivilaan (1936)
	Finland	accessed material from orchards at Viborg and concluded that *S. fructigena* likely prevalent	Woronin (1897, 1900)
	France	pome and stone fruits attacked causing mummification with severe losses like those in America and Germany; common occurrence of *S. fructigena* destruction of d'Ente prune and nectarines	Chaboussou (1945), Delacroix and Maublanc (1909), Molliard (1901)
	Germany /Bavaria	noted on apples in storage	Sprau (1948)
	Greece	mentioned on apples and pears	Sarejanni (1935, 1936, 1939)
	Holland	multiple observations	Berkhout (1923)
	Hungary	yield loss quantified, temporal development assessed and the importance of fruit wounding agents in organic apple orchards determined	Holb (2004b)
	Italy	*S. fructigena* cited as causing fruit rot; *S. fructigena* (for Sicily) noted as not controlled by any tested treatments; references to infection occurring through injures caused by *R. bacchus*.	Comes (1891) Montemartini (1924, 1936)
	Latvia	*S. fructigena* recorded on apple and pear	Smarods (1930)
	Lithuania		Valiuškaitė *et al.*(2006)
	Luxembourg		Batra (1991)
	Moldova		Paterilo (1976)
	Netherlands		Batra (1991) van Leeuwen (2000)
	Norway	mentioned on plums in a garden; details provided of additional widespread attacks; varietal differences noted in susceptibility; reported on apples, pears, plums	Jørstad (1923, 1938, 1945) Reuter (1898)

		and sweet cherries	
	Poland	reported on apple, pear, plum and *Pyrus mala baccata*	Garbowski and Juraszkówna (1933)
	Rumania (Romania)	*S. fructigena* documented on apples	Savulescu *et al.* (1941) Veresciaghin (1940)
	Russia		Truesdell (1922)
	Serbia	widespread throughout	Balaža (2000)
	Slovakia	present	Ondejková *et al.* (2010)
	Slovenia	present	Munda and Virščeк-Marn (2010)
	Spain	presence of *S. fructigena* noted; reported on plums—a new host for Spain at the time; cider apples infected	Benlloch (1931) Gallástegui (1942) Sardiña (1928) Unamuno (1929)
	Sweden	description, including illustrations, of blossom wilt of apple involving all forms under the name *M. fructigena*; diagnosis may be inaccurate with *S. laxa* f. mali as the true causal agent	Eriksson (1913)
	Switzerland	*S. fructigena* documented on apple, pear and quince	Muller-Thurgau and Osterwalder (1924)
	Ukraine		Woronin (1900)
	United Kingdom	in England, *S. fructigena* is common not only on apples, pears and quinces but also stone fruits; in Scotland, *S. fructigena* infected the fruit of apple, pear and peach	Alcock and Foister (1931)
	Yugoslavia	*S. fructigena* occurred each year on apples and pears; *M. fructigena* reported on apple, pear and plum; *S. fructigena* reported on apple and pear	Mijuskhovits (1950)
Asia	Armenia		Telerevnikova-Babayan (1981)
	Azerbaijan		Ibragimov and Abbasov (1976)
	China	Anhui, Beijing, Gansu, Guangxi, Henan, Hubei, Hunan, Jiangsu, Liaoning, Macau, Shaanxi, Shandong, Shanxi, Sichuan, Yunnan and Zhejiang	Wang *et al.*(1998)
	Georgia	recorded on grapes	Nagorny and Issarlischwili (1929)
	India, Pakistan and Kashmir	*S. fructigena* present on apples and pears specifically in Punjab region	Hafiz (1946) Sattar (1940)
	Iran	reported on pears along shores of Caspian Sea in one area causing 30% loss; also evident on quinces	Esfandiari (1947, 1949)
	Israel		Byrde and Willetts (1977)
	Japan and Manchuria	*S. fructigena* quite common in Japan on apple and pear fruits and detected on ripe quince, cherry, apricot, peach, plum, *P. mume*, *P. pseudo-cerasus*, grape and *E. macrophylla* fruits; material sent by Michuya Miura examined with presence of *S. fructigena* confirmed on peach from Japan and pear from S. Manchuria	Byrde and Willetts (1977) Miura (1929) Takahashi (1911) Wormald (1927)
	Jordan	unsubstantiated report	Map22 (2000)
	Kazakhstan	unsubstantiated report	Map22 (2000)

	Korea, DPR		Hakhoe (1972)
	Korea, Republic of		Hakhoe (1972)
	Lebanon	unsubstantiated report	Map22 (2000)
	Nepal	unsubstantiated report	Map22 (2000)
	Taiwan	unsubstantiated report	Map22 (2000)
	Turkey	*S. fructigena* documented on apple, pear, quince and peach	von Bremer *et al.* (1947), Byrde and Willetts (1977)
	Uzbekistan		Wormald (1954)
Africa	Egypt		Byrde and Willetts (1977) Mordue (1979)
	Morocco	*S. fructigena* documented on apple and pear	Bouhelier (1936), Byrde and Willetts (1977)
	South Africa	*S. fructigena* suggested as the causal agent of fruit mold (but with no evidence of fungus identity); South African conditions appear unfavorable to the fungus causing disease, from which appreciable damage only noted in exceptionally wet seasons. List of plant diseases occurring in South Africa revised, and *S. fructigena* included on apple (occasional occurrence on twigs), apricot (on mummified and fresh fruit; only reported during one exceptionally wet season in the western Cape Province), peach (rare) and plum (uncommon).	Doidge (1919), Doidge and Bottomley (1931)
North America	United States	In 1970s, *M. fructigena* found on pear fruits in Maryland; orchard destroyed to eradicate disease; no further records made of its occurrence in the U.S.	Batra (1979b)

Potential Distribution

If the fungus should become established, distribution of *M. fructigena* throughout the U.S. would be likely. According to a host map developed by USDA–APHIS–PPQ–CPHST for *M. polystroma*, a pest that shares known hosts with *M. fructigena* (Figure 4-2), the eastern half of the continental U.S. has a moderate to high risk of establishment. This map is based solely on the presence of susceptible hosts. Most areas of the western U.S. are at low risk, while portions of California, Washington and Oregon are at a moderate risk.

Because *M. fructigena* has many more known hosts than *M. polystroma*, the potential for *M. fructigena* distribution in the U.S. could prove higher and farther reaching than indicated by this map.

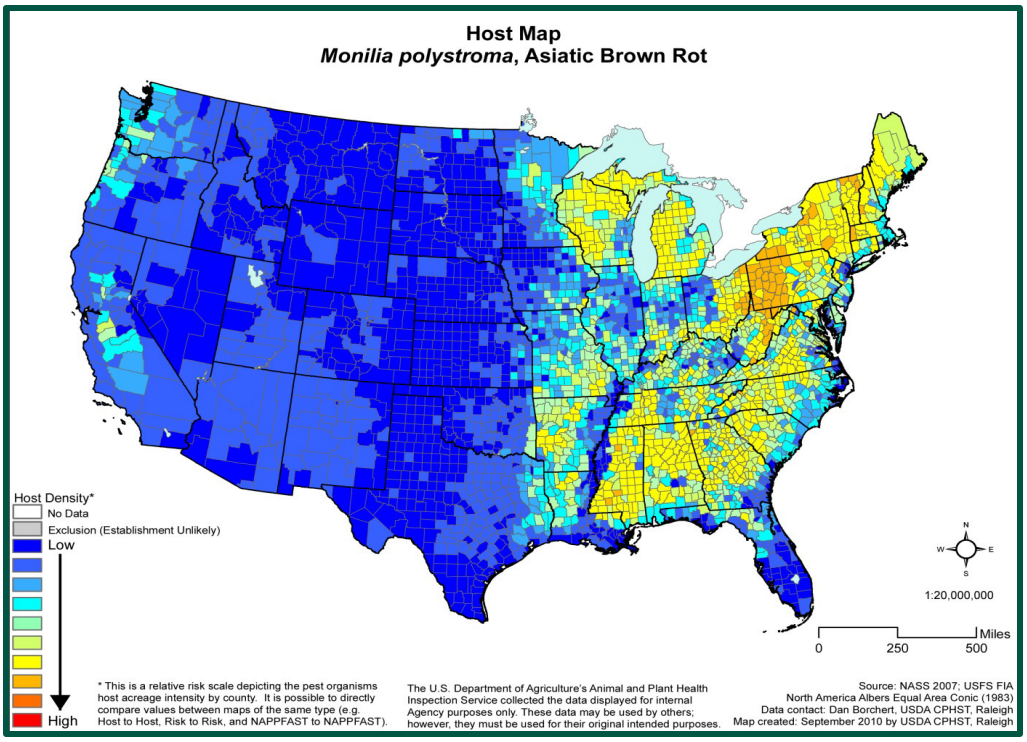


Figure 4-2 Host map for *Monilia polystroma* within the continental United States; values from low to high indicate increased risk based on host availability (map courtesy of USDA–APHIS–PPQ–CPHST. See www.nappfast.org for the most recent updates)

Chapter 5

Damage

Signs and Symptoms

The disease primarily affects fruit; however, twigs or branches can occasionally become infected (Duggar, 1909). Immature fruits are less prone to *M. fructigena* than mature fruits, with susceptibility increasing with maturation. Several possible explanations exist for this phenomenon: The incidence of brown rot has been negatively correlated with acidity and positively correlated with the pH of the fruit juice (Sharma and Kaul, 1988). Higher acidity in fruit juice is associated with younger fruits (Xu and Robinson, 2000). Additionally, low fruit juice sugar content and high respiration rates are associated with resistance to *M. fructigena* (Byrde and Willetts, 1977). Sugar levels are generally lower in younger fruits, and higher respiration rates are expected in response to wounding in young fruits (Xu and Robinson, 2000). Infection begins when the hyphae enter the fruit via a wound. The mycelium colonizes the fruit intercellularly (Sharma and Kaul, 1990). The symptoms first appear as a small, superficial circular brown spot that gradually extends outward (Byrde and Willetts, 1977). This spot grows until the entire fruit is infected; however, no diminution in size or sunken areas appear initially (Duggar, 1909). Before the fruit becomes completely decayed, conidiophores rupture its epidermis to form small yellowish tufts on the surface (Figures 5-1 and 5-2) (Byrde and Willetts, 1977). The conidiophores are often arranged in concentric bands around the source of infection yielding a characteristic appearance. Under moist conditions and on soft ripe fruit, the surface is practically covered with conidial tufts or vegetative mycelium, but under low relative humidity and/or with unripe fruits, no mycelium and few or no conidial tufts develop (Byrde and Willetts, 1977). Ultimately, the entire fruit becomes discolored, and water is lost to form a shriveled mummy (Figure 5-3). The dry rot that develops in fruits infected by brown rot fungi is referred to as mummification. The infection often spreads from diseased to healthy fruit within the same cluster where moisture is prevalent. Several mummies join together when the mucilage that exudes from fruits dries during the rotting process (Byrde and Willetts, 1977). Mummified fruits adhere to tree branches until spring or drop to the ground where they remain throughout the winter, partially or completely buried beneath soil or leaf litter (Byrde and Willetts, 1977).

Black Rot

Black rot, which affects fruits stored at or near maturity, begins as a brown rot but the color gradually darkens. Eventually the entire skin becomes black with dark rings around the lenticels (Byrde and Willetts, 1977; Wormald, 1954). Fungus fructifications are few or absent, although whitish tufts of mycelium appear on the surface. The apples do not shrink as in a typical brown rot, but remain firm with a shiny skin that is rubbery in texture. If exposed to dry air, the fruits shrink and become grooved and indented, but remain firm with shiny ebony-like surfaces. In Europe, black apple rot is almost invariably caused by *M. fructigena* (Wormald, 1954).

Figure 5-1 Apples infected with *Monilinia fructigena* (Photo courtesy of Radek Sotalar—the Czech Republic)

Figure 5-2 Mummified fruit with buff-colored sporodochia of *M. fructigena* (image courtesy of Malcolm Storey © 2011–2112 /www.discoverlife.org)

Figure 5-3 Mummified fruit still attached to the tree (photo courtesy of Sanja565658 Wikipedia Commons/ http://commons.wikimedia.org/wiki/Monilia_fructigena)

Impacts

Environmental

Introduction of this pathogen could negatively impact the environment. None of the known *M. fructigena* hosts present in the U.S. is listed as a species of concern or endangered (USFWS, 2013). If the *M. fructigena* pathogen is introduced in the U.S., chemical control programs may be initiated that could negatively impact non-target pests and the environment.

Economic

Brown rot caused by *M. fructigena* leads to economic losses each year in both orchards and storage (Byrde and Willetts, 1977), with severities varying greatly from one year to another, largely due to differences in weather. Overall, losses caused by *M. fructigena* in conventional and integrated apple orchards are low (≤ 5%) (Falconi and Mendgen, 1994; Moore, 1950; van Leeuwen *et al.*, 2000; Xu and Robinson, 2000); however, in organic and unsprayed orchards losses are 46% (Holb, 2004a) and 50–90% (Burchill and Edney, 1972; Garić *et al.*, 1990), respectively . Moore (1950) recorded an average rate of *M. fructigena* infection of approximately 9% in apple fruits during the growing season in conventional orchards. In a seriously affected cider apple orchard in the UK, 35.5% of fruit was infected with *M. fructigena* (Burchill and Edney, 1972), while a 7.3% loss of apple fruit was reported on unsprayed trees in Italy (Byrde and Willetts, 1977). Berrie (1989) indicated that the mean post-harvest losses in the cultivar Cox's Orange Pippin ranged from 0.1–0.6% in integrated apple orchards. More recently, Van Leeuwen *et al.* (2000) demonstrated a pre-harvest disease incidence of approximately 4.0% in the cultivar James Grieve over two years. In the cultivar Cox's Orange Pippin, pre-harvest disease incidences of 4.4 and 2.7% were reported in 1997 and 1998, respectively. Post-harvest yield losses averaged 1.5 to 2.0% for both cultivars (Larena *et al.*, 2005). According to Larena *et al*. (2005), the percentage of stone fruit affected by *M. fructigena* ranged from 10–15% in France, Italy and Spain. Holb and Scherm (2008) demonstrated that brown rot incidence in integrated orchards reached an average of 6.4% and in organic orchards reached a higher average incidence of 20.1%. Given climatic factors that are conducive for *M. fructigena* germination and the myriad of suitable hosts, this pathogen has the potential to cause economic losses in the U.S.

Pome and Stone Fruits

Apples are among the most profitable fruit crops in the U.S. The 2012 apple crop was valued at nearly $3.1 billion, up from more than $2.8 billion the

previous year (Table 5-2) (Geisler, 2012a). The utilized apple crop was nearly 9.0 billion pounds, down from more than 9.3 billion pounds in 2011 (NASS, 2013). Apples are grown in each state in the U.S., with 29 states producing apples commercially (Geisler, 2012a). Washington State produces approximately 70% of the apples grown in the U.S. (Geisler, 2012a). Other leading states include New York, Pennsylvania and California (Table 5-1) (Geisler, 2012a).

Table 5-1 Commercial apple production in the top 4 states in 2012 (NASS, 2013)

State	Value of utilized production ($1,000)	Percentage of total U.S. Production
Washington	2,250,850	71.73%
New York	249,790	7.95%
Pennsylvania	133,595	5.45%
California	77,750	3.31%

Most commercial pear production occurs in the northwest U.S.; Washington state and Oregon together grow approximately 75% of the nation's pears, followed by California (Geisler, 2012e). The total pear production for the U.S. for 2012 was estimated at 858,240 tons (Table 5-2) (NASS, 2013). The U.S. is a net exporter of pears. In 2012, the U.S. fresh pear exports were valued at nearly $188.7 million, and the organic fresh pear exports were valued at nearly $20.6 million (Geisler, 2012e). The combined value of all fresh pears increased 17% from 2011.

As of 2012, peaches are produced commercially in 23 states. The four major peach-producing states are California, South Carolina, Georgia and New Jersey (Brunke and Chang, 2002). California is an important producer of both fresh and processed peaches, while South Carolina and Georgia primarily produce fresh peaches (Brunke and Chang, 2002). The total peach production for 2012 was estimated at 978,260 tons, which is down from 1.03 million tons in the previous year (Table 5-2) (NASS, 2013).

The total U.S. nectarine production was valued at $144 million in 2012 with 188,900 tons produced (NASS, 2013). United States nectarine exports totaled 2,200 metric tons (MT) in 2011 at a value of over $8.5 million (FAS, 2012).

Cherries have been a popular fruit crop for consumption in the U.S. for many years (ERS, 2002). Sweet cherry production in the U.S. totaled 424,000 tons in 2012 and was valued at more than $843.3 million (Boriss *et al.*, 2006). Washington produced the most sweet cherries at 264,000 tons, followed by California (92,300 tons) and Oregon (56,000 tons) (NASS, 2013). Overall tart cherry production in the U.S. in 2012 was 42,550 tons valued at more than $50.5 million (Table 5-2) (NASS, 2013). Michigan is the primary tart cherry-

producing state. The U.S. is the third-largest producer of cherries in the world after the European Union and Turkey (FAS, 2012).

Table 5-2 The value and total production of commercial pome and stone fruits from 2012 (NASS, 2013)

Crops	Value ($1,000)	Total production (tons)
apples	3,088,915	4,530,550
nectarines	144,906	188,900
peaches	631,223	978,260
pears	437,113	858,130
sweet cherries	843,311	424,000
tart cherries	50,520	42,548

Chapter 6

Pathways

Natural Movement

Water is important in spreading conidia within the tree canopy; thus, short-range transport of conidia occurs via splash dispersal (Byrde and Willetts, 1977). Long-range dispersal likely depends on vector-borne or airborne mechanisms (Byrde and Willetts, 1977). *Monilinia fructigena* relies on wounds for infection. Insects are the major catalysts of brown rot infection, causing injuries or transporting spores to susceptible tissue. Birds can peck holes in fruit increasing the likelihood of brown rot infection. One incident of birds transporting infected mummified fruit from one place to another has been documented (Wormald, 1954). Primary infection of fruit by other fungi occasionally creates ruptures in the skin that serve as ports of entry for the brown rot fungi (Wormald, 1954).

Kable (1965) demonstrated that airborne conidia ensure a wide dispersal within an orchard. The aerial density of *M. fructigena* conidia increases continuously from the appearance of the first infected fruit to harvest (Holb, 2008). The density of airborne spores is related to the incidence of brown rot infection in fruit (Holb, 2008; Luo *et al.*, 2005; van Leeuwen, 2000). A diurnal fluctuation in spore concentration was observed with the highest spore concentrations during the afternoon (Bannon *et al.*, 2009). Xu *et al*. (2001a) demonstrated that airborne *M. fructigena* conidia deposited on apple surfaces can remain viable on the fruit surface for up to 20 days and infect the fruit under relatively low temperatures and high relative humidity. The number of *M. laxa* and *M. fructigena* conidia on peach surfaces also correlates with the incidence of latent infections in stone fruit (Gell *et al.*, 2008).

Human-Assisted Spread

A broad host range coupled with ideal climatic conditions creates multiple probable pathways into the U.S. Since 1984, *M. fructigena* has been intercepted numerous times at U.S. entryways (AQAS, 2013). Of those interceptions, 32 were found on contaminated fruit or infected seed belonging to the genera *Malus* or *Prunus* spp. Interceptions of apple and pear propagative materials are common:

The past ten years has seen 186 interceptions of *Malus* spp. and 56 interceptions of *Prunus* spp. on propagative material from China (AQAS, 2013). During the same period, 1,216 interceptions of *Malus* spp. and 203 interceptions of *Prunus* spp. have occurred from Europe(AQAS, 2013; CAPS, 2014b).

Movement of fruits or nursery stock infected with *M. fructigena* could introduce this pathogen into new regions. Fresh fruits may enter the U.S. only under USDA permit and are subject to scrutiny as specified in Title 7, Part 319.56 of the Code of Federal Regulations (Chang, 1986). Part 319.37 restricts the propagative material (including cut flowers) of its primary hosts (*Malus*, *Pyrus*, *Prunus*, *Chaenomeles*, *Cydonia* spp.) from most countries except under departmental permit for scientific purposes and subjects them to stringent entry requirements from certain other countries (Chang, 1986). Other hosts are subject to entry requirements, such as post-entry quarantine, to exclude the pathogen (Chang, 1986).

The primary risk of long-distance dispersal is the transport of infected trees or budwood to uninfested areas. Although large twig cankers may be evident, recent or dormant infections may not be detectable. Once planted or propagated, infected wood will eventually be exposed to the warm wet conditions required for conidia production and the wind and rain essential for conidia dispersal. Rotting or recently infected fruit may also transport this brown rot fungus into the U.S. However, the typical methods of fruit transportation and storage minimize the risk of establishment. Properly cleaned seed presents a low risk of transmission (NPB, 2002).

Chapter 7

Survey

Survey Types

Plant regulatory officials will conduct detection, delimiting and monitoring surveys for *M. fructigena*. A *Detection Survey* will be conducted to ascertain the presence or absence of *M. fructigena* in an area in which it is not known to occur. After a new detection in the United States, or when detection in a new area is confirmed, a *Delimiting Survey* should be conducted to define the extent and geographic location of the disease. In addition, when a control procedure is applied, its effectiveness should be measured via a *Monitoring Survey*.

Table 7-1 Decision table for selecting survey type

If you:	Use this type of survey:
are not sure whether the pest is present at a location	*Detection Survey* as described on page 7-1. Collect specimens and consult with the authorities listed in *Appendix D* to confirm identification.
know that the pest is present and need to define its geographic location	*Delimiting Survey* as described on page 7-4. Collect specimens and consult with the authorities listed in *Appendix D* to confirm identification.
have applied control measures and need to study their effectiveness	*Monitoring Survey* on page 7-8. Collect specimens and consult with the authorities listed in *Appendix D* to confirm identification.

Detection Survey

Detection surveys determine if a pest is present in a defined area and can be broad in scope to assess the presence of a pest or multiple pests over large areas or restricted to determine whether a specific pest or pests are present in a focused area.

Although negative results from a detection survey may not confirm the absence of a pest at a location, the surveys can provide reasonable confidence regarding pest occurrence.

Procedure

APHIS and state cooperators conduct pest detection surveys through the Cooperative Agriculture Pest Survey (CAPS) program, which is a part of the pest detection line item within USDA–APHIS–PPQ–PDEP. The state CAPS committee meets and develops the survey list for each state.

If the CAPS program determines that the pest should be surveyed, use the following procedure to conduct a detection survey for *M. fructigena*:

1. Prior to surveying, consider *M. fructigena*'s phenology to determine the time of survey
2. Focus the survey in locations where *M. fructigena* is more likely to occur including the following:
   - **A.** *Potential Distribution* on page 4-8 provides broad information on the geographical areas suitable for pest occurrence and is typically based on favorable environmental conditions and the presence of specific plant hosts as reported in states/counties
   - **B.** Within the potential distribution area, survey specific locations that have *Hosts* suitable for the pest species
   - **C.** Previous detection refers to information regarding commerce and previous interception records detailed in *Pathways*
3. The aforementioned information may be used to establish sentinel sites or targeted surveys for *M. fructigena*. Sentinel sites are locations regularly inspected along a surveyor's normal route. If sentinel sites are established for the pest species, use GPS to record the host plant locations and draw a map of the immediate area that includes reference points to aid others in finding the area if necessary. Once a sentinel site is established, the surveyor should re-inspect it on a regular basis (bimonthly or monthly) as permitted by their regular survey schedule. GIS can be used to map the sentinel site locations to promote even coverage, particularly in high-risk areas. Targeted surveys can be regularly conducted at nurseries and in areas of regular traffic from countries with known infections.
4. Survey coordinators should investigate commonalities to determine if the *M. fructigena* survey could be bundled with other ongoing surveys based on habitat, seasonality, taxonomic group, commodity/industry, priority and pathway
5. After determining the sites and design/bundling of the survey, conduct the survey using the CAPS-approved survey techniques for *M. fructigena*. Refer to the CAPS-Approved Survey Methodology for Negative Data Appendix M-1 for additional information.

A. Use visual inspection to examine host plants for symptoms. Refer to *Visual Inspection for Detection Survey* on page 7-4 for further information on inspection procedures.

6. Procure survey supplies.

7. Prior to beginning a survey, determine whether any pesticides have been recently applied rendering it unsafe to inspect the plant hosts and other substrates. Contact the property owner or manager and look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries. If pesticides have been applied, pest inspection should occur after the re-entry period. When visiting the area to conduct surveys or take samples, survey personnel must take strict measures to prevent pest contamination between properties during inspections. Confirm that the equipment and tools are clean. Determine and comply with all quarantine requirements that may be effective in the survey area.

8. Data entry forms are available from the CAPS Website for specific pests. If information on *M. fructigena* is not available here, use information from congeneric species or refer to *Data Collection* on page 7-6.

9. Morphological characteristics that may aid in preliminary identification of *M. fructigena* are described in *Identification* on page 3-1.

10. After a positive occurrence is suspected in the collected samples, submit the pest specimen(s) to the proper authority to confirm the detection. See *Sample Submission* on page on page E-1 and available *Taxonomic Support for Surveys* for further information.

A. To confirm disease, collect fruits showing typical symptoms. Take a minimum of 30 samples per orchard (5% confidence in detecting 10% disease prevalence). Place samples in labeled plastic bags detailing cultivar and date. Keep samples cool. Double bag the samples and deliver promptly to a diagnostic laboratory.

11. Data should be recorded for each survey site. Survey records and data recording formats should be consistent for standardizing the collection of information. If automated field collection services such as the Integrated Plant Health Information System (IPHIS) are used, ensure that all surveyors are trained in the technology prior to initiating the survey. Use the appropriate IPHIS templates for *M. fructigena*. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders prior to initiating the survey. After the survey is completed, all data should be entered into the designated state or national pest database. For example, if the detection data is entered in the National Agricultural Pest Information System (NAPIS) and publicly viewable from the Pest Tracker database

For additional information, refer to the CAPS survey guidelines (CAPS, 2014a).

Visual Inspection for Detection Survey

Use visual inspection as a tool when surveying for brown rot (*Monilinia fructigena*) in orchards, nurseries and residential landscapes.

Conduct a visual inspection in an orchard by looking for fruits with typical brown rot fungus symptoms. Examine any fruits displaying superficial, circular, brown spots associated with wounds growing outwards on the surface of the fruit. Mummified fruits on trees and on the orchard floor can also be collected. Traverse the orchard diagonally, across the backside and return to the starting point. Note the distribution of the problem including certain rows or low or high areas in the orchard. The absence of symptoms, however, does not necessarily mean *M. fructigena* is not present in the area inspected. Some infected fruits may not express symptoms, depending on the time and severity of the infection and in particular, less susceptible *Prunus* spp.

Delimiting Survey after Initial United States Detection

The objective of a delimitation survey is to determine the spatial extent of an exotic pest incursion following a detection. If *M. fructigena* is detected in the U.S., surveys will be conducted to determine the occurrence of an infection and its spread. After the initial detection, a Technical Working Group (TWG) is formed to prepare a delimitation survey plan to investigate the spread of *M. fructigena*. The TWG may consider the following information to recommend a delimitation survey plan for the introduced species.

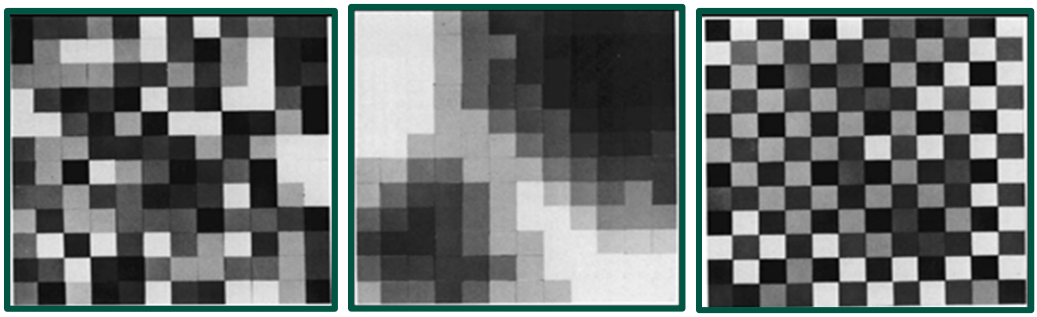
Delimitation Area

The total delimitation area may depend on information provided by the *Trace-Back and Trace-Forward Investigations*, the nearby host distribution, *Pathways* including the extent of natural and artificial dispersal, agency resources and logistics. An index (primary) site (Category A) is the property on which an initial detection of a disease or pathogen occurs. Each cultivated field, residential property and home garden within 15 m of the index field (Category A) must be sampled using a two-dimensional grid and submitted for laboratory analysis. Samples will be taken during the disease survey to determine if propagules are present. A designated laboratory will screen the samples. Plants may be visually surveyed, if necessary, to determine pathogen viability.

Generic Survey Techniques for Delimitation

Various sampling designs may be used to conduct a delimiting survey for plant pathogens including stratified random sampling (SRS), systematic and two-stage designs. Survey timing will depend upon the pathogen life cycle, the plant growth stage when infection is likely to occur and ecological parameters that support pathogen dispersal. Others considerations include the following:

1. Spatial patterns of diseased plants and of soil-borne plant pathogens in fields: A few examples are provided in Figure 7-1.
2. Hosts: If *M. fructigena* is detected in the U.S., the technical working group (TWG) should consider the preferred hosts of the pest near the detected area, the spatiotemporal distribution of these hosts and the host phenology suitable for the pest.
3. Pathways which include routes of natural and human-assisted dispersal
4. Logistics and available resources may include any related information helpful to the TWG for preparing the delimitation plan. Available resources can vary with the time and location of detection and the pest species.



A B C

Figure 7-1 Three spatial distributions of plant pathogens: (A) random, (B) aggregated and (C) regular (Nicot *et al.*, 1984)

Delimiting Survey

After determining the delimitation area, a specific survey design can be chosen. For this template, an SRS pattern is used.

- Divide field/area into uniform quadrants
  - Field dimensions and plant spacing are needed to divide the quadrants uniformly. Quadrants are represented by the squares in Figure 7-2.
- Quadrants are two dimensional regions whose size is defined as the total field area divided by number of sample sites. For example, a typical quadrants size could be 1 m × 1 m.
- Randomly select sample sites in each quadrant

- Each sample site is composed of a “cluster” of adjacent plants represented by the dots in Figure 7-2. Collect plants displaying signs or symptoms.

Any fruits displaying superficial, circular, brown spots associated with wounds growing outwards on the surface of the fruit or mummified fruits on trees and on the orchard floor can be collected

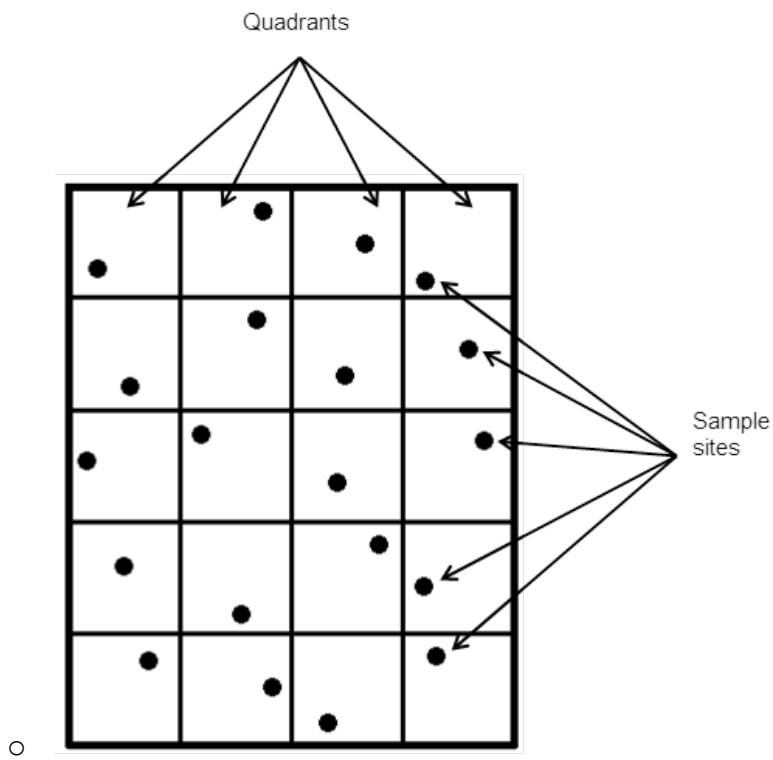


Figure 7-2 Stratified random sampling demonstrating uniform quadrants and sample sites

Data Collection

Flag the plant, tree or sampled location whenever possible, and draw a map of the immediate area, indicating reference points so that the areas can be found in the future if necessary. **Do not** rely solely on the flagging or other markers to re-locate a site as they may be removed. Record the GPS coordinates for each sampled area so that the area or plant may be re-sampled if necessary. Survey task forces should consist of an experienced survey specialist or plant pathologist familiar with *M. fructigena* and the symptoms of its damage.

Surveyors visiting sites to place holds or obtain samples should collect the following information:

- Date of collection or observations

- Collector's name
- Grower's field identification numbers
- Full name of business, institution, or agency
- Full mailing address including country
- Type of property (commercial nursery, hotel, natural field, residence)
- GPS coordinates of the host plant and property
- Host plant species and specific crop plant variety, if applicable
- Presence or absence of the pest
- Observations of signs and symptoms
- General conditions or any other relevant information

In the absence of inspection officials, take the following actions immediately if symptoms are noted

1. Mark the location
2. Take samples of symptomatic and asymptomatic plant parts and flag the location within the field
3. Notify the state or PPQ inspector
4. Place the samples from the infected plant inside two resealable plastic bags
5. Label the sealed bags with the following information:
   - **A.** Date
   - **B.** Name of person responsible
   - **C.** Location of sample collection
6. Keep bagged samples cool or refrigerated until the inspector arrives
7. **Do not** freeze the samples

After a positive occurrence is suspected in the collected samples, submit the pest specimen(s) to the proper authority to confirm the detection; see *Sample Submission* on page E-1.

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received *M. fructigena*-infected material. Speak to the growers or farm managers and obtain proper permission prior to entering private property.

Several actions should occur immediately upon confirmation that a nursery sample is positive for *M. fructigena*:

- Check nursery records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from affected nursery during the previous 6 months).
- Evaluate the situation, the location within the nursery and the severity.
- Check nursery records to identify potential sources of the infection including sources of seed outside the nursery.

Survey Records

Data should be recorded for each survey site. Survey records and data recording formats should be consistent for standardizing the collection of information. If automated field collection services such as the Integrated Plant Health Information System (IPHIS) are used, ensure that all surveyors are trained in the technology prior to initiating the survey. Use the appropriate IPHIS templates for *M. fructigena*. To reduce the burden on field data collectors, enter any known contact or address information into the database and hand-held data recorders prior to initiating the survey. After the survey is completed, all data should be entered into the designated state or national pest database.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. If *M. fructigena* is detected in the United States, a TWG will be assembled to provide guidance on using a monitoring survey to measure the effectiveness of applied treatments. Refer to *Control Procedures* on page 8-1 for further information regarding control options.

Procedure

Once *M. fructigena* has been confirmed from a particular field sample and control measures have been implemented, additional monitoring will be necessary. Use the following tools:

- Visual inspection of trees
- Collection of samples from potential hosts for several years and multiple times per season; refer to *Visual Inspection for Detection Survey* on page 7-4 further information concerning the inspection of host plants

Trace-Back and Trace-Forward Investigations

Trace-back and trace-forward investigations aid in prioritizing delimiting survey activities after an initial detection. Trace-back investigations attempt to determine the source of the infection. Trace-forward investigations attempt to define further potential dispersion through natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, efforts should be undertaken to determine the extent of the infection or the potentially infected areas in which to conduct further investigations.

Homeowner Properties

For positive detections on homeowner properties, ask the owner of the infected material to determine its point of origin (nursery, neighbors, *etc.*) and any possible sites of further distribution.

Nursery Properties

For nursery hosts, a list of facilities associated with potentially infected stock from nurseries testing positive for *M. fructigena* will be compiled. These lists will be distributed by the state to the field offices and are not to be shared with individuals outside the USDA–APHIS–PPQ regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is **prohibited**.

Each state is only authorized to see locations within their state, and sharing of confidential business information may be restricted between state and federal entities. Check the privacy laws with the State Plant Health Director for the state.

When notifying growers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received *M. fructigena*-infected material. Speak to the growers or farm managers and obtain proper permission prior to entering private property.

Several actions should occur immediately upon confirmation that a nursery host was infected with *M. fructigena*:

- Check nursery records to obtain names and addresses for all sales or distribution sites (if any sales or distribution has occurred from infected nursery during the previous 6 months).
- Evaluate the pest situation, including identification and inspection of the infected plant, the location within the nursery and the severity of infection.

- Check nursery records to identify potential sources of the infection inside or outside the nursery.

Analyzing Information

Use trace-back information gathered from the surveys and interactions to determine the origin of infection. With timely submitted records from landowners and growers, prioritized lists for further surveys can be prepared.

Cooperation with Other Surveys

Other surveyors regularly sent to the field should be trained to recognize outbreaks that could be associated with *M. fructigena*.

Chapter 8

Control Procedures

Overview of Emergency Programs

Plant Protection and Quarantine (PPQ) develops and makes control measures available to involved states. Environmental Protection Agency (EPA)-approved treatments will be recommended when available. If selected treatments are not labeled for use against the organism or in a particular environment, PPQ's FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) coordinator is available to explore the appropriateness in developing an emergency exemption under section 18, or a state special local need under section 24(c) of FIFRA, as amended. The PPQ FIFRA coordinator and pesticide-use coordinators are also available upon request to work with the EPA to expedite approval of a product that may not be registered in the United States, or to obtain labeling for a new use. Refer to *Resources* on page B-1 for information on contacting the coordinator.

Treatment Options

Treatments may include the following:

- *Cultural Control and Sanitary Measures* on page 8-1
- *Chemical Control* on page 8-4
- *Biological Control* on page 8-6
- *Host Resistance* on page 8-9

Cultural Control and Sanitary Measures

Losses due to *M. fructigena* rarely warrant specific control measures. Apart from avoiding susceptible cultivars in disease-prone districts, few control measures specifically target this fungus. Extensive literature is available on control strategies for the other two species, *M. fructicola* and *M. laxa*, that could be employed to control *M. fructigena*. Nonetheless, the best recommendation for

managing *M. fructigena* is the removal of mummified fruits via pruning, which can significantly reduce primary inoculum sources of brown rot (van Leeuwen *et al.*, 2002e; Wormald, 1954). This practice disrupts the primary inoculum source, which is released from these mummified fruits that are attached to the trees in the spring. During the season, this practice must be followed for all infected fruit to reduce sporulation and the number of airborne conidia in the orchard (Xu *et al.*, 2001f). Removing dropped fruit has proven effective at reducing the summer inoculum of apple diseases such as *M. fructigena*. Holb and Scherm (2007) established that the fruit dropped in early summer serves as a bridge between sporulation from overwintered fruit mummies in the spring and the first fruit with sporulating lesions in the tree during midsummer. Holb and Scherm (2007) indicated that removing these dropped fruits from the orchard floor significantly reduced disease incidence on the fruits in the tree. Other sanitary methods include the burning or deep-burying of mummified fruits and removal of wild host plants near orchards (CABI, 2014). Manuring and the application of potassium can reduce disease incidence in apricots (Byrde and Willetts, 1977; Vasudeva, 1930). Holb and Scherm (2008) also demonstrated that fruit damaged by *C. pomonella* should be removed during thinning in the beginning of the summer. Fruits that are clustered potentially harbor the larvae of *C. pomonella* and subsequently *can* infest each member of the fruit cluster through contact of each fruit.

Post-harvest losses due to brown rot that routinely occur during storage and transport (Hong *et al.*, 1997) are typically more severe than pre-harvest losses. However, post-harvest fungicides cannot be applied to stone fruit. Thus, damaged fruit should be removed from storage and transport containers to avoid fruit with incipient brown rot infections (Ogawa *et al.*, 1972; Wormald, 1954). Many authors consider the heat treatment of peach and nectarine fruits as a promising strategy to control post-harvest diseases such as brown rot (Jemric *et al.*, 2011; Karabulut and Baykal, 2004) and storage apple rot (Maxin *et al.*, 2012). Hot water treatment at 60 °C can reduce disease development without affecting fruit quality in nectarines and peaches (Casals *et al.*, 2010b). Based on work conducted by Spadoni *et al.* (2013), hot water treatment (60 °C for 30 and 60 seconds) could provide an effective and safe approach for managing *Monilinia* rots. The results indicate that conidial germination of *M. fructicola*, *M. fructigena* and *M. laxa* was completely inhibited by dipping fruit in hot water (55 °C) for 1 minute. However, the suppression of viable conidia or hyphae localized below the epidermal cells appeared more difficult (Spadoni *et al.*, 2013).

Other alternatives for controlling post-harvest brown rot include curing: Casals *et al*. (2010a) demonstrated that curing at 50 °C for 2 hours under 95–99% RH can adequately manage brown rot infections from the field in peaches and nectarines. However, this treatment did not protect fruit after the treatment process and prior

to cool storage. Thus, Casals *et al*. (2012) combined curing with either 1% chitosan or *Bacillus subtilis* (Ehrenberg) Cohn strain CPA-8 at 20 °C for 1 minute. Curing should eradicate the pre-existing *Monilinia* spp. infections initiated in the field, while the antagonist (chitosan or *B*. *subtilis*) protects the fruit during handling in packinghouses until used by the consumer (Casals *et al.*, 2012). Compared with the control, treatments using 1% chitosan or *B. subtilis* reduced brown rot incidence by 10 and 15%, respectively (Casals *et al.*, 2012).

Radiofrequency (RF) provides an alternative cultural method to control post-harvest disease caused by *Monilinia* spp. The RF efficacy depends on several factors with a number of obstacles that must be overcome before implementing RF treatment in commercial packinghouses. The most significant issue with RF heating is the heterogeneous temperature achieved within the fruit during treatment (Tang *et al.*, 2000). An RF heat treatment at 27.12 MHz, with 17 mm between the fruit and upper electrode proved effective in managing brown rot in peaches without affecting fruit quality (Casals *et al.*, 2010c). The RF treatment was further improved by immersing the fruit in water at 20 °C for 9 minutes (Sisquella *et al.*, 2013).

Integrating foliar pre-harvest calcium applications into controlled atmosphere and ultra-low oxygen storage conditions can reduce brown rot decay during long-term storage. However, the ability of these factors to reduce brown rot infections must not be overstated, and these treatments should not replace pre-harvest fungicide and insecticide applications (Holb *et al.*, 2012). The effectiveness of post-harvest treatments using high CO_2 levels (level species dependent, effective CO_2 ranges 10–40%) to control fungal decay in fruit has been well documented (Prusky *et al.*, 1997; Tian *et al.*, 2001). Elmer *et al*. (2007) demonstrated an incidence of brown rot of 40% in peach fruit treated with calcium pre-harvest, but 64% in fruit that received no calcium treatment. In large-capacity storage houses, fruit-to-fruit contact cannot be avoided, but the number of previously inoculated and injured fruit may be reduced via several methods such as careful fruit processing, sucrose polyester coating, individual film wrapping or modified atmosphere packaging (D'Aquino *et al.*, 2010; Díaz-Mula *et al.*, 2011).

Control of Wound Agents

Eradicating the wound agent is among the most effective methods of restricting the fruit-rot phase of brown rot disease (Byrde and Willetts, 1977). Various control options are available depending upon the wounding agent. If wounding results from weather-related conditions, an immediate application of a protectant fungicide is recommended (Byrde and Willetts, 1977). Sprays that induce russet formation on apples should be avoided as they could allow *M. fructigena* to invade the fruit (Byrde and Willetts, 1977).

Chemical Control

The key to successful management programs in apple orchards involves arthropod control, specifically *Cydia pomonella,* and fungal chemical control. In integrated orchards *C. pomonella* control is well set; however, in organic orchards *Bacillus thuringiensis*, confusion techniques and granulosis viruses are available for control with low efficacy (Holb and Scherm, 2008). Table 8-1 provides a list of insecticides and their modes of action for control of *C. pomonella*.

Table 8-1 Insecticides with modes of action used to control *Cydia pomonella*, codling moth (IRAC, 2013)

Class	Mode of action	Common name
avermectins	activates chloride channel	emamectin-benzoate
benzoylureas	inhibits chitin biosynthesis, type 0	diflubenzuron, flufenoxuron, lufenuron, novaluron, teflubenzuron, triflumuron
carbamates	inhibits acetylcholinesterase	carbaryl, methomyl
diacylhydrazines	ecdysone agonists	tebufenozide, methoxyfenozide
diamides	Modulates ryanodine receptor	flubendiamide, chlorantraniliprole
neonicotinoids	nicotinic acetylcholine receptor agonists	acetamiprid, thiacloprid
organophosphates	inhibits acetylcholinesterase	azynphos-methyl, chlorpyrifos, diazinon, malathion, parathion, phosmet, phosalone
oxadiazines	blocks voltage-dependent sodium channel	indoxacarb
phenoxy-phenoxy-ethyl carbamate	mimics juvenile hormone	fenoxycarb
pyrethroids	modulates sodium channel	lambda-cyhalothrin, beta-cyfluthrin, cypermethrin, deltamethrin, etofenprox,
spinosyns	modulates nicotinic acetylcholine receptor allosteric	spinosad, spinetoram

Protective fungicidal treatments control both blossom blight and fruit rot (Batra, 1991). The proper use of fungicides with systemic activity protects flowers and fruit, condenses the quantity of sporulation formed on the infected tissue and decreases sources of overwintering inoculum (Ogawa *et al.*, 1995). Copper fungicides were routinely used to control *Monilinia* spp. in the early and mid-1920s (Holb, 2006), but proved ineffective (Wormald, 1954). Bordeaux mixtures and similar substances (lime sulfur) were applied as winter treatments and significantly reduced the infection potential of the overwintering structures of this pathogen (Holb, 2005), but demonstrated phytotoxicity. Until the 1980s, benzimidazoles, specifically benomyl and thiophanate-methyl were used most often to control *Monilinia* spp., with (Ma *et al.*, 2003b). Protective fungicides

with nonspecific modes of action (captan, mancozeb, metiram, propineb, thiram, folpet, chlorotalonyl and ciram) were also used regularly (Baker *et al.*, 2011). New groups of fungicides with different modes of action have been registered due to the development of resistance by the fungi and the loss of efficacy with specific chemicals (Baker *et al.*, 2011; Brent and Hollomon, 2007). A common approach to manage fungicide resistance is to tank mix site-specific fungicides that are prone to developing resistance with multisite action materials (Russell, 1995; Staub, 1991)—for example, demethylation inhibitors (DMIs) tank-mixed with mixtures of elemental sulfur (Holb and Schnabel, 2008). Table 8-2 lists the fungicides and their modes of action for controlling *Monilinia* spp.

Table 8-2 List of fungicides with modes of action used to control *Monilinia* spp.

Class	Mode of action	Chemistry	Notes
benzimidazoles	interferes with spindle formation; blocks nuclear division (Clemons and Sisler, 1971; Davidse, 1973; Hammerschlag and Sisler, 1973)	benomyl, carbendazim (MBC), thiabendazole, thiophanate, thiophanate-methyl, fuberidazole (Erwin, 1973)	broad spectrum & systemic by xylem transfer (Russell, 1995)
dicarboximide	possibly protectant; interferes with osmotic signal transduction pathway (Yamaguchi and Fujimura, 2005)	iprodione (Lacroix *et al.*, 1974), vinclozolin (Pommer and Mangold, 1975), procymidone (Hisada *et al.*, 1977)	narrow activity spectrum includes *Botrytis*, *Sclerotinia* & *Monilia* (Brent and Hollomon, 2007)
DMI = triazole fungicides	inhibits C14-demethylase, which plays a role in sterol production (Wolfgang *et al.*, 2012)	metconazole (EPA, 2006), propiconazole, tebuconazole & tetraconazole	sterol biosynthesis inhibitor (SBI) class I; widely used many years (Wolfgang *et al.*, 2012)
keto-reductase-inhibitors (KRIs)	inhibits the C3-keto-reductase step in ergosterol biosynthesis (Debieu *et al.*, 2001)	hydroxyanilides, aminopyrazolinones	narrower activity spectrum than DMIs & amines, excellent botrycide, SBI class III (Debieu *et al.*, 2001)
succinate dehydrogenase inhibitor (SDHI)	inhibits fungal respiration (similar to Q_oI fungicides) by disrupting the tricarboxylic cycle & mitochondrial electron transport chain (Keon *et al.*, 1991).	**generation I:** carboxin (von Schmeling and Kulka, 1966) & oxycarboxin **generation II:** boscalid (Glättli *et al.*, 2011; Rheinheimer, 2007; Stammeler *et al.*, 2007), fluxapyroxad, penthiopyrad, isopyrazam & fluopyram (FRAC)	
quinone outside inhibitor (Q_oI)	inhibits fungal mitochondrial respiration by binding to cytochrome bc1 complex at Q_o site (Bartlett *et al.*, 2002; Sauter *et al.*, 1999; Ypema and Gold, 1999)	3 fungicide families: **strobilurins** (azoxystrobin, pyraclostrobin & trifloxystrobin), **fenamidone & famoxadone** (Reddy, 2013)	high-risk of resistance (Russell, 2005); reduced sensitivity of pathogens registered in apple orchards and vineyards (Brent and Hollomon, 2007)

Fungicides are not regularly applied during storage (Mari *et al.*, 2007); however, several fungicides, such as fludioxonyl, fenhexamid, tebuconazole, propiconazole and pyrimethanil, applied by dipping the fruit prior to storage have been registered in some countries including the U.S. (Karabulut *et al.*, 2010). Treatment can reduce the inoculum present on fruit surfaces (Karabulut *et al.*, 2010), but cannot affect the mycelium present inside fruits. In contrast, the European Union, Turkey and the U.S. do not allow the use of fungicides after harvest or in storage facilities (Karabulut and Baykal, 2004). Prior to 1996, fungicides such as benomyl, iprodione and triforine were registered in the U.S. for the post-harvest treatment of stone fruits to control brown rot (Feliciano *et al.*, 1992; Smilanick *et al.*, 1993). Due to fungicide resistance, benomyl can no longer be used, and iprodione was voluntarily removed from the market by Rhône-Poulenc Ag Co. in 1996 (Adaskaveg and Michailides, 1996).

Labeling

Although a proposed formulation may be approved for an effective eradication or control program, it may not be labeled, at the time of pest detection, for the specific use required. If a formulation is not labeled for the necessary use, one can request a federal crisis or quarantine exemption from the EPA under section 18 of FIFRA. For further information, refer to *Regulatory Procedures* on page 9-1. The prescribed formulation must be labeled for use on the site at which it is to be applied and must be registered for use in the state in which the eradication program is occurring. All applicable label directions must be followed, including requirements for personal protection equipment, maximum treatment rates, storage and disposal.

Biological Control

The biological control experiments discussed herein focus primarily on *M. fructicola* and *M. laxa*. Because *M. fructigena* is closely related to the two indicated species, these control strategies function similarly to manage *M. fructigena*. *Trichoderma* spp. are among the most widely demonstrated and utilized biological agents for controlling plant diseases (Elad, 1994; O'neill *et al.*, 1996). A.T.K. Corke (1977), indicated the presence of *T. viride* Persoon on mummified plum fruits treated with dinitro-*o*-cresol winter wash. Ale-Agha *et al*. (1974) then demonstrated that heat-killed *T. viride* spores suppressed *M. fructigena in vivo* (Byrde and Willetts, 1977). Other studies demonstrated the efficacy of various antagonists to control *Monilinia* spp. in stone fruit post-harvest (Karabulut *et al.*, 2003; Mari *et al.*, 2007; Zhou *et al.*, 2008); however, none are currently commercially available. *Pseudomonas cepacia* (ex Burkholder) Palleroni and Holmes, *P. fluorescens* Migula, *Bacillus thuringiensis* Berliner and

several isolates of *B. subtilis* have been tested for antagonism against *Monilinia* spp. The strains of *B. subtilis* proved most effective for controlling post-harvest peach brown rot (Pusey and Wilson, 1984). New shelf-stable and effective formulations of *B. subtilis* CPA-8 have been obtained via spray drying to manage brown rot on peach (Ya´nez-Mendizabal *et al.*, 2012). While analyzing the fermentation process on an industrial level, Yánez-Mendizábal *et al*. (2012) demonstrated that a low-cost medium (40 g/L defatted soy flour 44%, 5 g/L molasses plus mineral trace supplements) can support maximum *Bacillus* growth while maintaining effective biocontrol.

Aureobasidium spp. yeast is a potential biocontrol agent of post-harvest diseases in fruit. The potential increases significantly when combined with other routine post-harvest practices. Mari *et al*. (2012) identified and demonstrated the suitability of the L1 and L8 strains of *A. pullulans* (de Bary) G. Arnaud to control the three major species of *Monilinia* under critical post-harvest conditions. The efficacy of the L1 and L8 strains against brown rot was also examined under simulated commercial conditions (cold storage followed by fruit retail under ambient temperature) for nectarines stored at 0 °C for 21 days plus 9 days of shelf-life at 20 °C (Mari *et al.*, 2012). Low-temperature storage generally delays fungal growth; once fruits are removed from refrigeration, pathogens can resume growth, rapidly developing necrotic lesions (Mari *et al.*, 2012). No decay was observed on control fruits previously inoculated with *M. fructigena* under cold storage (21 days at 0 °C), while 49% of fruits developed infection after 7 days at 20 °C (Mari *et al.*, 2012). Both strains were effective in controlling brown rot after both cold storage and shelf-life and markedly reduced *M. fructigena* rots (above 90%), indicating that biocontrol of brown rot is feasible under commercial conditions with an appropriate formulation of the assayed antagonists (Mari *et al.*, 2012).

Penicillium frequentans Westling reduced the post-harvest brown rot caused by *M. laxa* and *M. fructigena* in laboratory assays (Guijarro *et al.*, 2006; Guijarro *et al.*, 2007). Guijarro *et al*. (2007) demonstrated that wettable powder formulations of the *P. frequentans* strain FOR_8 can be applied to fruit post-harvest to reduce brown rot during storage. Pre-harvest applications of the formulations decreased the pathogen inoculum density, which is particularly important because brown rot incidence in stone fruit caused by *Monilinia* spp. primarily depends on inoculum density combined with climatic factors (temperature and wetness duration) and fruit maturity (Gell unpublished; Luo and Michailides, 2001).

Epicoccum nigrum Link isolate 282 reduces peach twig blight and fruit rot caused by *Monilinia* spp. in field orchards (Larena *et al.*, 2005; Madrigal *et al.*, 1994; Melgarejo *et al.*, 1986; Pascual *et al.*, 1996). *Epicoccum nigrum* has been shown to produce morphological effects such as deformation of hyphae and germ tubes

in *M. laxa* (Madrigal and Melgarejo, 1995). Larena *et al*. (2004) developed a solid-state fermentation method for mass producing viable *E. nigrum* conidia. Several improvements have been made in the *E. nigrum* formulation to increase the efficacy of this biological control agent, one of which was the addition of non-toxic stabilizers to extend the shelf-life of the conidia. Stabilizers such as 50% polyethylene glycol 8000 (PEG 8000) and 1% KCl improved the viability of *E. nigrum* conidia over time by 57 and 71%, respectively (Larena *et al.*, 2007). Drying the *E. nigrum* conidia using 2.5% methylcellulose or 1% KCl plus silica powder further improved the shelf-life of the formulations (Larena *et al.*, 2007).

Resistance Inducers

Natural compounds with antimicrobial activity that elicit plant defense properties could provide alternatives to synthetic fungicides to control post-harvest disease in fruit (Bautista-Baños *et al.*, 2006). Such compounds include resistance inducers typically composed of pathogen or plant constituents or their analogs. The resistance inducers can potentially react with plant receptors to activate plant defenses, preventing pathogen infection (Elmer and Reglinski, 2006; Terry and Joyce, 2004). Feliziani *et al*. (2013) evaluated the ability of oligosaccharides, benzothiadiazole (BTH), chitosan and nettle macerate to inhibit the growth of *Monilinia laxa* and other fruit-rotting fungi. The effectiveness of these resistance inducers applied pre- and post-harvest was also evaluated.

Although pre-harvest treatment with BTH was unable to control brown rot, post-harvest BTH application (2 g/L) reduced the post-harvest decay of sweet cherry (Feliziani *et al.*, 2013). Benzothiadiazole mimics the effects of salicylic acid, which is involved in plant signal transduction systems and is required to activate the formation of defense compounds such as polyphenols and pathogenesis-related proteins (Durrant and Dong, 2004). Potassium bicarbonate is effective in reducing post-harvest brown rot of sweet cherries. However, symptoms of potassium bicarbonate phytotoxicity—pedicel browning and the formation of dark spots on the cherry surface—appeared after applications at concentrations equal to or greater than 9 g/L (Feliziani *et al.*, 2013). The pedicels of the sweet cherries treated with potassium bicarbonate dried early; thus, the dosage and the application time of potassium bicarbonate must be optimized to minimize the phytotoxicity symptoms for other stone and pome fruits (Feliziani *et al.*, 2013). Pre- and post-harvest applications of nettle macerate (10 g/L) reduced the infection indices for brown rot in sweet cherries exposed to cold storage followed by shelf life (20 ± 1 °C, 95–98% RH) by 63 and 80%, respectively. Pre- and post-harvest applications of fir extract (10 g/L) reduced the infection index by 57 and 68%, respectively. Pre- and post-harvest treatments with chitosan reduced brown rot infection indices by 63 and 67%, respectively (Feliziani *et al.*, 2013). In contrast, little information is available regarding the effects of pre- or post-harvest

applications of the commercial water-soluble chitosan formulation on sweet cherry post-harvest decay or the growth of *M. laxa*.

Host Resistance

Little evidence is available of breeding for resistance in the management of *Monilinia* spp. Several factors are considered significant in the resistance of fruit cultivars to brown rot including phenolic content, duration of flowering and skin thickness. Nevertheless, caution is warranted because few cultivars have undergone testing for resistance. Mechanisms involved in these factors include biochemical and cellular changes (Byrde and Willetts, 1977). The failure of *M. fructigena* conidia to infect wounds in fruits of some cider apple cultivars provides an example that has been ascribed to the oxidation of host phenolics by host polyphenol oxidase, followed by polymerization to brown oxidation products capable of precipitating the extracellular enzymes important to pathogenicity (Byrde, 1957; Byrde *et al.*, 1960). The genetic basis of host resistance to brown rot is poorly understood. However, many fruit tree cultivars have proven more or less resistant to *M. fructigena*. Cultivar susceptibility is high if the cultivars are late blooming and if the fruit is easily injured (Holb, 2006). Table 8-3 lists cultivars with apparent resistance to *M. fructigena*.

Table 8-3 Cultivars of pome and stone fruits “apparently resistant” to *Monilinia fructigena*

Fruit crop	Cultivars “apparently resistant” to *M. fructigena*	References
apples	La Claimanteuse, Beauty of Boskoop, Jonathan, Rote Jungfer, Rheinischer Bohnapfel, Ribston Pippin, Blenheim Orange, Edward VII, Slavyanka, Pepin, Giafrapnyi, Borsdorf Kitaika, Serinka, Titovka, Kekhura, Krasnyi Kal’vil, Tsigana, Kinula	Byrde (1956), Fawcett and Spencer (1967), Mittmann-Maier (1940), Rekhviashvili (1973), Skeivyte (1964), Sokolov (1962), Wormald (1954)
apricots	Neptun, Mamaia, Silvana, Sulina, Sirena	In Cociu cit. Soltesz (1990) Holb (2006)
peaches	Elberta, Red Bird, Stark Late Gold, Red Gold, La Gem, La Premiere, Cuberland, Pullars Cling	Brooks and Olmo (1955, 1957, 1966), Jordovic (1954), Kable (1974), Rekhviashvili (1973), Zehr (1974)
pears	Khechechuri, Bere-gri, Williams, Zamtris Klerzho	Rekhviashvili (1973)

Chapter 9

Regulatory Procedures

Use *Chapter 9 Regulatory Procedures* as a guide to the procedures that must be followed by regulatory personnel when conducting pest survey and control programs against *M. fructigena*.

Instructions to Officials

Agricultural officials must follow instructions for regulatory treatments or other procedures when authorizing the movement of regulated articles. Understanding the instructions and procedures is essential when explaining procedures to people interested in moving articles affected by the quarantine and regulations. Only authorized treatments can be used in line with labeling restrictions. During all field visits, ensure that proper sanitation procedures are followed.

Regulatory Actions and Authorities

After an initial suspect positive detection, an Emergency Action Notification may be issued to hold articles or facilities pending positive identification by a USDA–APHIS–PPQ-recognized authority and/or further instruction from the PPQ deputy administrator. If necessary, the deputy administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the *Federal Register*.

The Plant Protection Act of 2000 (Statute 7 USC 7701-7758) provides the authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under state authority.

State departments of agriculture normally work in conjunction with federal actions by issuing their own parallel hold orders and quarantines for intrastate movement. However, if the U.S. Secretary of Agriculture determines that an extraordinary emergency exists and that state measures are inadequate, intrastate regulatory action can be taken provided that the governor of the state has been

consulted and a notice has been published in the *Federal Register*. If intrastate action cannot or will not be taken by a state, PPQ may find it necessary to quarantine an entire state.

PPQ works in conjunction with state departments of agriculture to conduct surveys, enforce regulations and take control actions. PPQ employees must obtain permission of the property owner before entering private property. Under certain situations during a declared extraordinary emergency or if a warrant is obtained, PPQ can enter private property without owner permission. PPQ prefers to work with the state to facilitate access when permission is denied; however, each state government has varying authorities regarding entering private property.

A General Memorandum of Understanding (MOU) exists between PPQ and each state that specifies various areas in which PPQ and the state department of agriculture cooperate. For clarification, check with your State Plant Health Director (SPHD) or State Plant Regulatory Official (SPRO) in the affected state.

Tribal Governments

USDA–APHIS–PPQ also works with federally recognized Native American tribes to conduct surveys, enforce regulations and take control actions. Each tribe stands as a separate governmental entity (sovereign nation) with powers and authorities similar to state governments. Permission is required to enter and access tribal lands.

Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments, states that agencies must consult with Native American tribal governments about actions that may have substantial direct effects on tribes. Whether an action is substantial and direct is determined by the tribes. Effects are not limited to tribal land boundaries (reservations) and may include effects on off-reservation land or resources which tribes customarily use or even effects on historic or sacred sites in states where tribes no longer exist.

Consultation is a specialized form of communication and coordination between the federal and tribal governments. Consultation must be conducted early in the development of a regulatory action to ensure that tribes have opportunity to identify resources that may be affected by the action and to recommend the best ways to take actions on tribal lands or affecting tribal resources. Communication with tribal leadership follows special communication protocols. For more information, contact PPQ's Tribal Liaison.

To determine if there are federally recognized tribes in a state, contact the State Plant Health Director (SPHD). To determine if there are sacred or historic sites in

an area, contact the State Historic Preservation Officer (SHPO). For clarification, check with your SPHD or State Plant Regulatory Official (SPRO) in the affected state.

Overview of Regulatory Program after Detection

Once an initial US detection is confirmed, holds will be placed on the property by the issuance of an Emergency Action Notification. Immediately put a hold on the property to prevent the removal of any host plants of the pest.

Trace-back and trace-forward investigations from the property will determine the need for subsequent holds for testing and/or further regulatory actions. Further delimiting surveys and testing will identify positive properties requiring holds and regulatory measures.

Record-Keeping

Record-keeping and documentation are important for any holds and subsequent actions taken. Rely on receipts, shipping records and information provided by the owners, researchers or manager for information on destination of shipped plant material, movement of plant material within the facility and any management (cultural or sanitation) practices employed.

Keep a detailed account of the numbers and types of plants held, destroyed and/or requiring treatments in control actions. Consult a master list of properties, distributed with the lists of suspect nurseries based on trace-back and trace-forward investigations, or facilities within a quarantine area. Draw maps of the facility layout to located suspect plants and/or other potentially infected areas. When appropriate, take photographs of the symptoms, property layout and document plant propagation methods, labeling and any other information that may be useful for further investigations and analysis.

Keep all written records filed with the Emergency Action Notification documents, including copies of sample submission forms, documentation of control activities and related state-issued documents if available.

Issuing an Emergency Action Notification

Issue an Emergency Action Notification to hold all host plant material at facilities that have plant material suspected of direct or indirect connection to positive confirmations. Once an investigation determines the plant material is not infected

or testing determines there is no risk, the material may be released and the release documented on the EAN.

Establishing a Federal Regulatory Area or Action

Regulatory actions undertaken using Emergency Action Notifications continue to be in effect until the prescribed action is carried out and documented by regulatory officials. These may be short-term destruction or disinfection orders or longer term requirements for growers that include prohibiting the planting of host crops for a time. Over the long term, producers, shippers and processors may be placed under compliance agreements and permits issued to move regulated articles out of a quarantine area or property under an EAN.

Results analyzed from investigations, testing and risk assessment will determine the area to be designated for federal and parallel state regulatory actions. Risk factors will consider positive testing, positive associated and potentially infected exposed plants. Boundaries drawn may include a buffer area determined using risk factors and epidemiology.

Regulatory Records

Maintain standardized regulatory records and databases in sufficient detail to carry out an effective, efficient and responsible regulatory program.

Use of Chemicals

The PPQ *Treatment Manual* and these guidelines identify the authorized chemicals and describe the methods and rates of application and any special instructions. For further information refer to *Chemical Control* on page 8-4. Agreement by PPQ is necessary before using any chemical or procedure for regulatory purposes. No chemical can be recommended that is not specifically labeled for this pest. If a formulation is not labeled for the necessary use, one can request a federal crisis or quarantine exemption from the EPA under section 18 of FIFRA.

Chapter

10

Research Needs

There are many survey designs described in the scientific literature. However, no validated delimiting survey for *Monilinia fructigena* has been developed. This information would provide additional confidence in ascertaining the extent of the pathogen's movement in agricultural settings and provide details the steps necessary for adequate delimitation.

Literature Cited

M. fructigena

Adaskaveg, J. E., and T. J. Michailides. 1996. Cancellation of postharvest use of Rovral 50WP on stone fruit crops and other label changes of the fungicide. *in* D. P. S. D. K. A. C. University of California, ed. Central Valley Postharvest Newsletters, Davis.

Aderhold, R., and W. Ruhland. 1905. Zur Kenntnis der Obstbaum-Sklerotinien. Arbeit. Biol. Abt. Land und Forst. 4:427-442.

Alcock, N. L., and C. E. Foister. 1931. List of fungous diseases received by the Pathological Department of the Department of Agriculture for Scotland. transactions of the Botanical Society of Edinburg 30:338-350.

Ale-Agha, N., B. Dubos, C. Grosclaude, and J. L. Ricard. 1974. Antagonism between nongerminated spores of *Trichoderma viride* and *Botrytis cinerea*, *Monilia laxa*, *Monilia fructigena*, and *Phomopsis viticola*. Plant Disease Reporter 58:915-917.

AQAS. 2013. Agriculture Quarantine Activity Systems. *in*.

Baker, R., T. Candresse, E. Dormannsné Simon, G. Gilioli, J. C. Grégoire, M. J. Jeger, O. E. Karadjova, G. Lövei, D. Makowski, C. Manceau, M. Navajas, A. P. Puglia, T. Rafoss, V. Rossi, J. Schans, G. Schrader, G. Urek, J. C. van Lenteren, I. loutoglou, S. Winter, and M. Zlotinae. 2011. Pest risk assessment of *Monilinia farcical* for the EU territory and identification and evaluation of risk management options. European Food Safety Authority 9:155.

Balaža, J. 2000. *Monilia* spp. kao parazit voćaka. Biljni lekar:155-162.

Bannon, F., G. Gort, G. C. M. van Leeuwen, I. J. Holb, and M. J. Jeger. 2009. Diurnal patterns in dispersal of *Monilinia fructigena* conidia in an apple orchard in relation to weather factors. Agricultural and Forest Meteorology 149:8.

Bartlett, D. W., J. M. Clough, J. R. Godwin, A. A. Hall, M. Hamer, and B. Parr Dobrzanski. 2002. The strobilurin fungicides. Pest management science 58(7):649-662.

Batra, L. R. 1979a. First authenticated North American record of *Monilinia fructigena*, with notes on related species. Mycotaxon VIII(2):476-484.

Batra, L. R. 1979b. First authenticated North American record of *Monilinia fructigena*, with notes on related species. Mycotaxon 8:476-484.

Batra, L. R. 1991. World species of Monilinia (Fungi): Their ecology, biosystematics and control. J.

Cramer (Gebruder Borntraeger), Berlin - Stuttgart. 246 pp.

Bautista-Baños, S., A. Hernández-Lauzardo, M. Velázquez-del Valle, M. Hernández-López, E. Ait Barka, E. Bosquez-Molina, and C. Wilson. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection 25(2):108-118.

Benlloch, M. 1931. El moho de las frutas. Bol. Pat. veg. Ent. agric. Madr. 5:134-136.

Berkhout, C. M. 1923. De schimmelgeslachten *Monilia*, Oidium, Oospora en Torula, Scheveningen : Edauw & Johannissen, Utrecht.

Berrie, A. M. 1989. Storage rots of apple and pear in South East England 1980-1988: incidence and fungicide resistance. Pages 229-239 *In* I. Bulletin, (ed.). Integrated control of pome fruit diseases.

Boehm, E. W. A., Z. Ma, and T. J. Michailides. 2001. Species-specific detection of *Monilinia fructicola* from California stone fruits and flowers Phytopathology 91:428-439.

Boerema, H., H. A. Van Kesteren, M. M. J. Dorenbosch, and E. De Weert. 1971. Versl. Meded. plziektenk. Dienst Wageningen 145:142.

Boriss, H., H. Brunke, and M. Kreith. 2006. Commodity cherries profile. Iowa State University, Ames. Accessed on 30 April 2014, http://www.agmrc.org/commodities__products/fruits/cherry-profile/.

Bouhelier, R. 1936. Traitements en hiver. Fruits Primeurs 6:341-345.

Brent, K. J., and D. Hollomon. 2007. Fungicide resistance in crop pathogens: How can it be managed? *in*. Fungicide Resistance Action Committee 2007, Bristol.

Brooks, R. M., and H. P. Olmo. 1955. Register of new fruit and nut varieties List 10. Proceedings of the American Society of Horticulture Science 66:445-454.

Brooks, R. M., and H. P. Olmo. 1957. Register of new fruit and nut varieties. Proceedings of the American Society of Horticulture Science 70:557-584.

Brooks, R. M., and H. P. Olmo. 1966. Register of new fruit and nut varieties. Proceedings of the American Society of Horticulture Science 89(773-789):773.

Brunke, H., and M. Chang. 2002. Commodity peach profile. Iowa State University, Ames. Accessed on 23 April 2014, http://www.agmrc.org/commodities__products/fruits/peach-profile/.

Buchwald, N. F. 1943. Confirmation of *Monilinia* (*Sclerotinia) fructigena* (Aderh. et Ruhl.) Honey on hazel-nut *Corylus avellana*. Tidsskrift For Planteavl 47(3):521-538.

Burchill, R. T., and K. L. Edney. 1972. An assessment of some new treatments for the control of rotting of stored apples. Annals of Applied Biology 72(3):249-255.

Byrde, R. J. W. 1954. Observations on the sporulation of *Sclerotinia fructigena* on mummified apples and plums in late spring and summer. Annual Report Long Ashton Agricultural Horticultural Research Station 1953:163-166.

Byrde, R. J. W. 1956. The varietal resistance of fruits to brown rot I. Infection experiments with *Sclerotinia fructigena* Aderh. & Ruhl. on certain dessert, culinary and cider varieties of apple. Journal of Horticultural Science 31:188-195.

Byrde, R. J. W. 1957. The varietal resistance of fruits to brown rot; II. The nature of resistance in some varieties of cider apple. Journal of Horticultural Science 32:12.

Byrde, R. J. W., A. H. Fielding, and A. H. Williams. 1960. The role of oxidized polyphenols in the varietal resistance of apples to brown rot Pages 95-99 *in* J. B. Pridham, (ed.).Phenolics in plants in health and disease. Proceedings of a Plant Phenolics Group Symposium Bristol.

Byrde, R. J. W., and H. J. Willetts. 1977. The brown rot fungi of fruit; their biology and control (First). Pergamon Press, Oxford. 171 pp.

CABI. 2014. Invasive Species Compendium. CAB International Wallingford, UK.

CAPS. 2014a. 2015 National Survey Guidelines. Accessed

CAPS. 2014b. Cooperative Agricultural Pest Survey. Accessed on 1 May 2014, https://caps.ceris.purdue.edu/pest_lists.

Casals, C., P. A. G. Elmer, I. Vi˜nas, N. Teixidó, M. Sisquella, and J. Usall. 2012. The combination of curing with either chitosan or *Bacillus subtilis* CPA-8 to control brown rot infections caused by *Monilinia fructicola*. Postharvest Biology and Technology 64:7.

Casals, C., N. Teixidó, I. Viñas, S. Llauradó, and J. Usall. 2010a. Control of *Monilinia* spp. on stone fruit by curing treatments: Part I. The effect of temperature, exposure time and relative humidity on curing efficacy. Postharvest Biology and Technology 56(1):19-25.

Casals, C., N. Teixido, I. Vinas, E. Silvera, N. Lamarca, and J. Usall. 2010b. Combination of hot water, *Bacillus subtilis* CPA-8 and sodium bicarbonate treatments to control postharvest brown rot on peaches and nectarines. European Journal of Plant Pathology 128(1):51-63.

Casals, C., I. Vi˜nas, A. Landl, P. Picouet, R. Torresa, and J. Usalla. 2010c. Application of radio frequency heating to control brown rot on peaches and nectarines. Postharvest Biology and Technology 58:7.

Chaboussou, -.-.-.-. 1945. Sur deux rynchites (R. bacchus L. et R. aequatus L.) nuisibles aux arbres fruitiers en Agenais. C.R. Acad. Agric. Fr. 31:110-112.

Chang, L. W. H. 1986. Pests not known to occur in the United States or of limited distribution (PNKTO), No. 76: A fruit brown rot. United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Hyattsville. 1-14 pp.

Christoff, A. 1934. Some plant diseases new to Bulgaria. (II. Contribution). Bulletin Society of Botany de Bulgarie 6:37-48.

Christoff, A. 1938. The brown rot diseases of fruit trees in Bulgaria. Journal agricultural Experiment Station Bulgaria 8:3-32.

Clemons, G. P., and H. D. Sisler. 1971. Localization of the site of action of a fungitoxic benomyl derivative. Pesticide Biochemistry and Physiology 1:32-43.

Comes, O. 1891. Crittogamis Agraria. *in*. Napoli, Marghieri.

Corke, A. T. K. 1977. Private communication. Personal communication to R. J. W. Byrde and H. J. Willetts on

Côté, M. J., M. C. Tardif, and A. J. Meldrum. 2004. Identification of *Monilinia fructigena*, *M. fructicola*, *M. laxa* and *Monilia polystroma* on inoculated and naturally infected fruit using multiplex PCR. Plant Disease 88:1219-1225.

Croxall, H. E., C. A. Collingwood, and J. E. E. Jenkins. 1951. Observations on brown rot (*Sclerotinia fructigena*) of apples in relation to injury caused by earwigs (*Forficula auricularia*) Annals of Applied Biology 38(4):13.

D'Aquino, S., A. Palma, M. Schirra, A. Continella, E. Tribulato, and S. La Malfa. 2010. Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. Postharvest Biology and Technology 55(2):121-128.

Daff. 2008. Diagnostic protocol for *Monilinia fructigena* (Apple Brown Rot). Department of Agriculture, Forestry and Fisheries, Australian Government.

Davidse, L. C. 1973. Antimitotic activity of methyl benzimidazol-2-yl carbamate (MBC) in *Aspergillus nidulans*. Pesticide Biochemistry and Physiology 3:317-325.

Debieu, D. B., J., M. Hugon, C. Maloose, and P. Leroux. 2001. The hydroxyanilide fenhexamid, a new sterol biosynthesis inhibitor fungicide efficient against the plant pathogenic fungus *Botryotinia fuckeliana* (*Botrytis cinerea*). Pest management science 57:1060-1067.

Delacroix, G., and A. Maublanc. 1909. Maladies des plantes cultivées. Maladies parasitaires:260-263.

Díaz-Mula, H. M., D. Martínez-Romero, S. Castillo, M. Serrano, and D. Valero. 2011. Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality. Postharvest Biology and Technology 61(2):103-109.

Doidge, E. M. 1919. Diseases of stone fruit trees. Brown rot or fruit mold, *Sclerotinia fructigena* (Pers.) Schroet. . South African Fruit Growers 6:305.

Doidge, E. M., and A. M. Bottomley. 1931. A revised list of plant diseases occurring in South Africa Botanical Survey of South Africa, Memoir:1-78.

Duggar, B. M. 1909. Fungous diseases of plants.

Durrant, W. E., and X. Dong. 2004. Systemic acquired resistance. Annual Review Phytopathology 42:185-209.

Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. Crop Protection 13(1):35-38.

Elmer, P. A. G., and T. Reglinski. 2006. Biosuppression of *Botrytis cinerea* in grapes. Plant Pathology 55(2):155-177.

Elmer, P. A. G., T. M. Spiers, and P. N. Wood. 2007. Effects of pre-harvest foliar calcium sprays on

fruit calcium levels and brown rot of peaches. Crop Protection 26(1):11-18.

Emery, K. M. 2000. Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. Plant Disease 84:4.

EPA. 2006. EPA Pesticide fact sheet. Accessed on 29 April 2014

Eriksson, J. 1913. Zur Kenntnis der durch Monilia-Pilze hervorgerufenen Blüten- und Zweigdürre unserer Obstbäume. Mykol. Zbl. 2:65-78.

ERS. 2002. Cherries: sweet and tart, commodity highlight. Accessed on 30 April2014

Erwin, D. C. 1973. Systemic fungicides: disease control, translocation and mode of action. Annual review of Phytopathology 11:389-422.

Esfandiari, E. 1947. Diseases of cultivated plants and fruit trees of the sub-tropical regions of northern Iran. Publ. Trimest. Dep. gen. Prot. Pl. Tehran 5 1-21.

Esfandiari, E. 1949. Troisiéme liste des fungi rmassés en Iran. Ent. Phytopath. appl., Tehran, 1948 8:16.

Falconi, C. J., and K. Mendgen. 1994. Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 101(1):38-47.

FAS. 2012. Global Agricultural Trade System. Accessed on 30 April 2014, http://apps.fas.usda.gov/gats/ExpressQuery1.aspx.

Fawcett, C. H., and D. M. Spencer. 1967. Antifungal phenolic acids in apple fruits after infection with *Sclerotinia fructigena*. Annals of Applied Biology 60(1):87-96.

Feliciano, A., A. J. Feliciano, J. Vendrusculo, J. E. Adaskaveg, and J. M. Ogawa. 1992. Efficacy of ethanol in postharvest benomyl-DCNA treatments for control of brown rot of peach. Plant Disease 76:3.

Feliziani, E., M. Santini, L. Landi, and G. Romanazzi. 2013. Pre- and postharvest treatment with alternatives to synthetic fungicides to control postharvest decay of sweet cherry. Postharvest Biology and Technology 78(.).

Förster, H., and J. E. Adaskaveg. 2000. Early brown rot infections in sweet cherry fruit are detected by *Monilinia*-Specific DNA primers. Phytopathology 90:171-178.

FRAC. Crop Life. Accessed, www.frac.info

Fulton, C. E., and A. E. Brown. 1997. Use of SSU rDNA group-I intron to distinguish *Monilinia fructicola* from *M. laxa* and *M. fructigena*. FEMS Microbiology Letters 157:307-312.

Fulton, C. E., G. C. M. van Leeuwen, and A. E. Brown. 1999. Genetic variation among and within *Monilinia* species causing brown rot of stone and pome fruits. European Journal of Plant Pathology 105:495-500.

Gallástegui, I. 1942. The winter treatment of cider apples. Agricultura Madr. 11:17-19.

Garbowski, L., and H. Juraszkówna. 1933. Diseases of useful plants in the period 1926 to 1930. A summary of the reports of the Plant Protection Stations. Rocznik Ochrony Roślin 1:97-235.

Garić, R., S. Stamenkovic, and M. Canak. 1990. The effect of *Cydia molesta* on the incidence of *Monilinia* spp. on quince fruits. EPPO Bulletin 20:3.

Geisler, M. 2012a. Commodity apple proflie Iowa State University, Ames. Accessed, http://www.agmrc.org/.

Geisler, M. 2012e. Commodity pear profile. Iowa State University, Ames. Accessed on 23 April 2014, http://www.agmrc.org/commodities__products/fruits/pears/.

Gell, I., J. Cubero, and P. Melgarejo. 2007. Two different PCR approaches for universal diagnosis of brown rot and identification of *Monilinia* spp. in stone fruit trees. Journal of Applied Microbiology:9.

Gell, I., A. De Cal, R. Torres, J. Usall, and P. Melgarejo. 2008. Relationship between the incidence of latent infections caused by *Monilinia* spp. and the incidence of brown rot of peach fruit: factors affecting latent infection. European Journal of Plant Pathology 121(4):487-498.

Gell unpublished, I. *in*.

Glättli, A., T. Grote, and G. Stammler. 2011. SDH-inhibitors: History, biological performance and molecular mode of action. *In* H. B. D. H.W. Dehne, U. Gisi, K. H. Kuck, P.E. Russell, and H. Lyr, (ed.). Modern fungicides and antifungal compounds, Braunschweigh

Guijarro, B., I. Larena, P. Melgarejo, and A. De Cal. 2006. Effect of drying on conidial viability of *Penicillium frequentans,* a biological control agent against peach brown rot disease caused by *Monilinia* spp. . Biocontrol Science and Technology 16(3):257-269.

Guijarro, B., P. Melgarejo, R. Torres, N. Lamarca, J. Usall, and A. De Cal. 2007. Effects of different biological formulations of *Penicillium frequentans* on brown rot of peaches. Biological Control 42:86-96.

Hafiz, A. 1946. Brown rot of stone and pome fruits. Punjab Fruit Journal:145-146.

Hakhoe, H. g. S. P. 1972. A list of plant diseases, insect pests, and weeds in Korea 1-424 pp.

Hammerschlag, R. S., and H. D. Sisler. 1973. Benomyl and methyl-2-benzimidazolecarbamate (MBC): biochemical, cytological and chemical aspects of toxicity to *Ustilago maydis* and *Saccharomyces cerevesiae* Pesticide Biochemistry and Physiology 3:42-54.

Harada, Y. 1977. Studies on the Japanese species of Monilinia (Sclerotiniaceae). Bulletin of the Faculty of Agriculture, Hirosaki University (27):30-109.

Harada, Y., S. Nakao, M. Sasaki, Y. Sasaki, Y. Ichihashi, and T. Sano. 2004. *Monilia mumecola, a new brown rot fungus on Prunus mume* in Japan. Journal of General Plant Pathology:11.

Harrison, T. H. 1928. Brown rot of fruits, and associated diseases, in Australia

Part 1. History of the disease and determination of the causal organism. Journal Proceedings of the Royal Society of New South Wales 62:99-151.

Harrison, T. H. 1933. Brown rot of fruits and associated diseases of deciduous fruit trees. I. Journal and Proceedings of the Royal Society of New South Wales. 67:131-177.

Hashmi, M. H., G. Morgan-Jones, and B. Kendrick. 1972. Conidium ontogeny in hyphomycetes. *Monilia* state of *Neurospora sitophila* and *Sclerotinia laxa*. Canadian Journal of Botany

50:2.

Hily, J.-M., S. D. Singer, S. M. Villani, and K. D. Cox. 2011. Characterization of the cytochrome b (cyt b) gene from Monilinia species causing brown rot of stone and pome fruit and its significance in the development of QoI resistance. Pest management science 67:12.

Hisada, Y., T. Kato, and Y. Kawase. 1977. Systemic movement in cucumber plants and control of cucumber gray mould by a new fungicide, S-7131. Netherlands Journal of Plant Pathology 83(1):71-78.

Holb, I. 2004a. Yield loss and disease development of *Monilinia fructigena* (Aderh. & Ruhl.) Honey in an organic apple Journal of Agricultural Sciences 15:2.

Holb, I. J. 2004b. The brown rot fungi crops (Monilinia spp.): II. important features of their epidemiology. International Journal of Horticultural Science 10:17-35.

Holb, I. J. 2005. Effect of fungicide treatments and sanitation practices on brown rot blossom blight Incidence, phytotoxicity, and yield for organic sour cherry production. Plant Disease 89:7.

Holb, I. J. 2006. Possibilities of brown rot management in organic stone fruit production in Hungary. International Journal of Horticultural Science 12:5.

Holb, I. J. 2008. Monitoring conidial density of *Monilinia fructigena* in the air in relation to brown rot development in integrated and organic apple orchards. European Journal of Plant Pathology 120:12.

Holb, I. J., B. Balla, A. Vámos, and J. M. Gáll. 2012. Influence of preharvest calcium applications, fruit injury, and storage atmospheres on postharvest brown rot of apple. Postharvest Biology and Technology 67:29-36.

Holb, I. J., and H. Scherm. 2007. Temporal dynamics of brown rot in different apple management systems and importance of dropped fruit for disease development. Phytopathology 97(9):1104-1111.

Holb, I. J., and H. Scherm. 2008. Quantitative relationships between different injury factors and development of brown rot caused by *Monilinia fructigena* in integrated and organic apple orchards Phytopathology 98:7.

Holb, I. J., and G. Schnabel. 2008. The benefits of combining elemental sulfur with a DMI fungicide to control *Monilinia fructicola* isolates resistant to propiconazole. Pest management science 64(2):156-164.

Holst-Jensen, A., L. M. Kohn, K. S. Jakobsen, and T. Schumacher. 1997. Molecular phylogeny and evolution of *Monilinia* (Sclerotiniaceae) based on coding and non-coding rDNA sequences. American Journal of Botany 84(5):686-701.

Honey, E. E. 1928. The monilioid species of *Sclerotinia*. Mycologia 20(3):127-157.

Hong, C., B. A. Holtz, D. P. Morgan, and T. J. Michailides. 1997. Significance of thinned fruit as a source of the secondary inoculum of *Monilinia fructicola* in California nectarine orchards. Plant Disease 81(5):519-524.

Hrustic, J., M. Grahovac, M. Milhajlovic, G. Delibasic, M. Ivanovic, M. Nikolic, and B. Tanovic. 2012. Molecular detection of *Monilinia fructigena* as causal agent of brown rot on quince. Pestic. Phytomed. (Belgrade) 27(1):15-24.

Hu, M.-J., K. D. Cox, G. Schnabel, and C.-X. Luo. 2011. *Monilinia* species causing brown rot of peach in China. PLos one 6:14.

Hughes, K. J. D., C. E. Fulton, D. McReynolds, and C. R. Lane. 2000. Development of new PCR primers for identification of *Monilinia* species. EPPO Bulletin 30.

Ibragimov, G. R., and T. F. Abbasov. 1976. The occurrence of the ascus state of *Monilia fructigena* (Schroet.) Honey and *M. cydoniae* (Schell.) Honey in Azerbaijan. Mikologiya i fitopatologiya 10:219-222.

Ioos, R., and P. Frey. 2000. Genomic variation within *Monilinia laxa*, *M. fructigena*, and *M. fructicola*, and application to species identification by PCR. European Journal of Plant Pathology 106:373-378.

IRAC. 2013. Codling moth team update. Accessed on 30 July 2014, http://www.irac-online.org/.

Ivić, D., T. Fazinić, J. Cole, and A. Novak. 2013. *Monilinia* species identified on peach and nectarine in Croatia, with the first record of *Monilinia fructicola*. EPPO Bulletin 44:70-72.

Japp, O. 1908. Beitrage zur Pilzflora der osterreichischen Alpenlander. 1. Pilze aus Sudtirol und Karnten. Annales Mycologici 6(3):192-221.

Jemric, T., D. Ivic, G. Fruk, H. S. Matijas, B. Cvjetkovic, M. Bupic, and B. Pavkovic. 2011. Reduction of postharvest decay of peach and nectarine caused by *Monilinia laxa* using hot water dipping. Food and bioprocess technology 4(1):149-154.

Joessel, P. H. 1932. Quelques observations sur les traitements à envisager pour l'Abricotier et le Pêcher dans la vallée du Rhône. Revue de pathologie végétale et d'entomologie agricole 19:82.

Jordovic, M. 1954. Testing the susceptibity of peach varieties to the Monilia parasite Arh. poljopr. Nauk 7:96-99.

Jørstad, I. 1923. Beretning om planteskydommer i land- og hagebruket II. Frukttraer og baervekster:73.

Jørstad, I. 1938. *Monilia* på steinfrukt. Medlemsskr. Selsk. Havedyrkn. Venners 16:19-20.

Jørstad, I. 1945. Parasittsoppene på kultur- og nyttevekster i Norge. I. Sekksporesopper (Ascomycetes) og konidiesopper (Fungi imperfecti). Meldinger fra Statens Plantepatologiske Institutt 1:69-71.

Kable, P. F. 1965. Air dispersal of conidia of *Monilinia fructicola* in peach orchards. Australian Journal of experimental Agriculture and Animal Husbandry 5:6.

Kable, P. F. 1974. Reduce rots in stone fruit by post harvest dipping. Agricultural Gazette of New South Wales 85:43-44.

Karabulut, O. A., and N. Baykal. 2004. Integrated control of postharvest diseases of peaches with a yeast antagonist, hot water and modified atmosphere packaging. Crop Protection 23:5.

Karabulut, O. A., J. L. Smilanick, C. H. Crisosto, and L. Palou. 2010. Control of brown rot of stone fruits by brief heated water immersion treatments. Crop Protection 29:4.

Karabulut, O. A., J. L. Smilanick, F. Mlikota-Gabler, M. Mansour, and S. Droby. 2003. Near-harvest applications of *Metschnikowia fructicola*, ethanol, and sodium bicarbonate to control postharvest diseases of grape in Central California Plant Disease 87:6.

Kechakmadze, L. A., and I. Kikvadze. 1976. V. Diseases of feijoa. Zashchita Rastenii:43.

Keon, J. P. R., G. A. White, and J. A. Hargreaves. 1991. Isolation, characterization and sequence of a gene conferring resistance to the systemic fungicide carboxin from the maize smut pathogen, *Ustilago maydis* Current Genetics 19:475-481.

Kunze, G., and J. C. Schmidt. 1817. Mycologische Hefte I. *in*, Leipzig.

Lack, K. J. 1989. The spread of apple brown rot (*Monilinia fructigena*) by insects. Annals of Applied Biology 115(2):221-227.

Lacroix, L., C. Bic, L. Burgaud, M. Guillot, R. Leblanc, R. Riottot, and M. Sauli. 1974. Etude des propriétés antifongiques d'une nouvelle famille des dérivés de l'hydantoine et en particulier du 260190. R.P. Phytiatrie-Phytopharmacie 23:165-174.

Landgraf, F. A., and E. I. Zehr. 1982. Inoculum sources for *Monilinia fructicola* in South Carolina peach orchards. Phytopathology 72(2):6.

Lane, C. R. 2002. A synoptic key for differentiation of *Monilinia fructicola*, *M. fructigena* and *M. laxa*, based on examination of cultural characters. EPPO Bulletin 32(3):489-493.

Larena, I., A. De Cal, and P. Melgarejo. 2004. Solid substrate production of *Epicoccum nigrum* conidia for biological control of brown rot on stone fruits. International Journal of Food Microbiology 94(2):161-167.

Larena, I., A. De Cal, and P. Melgarejo. 2007. Effects of stabilizers on shelf-life of *Epicoccum nigrum* formulations and their relationship with biocontrol of postharvest brown rot by *Monilinia* of peaches. Journal of Applied Microbiology 102(2):570-582.

Larena, I., X. O. de Eribe, J. Usall, R. Torres, A. De Cal, M. Liñán, P. Melgarejo, P. Domenichini, A. Bellini, J. F. Mandrin, and J. Lichou. 2005. Biological control of postharvest brown rot (*Monilinia* spp.) of peaches by field applications of *Epicoccum nigrum*. Biological Control 32(2):305-310.

Lesik, K. 2013. Monilinia species causing fruit brown rot, blossom and twig blight in apple orchards in Belarus. Proceedings of the Latvian Academy of Sciences 67:192-194.

Lovisolo, O. 1955. *Monilia* species on fruit tress.1. Reports of new hosts and observations on some of those already noted. Soil. Staz. Pat. Veg. Roma 13:7-40.

Luo, Y., and T. J. Michailides. 2001. Factors affecting latent infection of prune fruit by *Monilinia fructicola*. Phytopathology 91(9):864-872.

Luo, Y., T. J. Michailides, D. P. Morgan, W. H. Krueger, and R. P. Buchner. 2005. Inoculum dynamics, fruit infection, and development of brown rot in prune orchards in California. Phytopathology:5.

Ma, Z., Y. Luo, and T. J. Michailides. 2003a. Nested PCR assays for detection of *Monilinia fructicola* in stone fruit orchards and *Botrytiosphaeria dothidea* from pistachios in California Journal of Phytopathology 151:312-322.

Ma, Z., A. Yoshimura, and T. J. Michailides. 2003b. Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. Applied and Environmental Microbiology 69(12):9.

Madrigal, C., and P. Melgarejo. 1995. Morphological effects of *Epiccocum nigrum* and its antibiotic flavipin on *Monilinia laxa*. Canadian Journal of Botany 73:7.

Madrigal, C., S. Pascual, and P. Melgarejo. 1994. Biological control of peach twig blight (*Monilinia laxa*) with *Epicoccum nigrum*. Plant Pathology 43(3):554-561.

Malkoff, K. 1908. Erster Beitrag zur Kenntnis der Pilzflora Bulgariens. Annales Mycologici 6:29-36.

Map22. 2000. *Monilinia fructigena* (Distribution map).

Marchal, E., and E. Marchal. 1921. Contribution a Letude des champignons fructicoles de Belgique. Bulletin de la Société Royale de Botanique de Belgique / Bulletin van de Koninklijke 54:109-139.

Mari, M., C. Martini, M. Guidarelli, and F. Neri. 2012. Postharvest biocontrol of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. Biological Control 60:132-140.

Mari, M., R. Torres, L. Casalini, N. Lamarca, J. F. Mandrin, J. Lichou, I. Larena, A. De Cal, P. Melgarejo, and J. Usall. 2007. Control of post-harvest brown rot on nectarine by *Epicoccum nigrum* and physico-chemical treatments. Journal of the Science of Food and Agriculture 87:6.

Matheny, W. A. 1913. A comparison of The American brown-rot fungus with *Sclerotinia fructigena* and *S. cinera* of Europe. Botanical Gazette 56:418-432.

Maxin, P., R. W. Weber, H. L. Pedersen, and M. Williams. 2012. Control of a wide range of storage rots in naturally infected apples by hot-water dipping and rinsing. Postharvest Biology and Technology 70:25-31.

Melgarejo, P., R. Carrillo, and E. M. Sagasta. 1986. Potential for biological control of *Monilinia laxa* in peach twigs. Crop Protection 5(6):422-426.

Mijuskhovits, M. 1950. Diseases of plants recorded in Montenegro in 1949. Plant Protection, Belgrade (1).

Mittmann-Maier, G. 1940. Gartenbauwissenschaft 15:334-361.

Miura, M. 1929. Flora of Manchuria and East Mongolia. Part III. . Cryptogams, Fungi:93-98.

Molliard, M. M. 1901. Sur une epidémie de Rot Brun aux environs de Paris. Journal of the Royal Horticultrual Society 26.

Montemartini, L. 1924. Rassegna crittogamica per l'anno, 1921, 1922, 1923. Atti Ist. bot. Univ. Pavia 1:9- 63.

Montemartini, L. 1936. New observations on the parasites and diseases of plants grown in western Sicily: three-year period 1934-1936. Riv. Pat. veg 26:355-377.

Moore, M. H. 1950. Brown rot of apples: fungicide trials, and studies of the relative importance of different wound agents. Journal of Horticultural Science 25:9.

Moore, M. H., and P. W. Talboys. 1953. Note on infection of blackberry fruits by the brown rot fungus, *Monilia fructigena*. 136-137 pp.

Mordue, J. E. M. 1979. IMI descriptions of fungi and bacteria: *Sclerotinia fructigena* (No. 617) CAB International 62:1-2.

Muller-Thurgau, H., and A. Osterwalder. 1924. Foreign Title: Bericht der Schweizerischen Versuchsanstalt fur Obst-, Wein- und Gartenbau in Wädenswil fur die Jahre 1921-1923, iv. A. 6. Ueber die durch *Cercospora macrospora* verursachte Blattkrankheit an Pensées

Title: Report of the Swiss Experiment Station for fruit growing, viticulture, and horticulture in Wadenswil for the years 1921 to 1923. iv. A. 6. The leaf disease of pansies caused by *Cercospora macrospora*. Landwitschaftliches Jahrbuch der Schweiz 38:616-617.

Munda, A., and M. Virščeк-Marn. 2010. First report of brownrot caused by *Monilinia fructicola* affecting peach orchards in Slovenia. Plant Disease 94:1116.

Nagorny, P. I., and S. J. Issarlischwili. 1929. Reprentatives of the fungal flora of the vine hiterto unknown in the Caucasus. Bulletin Inst. Exp. Agric. Georgia, Tiflis 1:5-18.

NASS. 2013. Noncitrus fruits and nuts 2012 preliminary summary. Accessed on 7 April 2014, http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-01-25-2013.pdf.

Negru, A., E. Mircea, and A. Crisan. 1957. Studii Cerc. Agron. Cluj 8:93-98.

Nicot, P. C., D. I. Rouse, and B. S. Yandell. 1984. Comparison of statistical methods for studying spatial patterns of soilborne plant pathogens in the field Phytopathology 74:4.

NPB. 2002. Preventing the introduction of plant pathogens: Into the United States: The role and application of the "systems approach". 1-98 pp.

O'neill, T., A. Niv, Y. Elad, and D. Shtienberg. 1996. Biological control of *Botrytis cinerea* on tomato stem wounds with*Trichoderma harzianum*. European Journal of Plant Pathology

102(7):635-643.

Ogawa, J. M., W. E. Yates, B. T. Manji, and R. E. Cowden. 1972. Ground and aircraft applications of thiophanate-methyl on control of stone fruit brown rot blossom blight. Pages 781-781 *in*Phytopathology. American Phytopathological Society 3340 Pilot Knob Rd., St Paul, MN 55121.

Ogawa, J. M., E. I. Zehr, G. W. Bird, D. F. Richie, K. Uriu, and J. K. Uyemoto. 1995. Compendium of stone fruit diseases. APS Press:1-98. Accessed

Ondejková, N., M. Hudecová, and K. Bacigálová. 2010. First report on *Monilinia fructicola* in the Slovak Republic. Plant Protection Science 46:181-184.

Pascual, S., N. Magan, and P. Melgarejo. 1996. Improved biological control of peach twig blight by physiological manipulation of Epicoccum nigrum. Pages 411-412 *in*Brighton Crop Protection Conference: Pests & Diseases-1996. Volume 1. Proceedings of an International Conference, Brighton, UK, 18-21 November, 1996. British Crop Protection Council.

Paterilo, G. A. 1976. Sadovodstvo. Vinogradarstvo I Vinodelie Moldavii:41-42.

Persoon, C. H. 1796. Observationes Mycologicae. *in* Lipsiae, ed.

Persoon, C. H. 1801. Synopsis methodica fungorum. *in*, Gottingae.

Persoon, C. H. 1822. Mycologia europaea.

Petro´czy , M., A. Szigethy, and L. Palkovics. 2012. *Monilinia* species in Hugary: morphology, culture characteristics, and molecular analysis Trees 26:153-164.

Pommer, E. H., and D. Mangold. 1975. Vinclozolin (BAS 352F) ein neuer Wirkstoff zur Bekämpfung von Botrytis cinerea. Meded. Fak. Landbouwwet. Rijksuniv. Gent 40:713-722.

Protection, K. S. o. P. 1972. A list of plant diseases, insect pests, and weeds in Korea (Chinese, English, Japanese, Korean). Pages 424 *in*, Seoul.

Prusky, D., A. Perez, Y. Zutkhi, and R. Ben-Arie. 1997. Effect of modified atmosphere for control of black spot, caused by *Alternaria alternata*, on stored persimmon fruits. Phytopathology 87(2):203-208.

Pusey, P. L., and C. L. Wilson. 1984. Postharvest biological control of stone fruit brown rot by *Bacillus subtilis* Plant Disease:4.

Reade, J. M. 1908. Preliminary notes on some species of *Sclerotinia*. *in* S. M. Universalis, ed. Annals mycologici. Verlag Von R. Friedlaender and Sohn, Berlin.

Reddy, P. P. 2013. Strobilurin fungicides. Pages 185 - 200 *in* Recent advances in crop protection. Springer India.

Rekhviashvili, L. M. 1973. Trudy Inst. Zashch. Rast. Tbilisi 23:261-265.

Reuter, E. 1898. In Norwegen im Jahre 1896 aufgetretene Krankheits-Erscheinungen. Zeitschrift für Pflanzenkrankheiten 8:209-214.

Rheinheimer, J. 2007. Succinate dehydrogenase inhibitors *In* W. K. a. U. Schirmer, (ed.). Modern Crop Protection. Wiley-VCG, Weinheim.

Roberts, J. W., and J. C. Dunegan. 1932. Peach brown rot. Technical Bulletin U.S. Department of Agriculture 328:1-60.

Russell, P. E. 1995. Fungicide resistance: occurrence and management. Journal of Agricultural Science, Cambridge 124:6.

Russell, P. E. 2005. Centenary review: A century of fungicide evolution. Journal of Agricultural Science 143:11-25.

Sardiña, J. R. 1928. Los preparados coloidales en terapéutica vegetal. Bol. Pat. veg. Ent. agric. Madr. 3:24-30.

Sarejanni, J. A. 1935. Sur le *Sclerotinia* de l'amandier. Annales de I'Institut Phytopathologique Benaki 1:57-60.

Sarejanni, J. A. 1936. Notes phytopathologiques II. Annls. Inst. phytopath. Benaki 2:86-92.

Sarejanni, J. A. 1939. Catalogue commenté des champignons rencontrés sur les plantes cultivées en Grèce. Pages 41-66 *in*Annales de l'Institut Phytopathologique Benaki.

Sattar, A. 1940. Some important diseases of apples in the Punjab and their control. Punjab Fruit Journal 4:719-721.

Sauter, H., W. Steglich, and T. Anke. 1999. Strobilurins: evolution of a new class of active substances. Angewandte Chemie International Edition 38(10):1328-1349.

Savulescu, T., C. Sandu-Ville, A. Hulea, and A. Hulpoi. 1941. Starea fitosanitară în România în anul 1937-1938. Institutul de cercetări agronomice al României, Nr.ri a:106.

Schellenberg, H. C. 1923. Infektionsversuche mit Vertretern der Gattung *Sclerotinia*. Verhandl. Schweiz. Naturf. Ges. 104:161-162.

Schröter, J. 1893. Discomycetes in Kryptogamenflora von Schlesien. II. *in*. Bd.

Sharma, R., and J. Kaul. 1988. Susceptibility of apples to brown rot in relation to qualitative characters. Indian Phytopathology 41(3):410-415.

Sharma, R. L., and J. L. Kaul. 1990. Mode of entry and histopathological changes induced by *Monilinia* species in apple fruit. Indian Phytopathology 43:4.

Sisquella, M., C. Casals, P. Picouet, I. Viñas, R. Torres, and J. Usall. 2013. Immersion of fruit in water to improve radio frequency treatment to control brown rot in stone fruit. Postharvest Biology and Technology 80:31-36.

Skeivyte, S. A. 1964. Liet. TSR Mokslu Akad. Darb. Ser. C:55-62.

Smarods, J. 1930. Larvija novērotas kulturaugiem kaitigās sēnes. Lauksaimn. Mēnešr. 10:31.

Smilanick, J. L., R. Denis-Arrue, J. R. Bosch, A. R. Gonzalez, D. Henson, and W. J. Janisiewicz. 1993. Control of postharvest brown rot of nectarines and peaches by *Pseudomonas* species.

Crop Protection 12(7):513-520.

Snyder, C. L. 1997. Genetic variation between strains of *Monilinia fructicola* and *Monilinia laxa* isolated from cherries in Michigan Michigan State University, East Lansing.

Snyder, C. L., and A. L. Jones. 1999. Genetic variation between strains of *Monilinia fructicola* and *Monilinia laxa* isolated from cherries in Michigan. Canadian Journal of Plant Pathology 21(1):70-77.

Sokolov, A. M. 1962. Sb. Nauch. Trud. ivanov. sel.'-khoz. Inst.:63-67.

Sorauer, P. 1897. Ueber die jetzige Monilia-Epidemie der Kirschbaume. Prakt. Ratg. Obstb 12:281-282.

Spadoni, A., F. Neri, P. Bertolini, and M. Mari. 2013. Control of *Monilinia* rots on fruit naturally infected by hot water treatment in commercial trials. Postharvest Biology and Technology 86:280-284.

Sprau, F. 1948. Lagerschäden an Kernobst. Pflanzenschutz 1:11-14.

Stammeler, G., H. D. Brix, A. Glaettli, M. Semnar, and U. Schoefl. 2007. Biological properties of the carboxamide boscalid including recent studies on its mode of action. Glasgow, UK.

Staub, T. 1991. Fungicide resistance: Practical experience with antiresistance strategies and the role of integrated use. Annual review of Phytopathology 29(1):421-442.

Szodi, S., H. Komjati, and G. Turoczi. 2012. Characterization of *M. laxa* and *M. fructigena* isolates from Hungary with MP-PCR. Horticultural Science (Prague) 39:116-122.

Takahashi, Y. 1911. On the *Sclerotinia* diseases of rosaceous fruit trees in Japan. Miyabe-Festschrift:135-155.

Tang, J., J. N. Ikediala, S. Wang, J. D. Hansen, and R. P. Cavalieri. 2000. High-temperature-short-time thermal quarantine methods. Postharvest Biology and Technology 21(1):129-145.

Telerevnikova-Babayan, D. N. 1981. Some data on fungus diseases of rare and disappearing plants of the Armenian SSR. Biologicheski Zhurnal Armenii 34:1099-1106.

Terry, L. A., and D. C. Joyce. 2004. Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. Postharvest Biology and Technology 32(1):1-13.

Tian, S., Q. Fan, Y. Xu, Y. Wang, and A. Jiang. 2001. Evaluation of the use of high CO2 concentrations and cold storage to control of *Monilinia fructicola* on sweet cherries. Postharvest Biology and Technology 22(1):53-60.

Truesdell, H. W. 1922. Plant pathology in Crimea. Pages 533-535 *In* L. L. Harter, P. Spaulding, and G. M. Reed, (eds.). Phytopathology.

Tzavella-Klonari, K. 1985. Attack of hazel-nut fruits by the fungus *Monilia fructigena*. Annales de l'Institut Phytopathologique Benaki 14:171-173.

Unamuno, L. M. 1929. New data relating to the study of the parasitic and saprophytic fungi of the

environs of Durango (Vizcaya). BD. R. Soc. Espanola Hist. nat. 29:113-125.

USDA–ARS–SMML. 2014. Fungal databases: *Monilinia fructigena*. United States Department of Agriculture, Agricultural Research Service, Systematic Mycology and Microbiology Laboratory.Accessed on 9 April 2014, http://nt.ars-grin.gov/fungaldatabases/new_allView.cfm?whichone=FungusHost&thisName=Monilinia fructigena&organismtype=Fungus&fromAllCount=yes.

USFWS. 2013. Endangered species, flowering plants. Accessed on 7 April 2014, http://www.fws.gov/endangered/species/us-species.html.

Valiuškaitė, A., N. Kviklienė, D. Kviklys, and J. Lanauskas. 2006. Post-harvest fruit rot incidence depending on apple maturity. Agronomy Research 4(special):427-431.

van Brouwershaven, I. R., M. L. Bruil, G. C. M. van Leeuwen, and L. F. F. Kox. 2010. A real-time (TaqMan) PCR assay to differentiate *Monilinia fructicola* from other brown rot fungi of fruit crops. Plant Pathology 59:8.

van Leeuwen, G. C. M. 2000. The brown rot fungi of fruit crops (*Monilinia* spp.), with special reference to *Monilinia fructigena* (Aderh. & Ruhl.) Honey. , Wageningen University, Wageningen.

van Leeuwen, G. C. M., R. P. Baayen, I. J. Holb, and M. J. Jeger. 2002a. Distinction of the Asiatic brown rot fungus *Monilia polystroma* sp. nov. from *M. fructigena*. Mycological Research 106(4):444-451.

van Leeuwen, G. C. M., I. J. Holb, and M. J. Jeger. 2002e. Factors affecting mummification and sporulation of pome fruit infected by *Monilinia fructigena* in Dutch orchards. Plant Pathology 51:6.

van Leeuwen, G. C. M., A. Stein, I. J. Holb, and M. J. Jeger. 2000. Yield loss in apple caused by *Monilinia fructigena* (Aderh. & Ruhl.) Honey, and spatio-temporal dynamics of disease development. European Journal of Plant Pathology 106(6):519-528.

van Leeuwen, G. C. M., and H. A. van Kesteren. 1998. Delineation of the three brown rot fungi of fruit crops (*Monilinia* spp.) on the basis of quantitative characteristics. Canadian Journal of Botany 76:2042-2050.

Vasudeva, S. 1930. On the occurrence of “false sectors” in cultures of *Fusarium fructigenum* Fr. Transactions of the British Mycological Society 15(1):96-IN94.

Veresciaghin, B. 1940. Bolile cryptogamice pe ramurile si tulpinile pomilor roditori. Bulletin agric. Basarabia 1:11-12.

Vielwerth, V. 1938. Report on the agencies injurious to cultivated plants in western and central Slovakia. Ochrana Rostlin 14:8-16.

von Bremer, G., H. Ismen, and M. Özkan. 1947. Beiträge zur Kenntnis der parasitischen Pilze der. Türkei. I. Revue de la Faculte des Sciences de I'Universite d'Istanbul, Ser. B 17:259-276.

von Schmeling, B., and M. Kulka. 1966. Systemic fungicidal activity of 1,4-oxathiin derivatives Science 152:659-660.

von Thümen, F. 1875. *Oidium fructigenum* Oessterr. Landw. Wochenblott. Der Grind oder Schimmel des Obstes 41:484.

Walker, J., and R. W. McLeod. 1970. New records of plant diseases in New South Wales. The Agricultural Gazette of New South Wales 81:452-458.

Wallroth, C. F. W. 1833. Flora Cryptogamica Germaniae J.L. Schrag, Nü rnberg. 923 pp.

Wang, Y., G. Wang, Q. Zeng, and Z. Zhang. 1998. Studies on the investigation and integrated control of brown rot disease of peach Journal of Yunnan Agricultural University 13:29-32.

Weber, A. 1926. Sprøjtning af frugttraeer og frugtbuske mod snyltesvampe samt disses biologi. Tidsskr. Planteavl. 32:219-318.

Weber, A. 1938. Aeblesygdomme i lagerrum. Tidsskr. Planteavl. 43:337-345.

Whetzel, H. H. 1945. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. Mycologia 37(6):648-714.

Wolfgang, K., U. Schirmer, P. Jeschke, and M. Witschel (eds.). 2012. Modern crop protection compounds, 2nd edition. Wiley. 1-1498 pp.

Wormald, H. 1920. The 'Brown Rot' diseases of fruit trees, with special reference to two biologic forms of *Monilia cinerea*, Bon. I. Annals of Botany 34:143-171.

Wormald, H. 1927. Further studies of the brown rot fungi II. A contribution of our knowledge of the distribution of the species of *Sclerotinia* causing brown rot. Annals of Botany 41:287-299.

Wormald, H. 1939. Notes on plant diseases in 1938. 167-172 pp.

Wormald, H. 1940. Host plants of the brown rot fungi in Britain. Transactions of the British Mycological Society 24(1):20-28.

Wormald, H. 1954. The brown rot diseases of fruit trees. Her Majesty's Stationery Office, London. 1-113 pp.

Woronin, M. 1897. Kurze Notiz über *Monilia fructigena* Pers. . Zeitschrift für Pflanzenkrankheiten 7:196-198.

Woronin, M. 1900. Über *Sclerotinia cinerea* und *Sclerotinia fructigena*. Mem. Acad. VIIIe sér 10:1-38.

Xu, X. M., L. Guerin, and J. D. Robinson. 2001a. Effects of temperature and relative humidity on conidial germination and viability, colonization and sporulation of *Monilinia fructigena*. Plant Pathology 50(5):561-568.

Xu, X. M., and J. D. Robinson. 2000. Epidemiology of brown rot (*Monilinia fructigena*) on apple: infection of fruits by conidia. Plant Pathology 49:5.

Xu, X. M., J. D. Robinson, A. M. Berrie, and D. C. Harris. 2001f. Spatio-temporal dynamics of brown rot (*Monilinia fructigena*) on apple and pear. Plant Pathology 50(5):569-578.

Ya´nez-Mendizabal, V., I. Vin˜ as , J. Usall, R. Torres, C. Solsona, M. Abadias, and N. Teixido.

2012. Formulation development of the biocontrol agent *Bacillus subtilis* strain CPA-8 by spray-drying. Journal of Applied Microbiology 112:12.

Yamaguchi, I., and M. Fujimura. 2005. Recent topics on action mechanisms of fungicides. Nippon Noyaku Gakkaishi 30(2):67-74.

Yánez-Mendizábal, V., I. Viñas, U. J., R. Torres, C. Solsona, and N. Teixidó. 2012. Production of the postharvest biocontrol agent *Bacillus subtilis* CPA-8 using low cost commercial products and by-products. Biological Control 60:10.

Ypema, H., and R. Gold. 1999. Kresoxim-methyl: modification of a naturally occurring compound to produce a new fungicide. Plant Disease 83(1):4-19.

Zehr, E. I. 1974. Fungicide and nematicide tests: results of 1971. American Phytopathological Society.

Zhou , T., K. E. Schneider, and X. Z. Li. 2008. Development of biocontrol agents from food microbial isolates for controlling post-harvest peach brown rot caused by *Monilinia fructicola*. International Journal of Food Microbiology 126:6.

Appendix

A

How to Use the Guidelines

Use *New Pest Response Guidelines: Monilinia Fructigena (Alderhold & Ruhland) Honey ex Whetzel 1945, Apple Brown Rot* when designing a program to detect, monitor, control, contain or eradicate an outbreak of this pest in the United States and collaborating territories.

The United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the guidelines through discussion, consultation or agreement with staff members at the USDA–Agricultural Research Service and advisors at universities.

Any new detection may require the establishment of an incident command system to facilitate emergency management. This document is meant to provide the information necessary to launch a response to an *M. fructigena* detection.

If *M. fructigena* is detected, a site-specific action plan will be based on the guidelines. As the program develops and new information becomes available, the guidelines will be updated.

Users

The guidelines are intended as a field reference for the following users who have been assigned responsibilities for a plant health emergency involving apple brown rot:

- PPQ personnel
- Emergency response coordinators
- State agriculture department personnel
- Others concerned with developing local survey or control programs

Contacts

When an emergency program for *M. fructigena* has been implemented, the success of the program depends on the cooperation, assistance and understanding of other involved groups. The appropriate liaison and information officers should distribute news of the program's progress and developments to interested groups including the following:

- Academic entities with agricultural interests
- Agricultural interests in other countries
- Commercial interests
- Grower groups such as specific commodity or industry groups
- Land-grant universities and cooperative extension services
- National, state and local news media
- Other federal, state, county and municipal agricultural officials
- Public health agencies
- The public
- State and local law enforcement officials
- Tribal governments

Initiating an Emergency Pest Response Program

An emergency pest response program consists of detection and delimitation and may be followed by programs in regulation, containment, eradication and control. The New Pest Advisory Group (NPAG) will evaluate the pest. After assessing the risk to U.S. plant health and consulting with experts and regulatory personnel, NPAG will recommend a course of action to PPQ management.

Follow this sequence when initiating an emergency pest response program:

1. A new or reintroduced pest is discovered and reported
2. The pest is examined and pre-identified by regional or area identifier
3. The pest's identity is confirmed by a national taxonomic authority recognized by the USDA–APHIS–PPQ National Identification System

4. Published New Pest Response Guidelines are consulted or a new NPAG is assembled to evaluate the pest
5. Depending on the urgency, official notifications are made to the National Plant Board, cooperators and trading partners
6. Based on information provided, PPQ Leadership may immediately determine the efficacy of a federal response in which one or all of the following actions may take place: delimitation, regulation, containment, eradication and/or control
7. If additional information is required, the following actions may occur:
   - **A.** A delimiting survey is conducted at the site(s) of detection
   - **B.** Trace-back and trace-forward investigations are conducted
   - **C.** An incident assessment team may be sent to evaluate the site
   - **D.** State departments of agriculture are consulted
8. A recommendation is made, based on the assessment of surveys, other data and recommendation of the incident assessment team or the NPAG as follows:
   - **A.** Take no action
   - **B.** Regulate the pest and its hosts
   - **C.** Contain the pest
   - **D.** Suppress the pest
   - **E.** Eradicate the pest
9. If appropriate, a control strategy is selected
10. A PPQ Deputy Administrator authorizes a response
11. A command post is selected and the incident command system is implemented
12. State departments of agriculture cooperate with parallel actions using a unified command structure
13. Trace-back and trace-forward investigations are conducted
14. Field identification procedures are standardized
15. Data reporting is standardized
16. Regulatory actions are taken
17. Environmental assessments are completed as necessary
18. Treatment is applied for required pest generational time
19. Environmental monitoring surveys are conducted to evaluate program success
20. Pest monitoring surveys are conducted to evaluate program success
21. Programs are designed for eradication, containment or long-term use

Preventing an Infection

Federal and state regulatory officials must conduct inspections and apply

prescribed measures to ensure that pests do not spread within or between properties. Federal and state regulatory officials conducting inspections should follow the necessary sanitation guidelines before entering and upon leaving each property to prevent contamination.

Scope

The guidelines are divided into the following chapters:

1. *Introduction* on page 1-1
2. *Taxonomy* on page 2-1
3. *Identification* on page 3-1
4. *Biology* on page 4-1
5. *Damage* on page 5-1
6. *Pathways* on page 6-1
7. *Survey* on page 7-1
8. *Control Procedures* on page 8-1
9. *Regulatory Procedures* on page 9-1
10. *Research Needs* on page 10-1

The guidelines also include appendices and a list of literature cited.

Authorities

The regulatory authority for taking the actions listed in the guidelines is contained in the following authorities:

- Plant Protection Act of 2000 (Statute 7 USC 7701-7758)
- Executive Order 13175, Consultation and Coordination with Indian and Tribal Governments
- Fish and Wildlife Coordination Act
- National Historic Preservation Act of 1966
- Endangered Species Act
- Endangered and Threatened Plants (50 CFR 17.12)
- National Environmental Policy Act

Program Safety

The safety of the public and program personnel is a priority in pre-program planning and training and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decision Making

The USDA–APHIS–PPQ–Center for Plant Health, Science and Technology (CPHST) provides technical support to emergency pest response program directors concerning risk assessments, survey methods, control strategies, regulatory treatments and other aspects of the pest response programs. PPQ managers consult with state departments of agriculture in developing guidelines and policies for pest response programs.

How to Obtain the Guidelines

The guidelines are a portable electronic document that is updated periodically. Download the current version from its source and then use Adobe Reader® to view it on your computer screen. You can print the guidelines for convenience; however, links and navigational tools are only functional when the document is viewed in Adobe Reader®. Remember that printed copies of the guidelines are obsolete once a new version has been issued.

Conventions

Conventions are established by custom and are widely recognized and accepted. Conventions used in the guidelines are listed in this section.

Advisories

Advisories are used throughout the guidelines to bring important information to your attention. Please carefully review each advisory. The definitions have been updated to coincide with the American National Standards Institute (ANSI) and are formatted as follows:

Example	Example provides an example of the topic.
Important	Important indicates information that is helpful.

CAUTION

CAUTION indicates that people could possibly be endangered and slightly hurt.

DANGER

DANGEROUS indicates that people could easily be hurt or killed.

NOTICE

NOTICE indicates a possibly dangerous situation where goods might be damaged.

WARNING

WARNING indicates that people could possibly be hurt or killed.

Boldfacing

Boldfaced type is used to highlight negative or important words. These words are **never**, **not**, **do not**, **other than** and **prohibited**.

Lists

Bulleted lists indicate information listed in no particular order. Numbered lists indicate that information will be used in a particular order.

Disclaimers

All disclaimers are located on the page that follows the cover.

Control Data

Information placed at the top and bottom of each page helps users keep track of where they are in the guidelines. At the top of the page is the chapter. At the bottom of the page is the year, edition, title and page number. PPQ–Pest Detection and Emergency Programs (PDEP) is the unit responsible for the content of the guidelines.

Decision Tables

Decision tables are used throughout the guidelines. The first and middle columns in each table represent conditions, and the last column represents the action to take after considering all conditions listed for that row. Begin with the column headings and move left-to-right. If the condition does not apply, then continue one row at a time until you find the condition that does apply.

Table A-1 How to use decision tables

If you:	And if the condition applies:	Then:
read this column cell and row first	continue in this cell	TAKE the action listed in this cell
find the previous condition does not apply, then read this column cell	continue in this cell	TAKE the action listed in this cell

Footnotes

When space allows, figure and table footnotes are located directly below the associated figure or table. However, for multi-page tables or tables that cover the length of a page, footnote numbers and footnote text cannot be listed on the same page. If a table or figure continues beyond one page, the associated footnotes will appear on the page following the end of the figure or table.

Heading Levels

Within each chapter and section there can be four heading levels; each heading is green and is located within the middle and right side of the page. The first-level heading is indicated by a horizontal line across the page with the heading following directly below. The second-, third- and fourth-level headings each have a font size smaller than the preceding heading level. The fourth-level heading runs in with the text that follows.

Hypertext Links

Figures and tables are cross-referenced in the body of the guidelines and are highlighted in blue hypertext type.

Italics

The following items are italicized throughout the guidelines:

- Cross-references to headings and titles
- Names of publications
- Scientific names

Numbering Scheme

A two-level numbering scheme is used in the guidelines for pages, tables and figures. The first number represents the chapter. The second number represents the page, table or figure. This numbering scheme allows for identification and updating. Dashes are used in the page numbering to differentiate page numbers from decimal points.

Transmittal Number

The transmittal number contains the month, year and a consecutively issued number (beginning with -01 for the first edition and increasing consecutively for each update to the edition). The transmittal number is only changed when the specific chapter sections, appendices, tables or index is updated. If no changes are made, then the transmittal number remains the unchanged. The transmittal number only changes when a new guidelines edition is issued or changes are made to the entire guidelines.

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How to Cite the Guidelines

Cite the guidelines as follows:

U.S. Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine. 2015. New Pest Response Guidelines: *Monilinia Fructigena* (Alderhold & Ruhland) Honey ex Whetzel 1945, Apple Brown Rot. Washington, D.C.: Government Printing Office.
http://www.aphis.usda.gov/import_export/plants/manuals/online_manuals.shtml

How to Find More Information

Contact USDA–APHIS–PPQ–PDEP–Emergency Management for more information regarding the guidelines. Refer to *Resources* on page B-1 for contact information.

Appendix

B

Resources

Use *Appendix B Resources* to find the Website addresses, street addresses and telephone numbers for the resources mentioned in the guidelines.

- Center for Plant Health, Science and Technology (USDA–APHIS–PPQ–CPHST)

- Pest Detection and Emergency Programs, Emergency Management (USDA–APHIS–PPQ–PDEP–EM)

- PPQ Treatment Manual

- Plant, Organism and Soil Permits (APHIS–PPQ)

- National Program Manager for Native American Program Delivery and Tribal Liaison (USDA–APHIS–PPQ)
  14082 S. Poston Place
  Tucson, AZ 85736
  Telephone: (520) 822-5440

- Biological Control Coordinator (USDA–APHIS–CPHST)

- FIFRA Coordinator (USDA–APHIS–PPQ–PDEP)
  4700 River Road
  Riverdale, MD 20737
  Telephone: (301) 851-2243

- Environmental Compliance Coordinator (USDA–APHIS–PPQ–PDEP)
  4700 River Road
  Riverdale, MD 20737
  Telephone: (301) 851-2345

- PPQ Forms
- List of State Plant Health Directors (SPHD)
- List of State Plant Regulatory Officials (SPRO)
- National Climatic Center, Database Administration
  Box 34
  Federal Building
  151 Patton Ave
  Asheville, NC 28801-5001
- CAPS Survey Manual
- GenBank®
- iPhyClassifier

Appendix

C

Forms

PPQ Form 391, Specimens for Determination **C-2**

PPQ Form 523, Emergency Action Notification **C-6**

PPQ Form 391, Specimens for Determination

This report is authorized by law (7 U.S.C. 147a). While you ar not required to respond your cooperation is needed to make an accurate record of plant pest conditions. *See reverse for additional OMB information.*

FORM APPROVED
OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 - assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001.
Pest Data Section – Complete Items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete Items 17 and 18 if a trap was used.

FOR IIBIII USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER
2. DATE — MO | DA | YR
3. SUBMITTING AGENCY — ☐ State Cooperator ☐ PPQ ☐ Other _____

SENDER AND ORIGIN

4. NAME OF SENDER
6. ADDRESS OF SENDER
ZIP

INTERCEPTION SITE

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*
7. NAME AND ADDRESS OF PROPERTY OR OWNER
COUNTRY/ COUNTY

PURPOSE

8. REASON FOR IDENTIFICATION *("x" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)
B. ☐ Damaging Crops/Plants
C. ☐ Suspected Pest of Regulatory Concern *(Explain in* REMARKS*)*
D. ☐ Stored Product Pest
E. ☐ Livestock, Domestic Animal Pest
F. ☐ Possible Immigrant *(Explain in* REMARKS*)*
G. ☐ Survey *(Explain in* REMARKS*)*
H. ☐ Other *(Explain in* REMARKS*)*

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

HOST DATA

10. HOST INFORMATION
NAME OF HOST *(Scientific name when possible)*

11. QUANTITY OF HOST
NUMBER OF ACRES/PLANTS
PLANTS AFFECTED *(Insert figure and indicate* ☐ Number ☐ Percent*):*

12. PLANT DISTRIBUTION
☐ LIMITED
☐ SCATTERED
☐ WIDESPREAD

13. PLANT PARTS AFFECTED
☐ Leaves, Upper Surface
☐ Leaves, Lower Surface
☐ Petiole
☐ Stem
☐ Trunk/Bark
☐ Branches
☐ Growing Tips
☐ Roots
☐ Bulbs, Tubers, Corms
☐ Buds
☐ Flowers
☐ Fruits or Nuts
☐ Seeds

PEST DATA

14. PEST DISTRIBUTION
☐ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD
17. TYPE OF TRAP AND LURE
18. TRAP NUMBER

19. PLANT PATHOLOGY – PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY
☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE
☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

SIGNATURE
DATE

FOR IIBIII USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

PPQ FORM 391
(AUG 02)
Previous editions are obsolete.

This is a 6-Part form. Copies must be disseminated as follows:
☐ PART 1 – PPQ ☐ PART 2 – RETURN TO SUBMITTER AFTER IDENTIFICATION ☐ PART 3 – IIBIII OR FINAL IDENTIFIER
☐ PART 4 – INTERMEDIATE IDENTIFIER ☐ PART 5 – INTERMEDIATE IDENTIFIER ☐ PART 6 – RETAINED BY SUBMITTER

Figure C-1 Example of PPQ Form 391, Specimens for Determination, side 1

PPQ Form 391, Specimens for Determination (cont.)

OMB Information

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0010. The time required to complete this information collection is estimated to average .25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

Instructions

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS
1	1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number **EXAMPLE** In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001 2. Enter the collection number
2	Enter date
3	Check block to indicate Agency submitting specimens for identification
4	Enter name of sender
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)
6	Enter address
7	Enter name and address of property owner
8A-8L	Check all appropriate blocks
9	Leave Blank
10	Enter scientific name of host, if possible
11	Enter quantity of host and plants affected
12	Check block to indicate distribution of plant
13	Check appropriate blocks to indicate plant parts affected
14	Check block to indicate pest distribution
15	• Check appropriate block to indicate type of specimen • Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Enter X in block to indicate isolated or general plant symptoms
20	Enter X in appropriate block for weed density
21	Enter X in appropriate block for weed growth stage
22	Provide a brief explanation if Prompt or URGENT identification is requested
23	Enter a tentative determination if you made one
24	Leave blank

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

1. Send Original along with the sample to your Area Identifier.
2. Retain and file a copy for your records.

Figure C-2 Example of PPQ Form 391, Specimens for Determination, side 2

Purpose

Submit PPQ Form 391, Specimens for Determination, along with specimens for positive or negative identification.

Instructions

Follow the instructions in on page C-3. Inspectors must provide all relevant collection information with samples. This information should be shared within both the state and the regional office program contact. If a sample tracking database is available at the time of detection, please enter the collection information in the system as quickly as possible.

Distribution

Distribute PPQ Form 391 as follows:

1. Send the original with the sample to your area identifier.
2. Keep and file a copy for your records.

Table C-1 Instructions for completing PPQ Form 391, Specimens for Determination

Block	Description	Instructions
1	COLLECTION NUMBER	1. ASSIGN a collection number for each collection as follows: 2-letter state code-5-digit sample number (survey identification number in parentheses); example: PA-1234 (0402010001) 2. CONTINUE consecutive numbering for each subsequent collection 3. ENTER the collection number
2	DATE	ENTER the date of the collection
3	SUBMITTING AGENCY	PLACE an X in the PPQ block
4	NAME OF SENDER	ENTER the sender's or collector's name
5	TYPE OF PROPERTY	ENTER the type of property from which the specimen was collected (farm, feed mill, nursery, etc.)
6	ADDRESS OF SENDER	ENTER the sender's or collector's address
7	NAME AND ADDRESS OF PROPERTY OR OWNER	ENTER the name and address of the property from which the specimen was collected
8A–8H	REASONS FOR IDENTIFICATION	PLACE an X in the correct block
9	IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE GIVE A BRIEF EXPLANATION UNDER "REMARKS"	LEAVE BLANK; ENTER remarks in *Block 22*
10	HOST INFORMATION, NAME OF HOST	If known, ENTER the scientific name of the host
11	QUANTITY OF HOST	If applicable, ENTER the number of acres planted with the host
12	PLANT DISTRIBUTION	PLACE an X in the applicable box
13	PLANT PARTS AFFECTED	PLACE an X in the applicable box
14	PEST DISTRIBUTION: FEW/COMMON/ABUNDANT/ EXTREME	PLACE an X in the appropriate block
15	INSECTS/NEMATODES/ MOLLUSKS	PLACE an X in the applicable box to indicate type of specimen
	NUMBER SUBMITTED	ENTER the number of specimens submitted as ALIVE or DEAD under the appropriate stage
16	SAMPLING METHOD	ENTER the type of sample
17	TYPE OF TRAP AND LURE	ENTER the type of sample
18	TRAP NUMBER	ENTER the sample numbers
19	PLANT PATHOLOGY-PLANT SYMPTOMS	If applicable, check the appropriate box; otherwise LEAVE BLANK
20	WEED DENSITY	If applicable, check the appropriate box; otherwise LEAVE BLANK
21	WEED GROWTH STAGE	If applicable, check the appropriate box; otherwise LEAVE BLANK
22	REMARKS	ENTER the name of the office or diagnostic laboratory forwarding the sample; include a contact name, email address, phone number of the contact and the date forwarded to the state diagnostic laboratory or USDA-APHIS-NIS
23	TENTATIVE DETERMINATION	ENTER the preliminary diagnosis
24	DETERMINATION AND NOTES (**Not** for field use)	LEAVE BLANK; to be completed by the official identifier

PPQ 523 Emergency Action Notification

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED - OMB NO. 0579-0102

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

EMERGENCY ACTION NOTIFICATION

SERIAL NO.

1. PPQ LOCATION
2. DATE ISSUED
3. NAME AND QUANTITY OF ARTICLE(S)
4. LOCATION OF ARTICLES
5. DESTINATION OF ARTICLES
6. SHIPPER
7. NAME OF CARRIER
8. SHIPMENT ID NO.(S)
9. OWNER/CONSIGNEE OF ARTICLES

Name:

Address:

PHONE NO. FAX NO.

SS NO. TAX ID NO.

10. PORT OF LADING
11. DATE OF ARRIVAL
12. ID OF PEST(S), NOXIOUS WEEDS, OR ARTICLE(S)
12a. PEST ID NO.
12b. DATE INTERCEPTED
13. COUNTRY OF ORIGIN
14. GROWER NO.
15. FOREIGN CERTIFICATE NO.
15a. PLACE ISSUED
15b. DATE

Under Sections 411, 412, and 414 of the Plant Protection Act (7 USC 7711, 7712, and 7714) and Sections 10404 through 10407 of the Animal Health Protection Act (7 USC 8303 through 8306), you are hereby notified, as owner or agent of the owner of said carrier, premises, and/or articles, to apply remedial measures for the pest(s), noxious weeds, and or article(s) specified in Item 12, in a manner satisfactory to and under the supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action specified in Item 16 and shall be completed within the time specified in Item 17.

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

☐ TREATMENT:

☐ RE-EXPORTATION:

☐ DESTRUCTION:

☐ OTHER:

Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent cost of any care, handling, application of remedial measures, disposal, or other action incurred in connection with the remedial action, destruction, or removal.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):
18. SIGNATURE OF OFFICER:

ACKNOWLEDGMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION

I hereby acknowledge receipt of the foregoing notification.

SIGNATURE AND TITLE:

DATE AND TIME:

19. REVOCATION OF NOTIFICATION

ACTION TAKEN:

SIGNATURE OF OFFICER:

DATE:

PPQ FORM 523 (JULY 2002) Previous editions are obsolete.

Figure C-3 Example of PPQ 523 Emergency Action Notification

Purpose

Issue a PPQ 523 Emergency Action Notification (EAN) to hold all host plant material at facilities that house the suspected plant material directly or indirectly connected to positive confirmations. Once an investigation determines that the plant material is not infected or testing determines there is no risk, the material may be released and the release documented on the EAN.

The EAN may also be issued to hold plant material in fields pending positive identification of suspect samples. When a decision is made to destroy plants, or in the case of submitted samples, once positive confirmation is received, the same EAN that placed plants on hold also documents any actions taken, such as destruction and disinfection. More action may be warranted if other fields test positive for this pest.

Instructions

If plant lots or shipments are held as separate units, issue separate EANs for each unit of suspected and associated plant material. The EANs are issued under the authority of the Plant Protection Act of 2000 (state 7 USC 7701-7758). States are advised to issue their own hold orders parallel to the EAN to prevent intrastate movement of plant material.

When using an EAN to hold articles, the EAN language must clearly specify actions to be taken. An EAN issued for positive testing and positive associated plant material must clearly state that the material must be disposed of, or destroyed, and the areas disinfected. Include language that these actions will occur at the owner's expense and will be supervised by a regulatory official. If the EAN is used to issue a hold order for further investigations and testing of potentially infected material, use the same EAN to document any disposal, destruction and disinfection orders resulting from the investigations or testing.

Appendix

D

Taxonomic Support for Surveys

Background

The National Identification Services (NIS) coordinates the identification of plant pests in support of the USDA's regulatory programs. Accurate and timely identifications are the foundation of quarantine action decisions and are essential in the effort to safeguard the nation's agricultural and natural resources.

The NIS employs and collaborates with scientists who specialize in various plant pest groups, including weeds, insects, mites, mollusks and plant diseases. These scientists are stationed at a variety of institutions around the country, including federal research laboratories, plant inspection stations, land-grant universities and natural history museums. Additionally, the NIS Molecular Diagnostics Laboratory is responsible for providing biochemical testing to support the agency's pest monitoring programs.

On 13 June 2007, the PPQ Deputy Administrator issued PPQ Policy No. PPQ-DA-2007-02, which established the role of PPQ NIS as the point of contact for all domestically detected confirmations and communications regarding introduced plant pests. The position of Domestic Diagnostics Coordinator (DDS) was established to administer the policy and coordinate domestic diagnostics for the NIS. Any questions regarding sample routing or communication of results can be directed to the PPQ Survey Field Operations Manager (Brian Kopper: phone (919) 855-7318; e-mail, brian.j.kopper@aphis.usda.gov) or the Domestic Diagnostics Coordinator

Taxonomic Support and Survey Activity

Taxonomic support for pest surveillance is fundamental to conducting quality surveys. A misidentification or incorrectly screened target pest can yield a missed opportunity for early detection when control strategies are more viable and cost effective. The importance of good sorting, screening and identification during domestic survey activity cannot be overemphasized.

Fortunately most states have, or have access to, good taxonomic support. Taxonomic support should be considered in cooperative agreements as another cost of conducting surveys. Taxonomists and laboratories within the state often

require supplies, develop training materials or hire technicians to meet their screening and identification needs. When considering whether to survey for a particular pest during a given year, consider the challenges of taxonomic support.

Sorting and Screening

For survey activities, the proper sorting and screening of samples prior to examination by an identifier will result in improved turn-around times for identification.

Sorting

Sorting is the first level of activity to ensure samples submitted are of the correct target group for the pests being surveyed. Select those plant samples that are symptomatic if appropriate. A minimum level of sorting is expected of surveyors depending on the target group, training, experience or demonstrated ability.

Screening

Screening for plant pathogens is performed by the laboratory diagnostician.

Check individual survey protocols to determine if samples should be sorted, screened or sent in their entirety (raw) before submitting for identification. If not specified in the protocol, assume that samples should be sorted to some degree.

Resources for Sorting, Screening and Identification

Sorting, screening and identification resources and aids useful to CAPS and PPQ surveys are best developed by taxonomists knowledgeable in the taxa that include the target pests and the established or native organisms in the same group that are likely in the samples and can be confused with the target. These aids are often regionally based and can be in the form of dichotomous keys, picture guides or reference collections. The NIS encourages the development of these resources, and when aids are complete, posts them in the CAPS Website for the benefit of others. Please see the following Website for some available screening aids: https://caps.ceris.purdue.edu/node/34.

Other Entities for Taxonomic Assistance in Surveys

When taxonomic support within a state is inadequate for a particular survey, other entities may assist including PPQ identifiers, universities and state departments of agriculture from other states and independent institutions. Check with the PPQ regional CAPS coordinators regarding the availability of taxonomic assistance.

Universities and State Departments of Agriculture

Depending on the taxonomic group, a few cases involve two entities that are interested in receiving samples from other states. Arrangements for payment, if required for these taxonomic services, can be made through cooperative agreements. The National Plant Diagnostic Network (NPDN) also has several regional hub laboratories that can provide service identifications of plant diseases in their respective regions. PPQ currently has arrangements with to state departments of agriculture (Oregon and Washington) and one university (Mississippi State University) through Farm Bill funding to provide taxonomic services to other states should they desire it. Contact your CAPS NOM for more information.

Independent Institutions

The Raleigh PPQ Field Operations office has set up multi-state arrangements for the Carnegie Museum of Natural History to identify insects from trap samples. They prefer to receive unscreened material and work on a fee basis per sample.

PPQ Port Identifiers

There are over 70 identifiers in PPQ that are stationed at ports of entry to primarily identify pests encountered in international commerce including conveyances, imported cargo, passenger baggage and propagative material. In some cases, these identifiers process survey samples generated during PPQ-conducted surveys and occasionally those from CAPS surveys. They can also enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to their being forwarded for confirmation by an NIS-recognized authority. The list of PPQ port identifiers and their areas of coverage can be found on the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

PPQ Domestic Identifiers

PPQ has a limited number of domestic identifiers normally stationed at universities who are primarily responsible for survey samples. Domestic identifiers can handle unscreened or partially screened samples with prior arrangement through the PPQ CAPS NOM. They can also act as an intermediary alternative to sending an unknown suspect to, for example, the ARS Systematic Entomology Lab (SEL) depending on their specialty and area of coverage. In addition, these identifiers can enter the PPQ form 391 for a suspect CAPS target or other suspect new pests into our PestID database prior to forwarding the sample for confirmation by an NIS-recognized authority.

Craig A. Webb, Ph.D.
Domestic Plant Pathology Identifier
USDA–APHIS–PPQ
Department of Plant Pathology
Kansas State University
4024 Throckmorton Plant Sciences
Manhattan, KS 66506-5502
Cell: (785) 633-9117
Office: (785) 532-1349
Fax: (785) 532-5692
e-mail: craig.a.webb@aphis.usda.gov

Specialty: Molecular diagnostics (citrus health, sudden oak death, cyst nematode screening)

Area of coverage: Primarily western U.S.

ATTENTION SAMPLE SUBMITTERS: When sending domestic samples to domestic identifiers, you must notify them first by e-mail or phone that you plan to send samples, describing what type and how many. Once notification has been sent, forward an e-mail to them with a tracking number for the express carrier through whom the samples were forwarded. If you plan to send a domestic sample to a national specialist, notify the Coordinated Agricultural Project National Operations Manager (CAPS NOM) or the National Domestic Diagnostics Coordinator prior to sending the sample.

Final Confirmations

If identifiers or laboratories at the state, university or institution level suspect the detection of a CAPS target, a plant pest new to the United States or a quarantine pest of limited distribution in a new state, the specimens should be forwarded to an NIS-recognized taxonomic authority for final confirmation. State cooperator and university taxonomists can go through a PPQ area identifier or the appropriate domestic identifier that covers their area to place the specimen into the PPQ system. They will then send the specimen to the NIS-recognized authority for that taxonomic group. In some cases, domestic identifiers can make final confirmation depending on their ID authority, accreditation and proficiency testing.

State-level taxonomists, who are reasonably certain that they have a new United States record, CAPS target or federal quarantine pest, can send the specimen directly to the NIS-recognized authority, but must notify their State Survey Coordinator (SSC), PPQ Pest Survey Specialist (PSS), State Plant Health Director (SPHD) and State Plant Regulatory Official (SPRO).

Before forwarding these suspect specimens to identifiers or to the NIS-recognized authority for confirmation, please complete a PPQ form 391 with the tentative determination. In addition, fax a copy of the completed PPQ Form 391 to ‘Attention: Domestic Diagnostics Coordinator’ at (301) 851-2115, or send a PDF

file in an e-mail to aphis-ppq.nis.urgents@aphis.usda.gov with the overnight carrier tracking number.

The addresses of the NIS-recognized authorities to which suspect specimens are to be sent can be found at the following Website: http://inside.aphis.usda.gov/ppq/php/manual/mac/identifiers_co-lat_natl_spec.pdf.

Only use the 'Urgent' listings for suspected new United States or state records of a significant pest, and the 'Prompt' listings for all others.

When the specimen is forwarded to a specialist for final confirmation, use an overnight carrier, insure proper and secure packaging and include a hard copy of the PPQ form 391 marked 'Urgent' or 'Prompt' as previously described.

Please contact the National Operations Manager assigned to this new pest response by calling (919) 855-7335.

Digital Images for Confirmation of Domestic Detections

For the aforementioned confirmations, send specimens, not digital images. For entry into the National Agricultural Pest Information System (NAPIS), digital imaging confirmations can be used for new county records of widespread pests by state taxonomists or identifiers with their prior approval. These scientists always have the prerogative to request that the specimens be sent. Pests with PPQ regulatory programs may require specimens to be sent to SEL for new county records depending on the species.

Communication of Results

If no suspect CAPS target, program pests or new detections are found, communication of these identification results can be sent by the domestic identifiers or taxonomists at other institutions directly back to the submitter. The information can be presented in a spreadsheet, in a hardcopy of PPQ form 391 or other informal means labelled with the species or 'no CAPS target or new suspect pest species found.' Good record keeping by the intermediate taxonomists performing these identifications is essential.

All confirmations received from the NIS-recognized authorities, positive or negative, are communicated by the NIS to the PPQ Pest Detection and Emergency Programs (PDEP) staff at PPQ headquarters. The PDEP then notifies the appropriate PPQ program managers and the SPHD and SPRO simultaneously. One of these contacts should forward the results to the originating laboratory, diagnostician, identifier and/or submitter of the specimen or sample.

Data Entry in NAPIS

For survey data entered into NAPIS, new country and state records should be confirmed by an NIS-recognized authority, while for others that are more widespread, use the identifications from PPQ identifiers or state taxonomists. When in doubt, contact the PPQ Domestic Survey Coordinator.

Appendix

E

Sample Submission

Taxonomic support for insect surveys requires that samples be competently and consistently sorted, stored, screened (in most cases) and submitted to the identifier.

Sampling

When possible, submit adequate quantities of suspect leaf material (preferably 12 or more leaves per sample) to ensure sufficient material for possible downstream diagnostic techniques.

Storage

Refrigerate samples while awaiting shipment to the diagnostic laboratory. Place leaves **without paper towels** in a sealed and labeled resealable plastic bag.

Documentation

Each sample should be documented in and accompanied by its own completed PPQ form 319, *Specimens for Determination*. You should maintain a partially pre-filled electronic copy of this form on your computer with your address and other information to save time. Please ensure all applicable fields are completed and that the bottom field (block 24, *Determination and Notes*) is left blank for completion by the identifier. Include the phone number and/or e-mail address of the submitter. Other documentation in the form of notes, images, *etc.* can be included if useful to the determination. A method for cross-referencing the sample with the accompanying form is critical. For example, write the collection number on both Form 391 and the sample bag.

Packing

To provide extra insurance against accidental release during shipment, specimens should be double bagged—*i.e.,* first place the specimen in a self-locking plastic bag and place that bag within a second self-locking plastic bag. FORM 391 **SHOULD NOT** BE PLACED IN THE BAG HOLDING THE SAMPLE! RATHER, IT SHOULD BE PLACED INSIDE THE OUTER BAG.

Place the double-bagged samples in a sturdy cardboard box or heavy Styrofoam container to prevent damage to the samples during shipping and handling. Ideally, samples should be packed with cold packs or ice to maintain their integrity during the shipping process. Thoroughly seal all container seams with shipping tape.

Shipping

The identifying laboratory should be contacted prior to forwarding samples. It is helpful to know how many samples are being forwarded, the type of samples (*e.g.,* SOD-suspect camellia leaves), when the samples will be shipped and the package tracking number.

Label the shipping box as ‘URGENT’ and send via overnight express courier (FedEx, UPS, Airborne, DHL, *etc.*) to the appropriate identifier.

Appendix

F

Environmental Compliance

Overview

Program managers of federal emergency response or domestic pest control programs must ensure that their programs comply with all federal acts and executive orders pertaining to the environment as applicable. Two primary federal acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may begin.

Program managers should also seek guidance and advice as needed from Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development (PPD) staff. ERAS is available to provide guidance to program managers and prepare drafts of applicable environmental documentation.

In preparing draft NEPA documentation, ERAS may also perform and incorporate assessments that pertain to other acts and executive orders described below as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), will assess ERAS in the development of documents and will implement any environmental monitoring.

Leaders of the programs are strongly advised to meet with ERA and/or ECT early in the development of a program to conduct a preliminary review of applicable environmental statutes as requested by program managers or as suggested to address concerns over controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from the ECT will work with the program manager to develop an environmental monitoring plan, conduct training to carry out the plan, provide day-to-day guidance on monitoring and provide an interpretive report of monitoring activities.

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires all federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decision maker before taking action and to tell the public of the decision. Actions that are excluded from this examination, that normally require an environmental assessment and environmental impact statements, are codified in APHIS' NEPA implementing procedures located in 7 CFR 372.5.

The three types of NEPA documentation are categorical exclusions, environmental assessments and environmental impact statements.

Categorical Exclusion

Categorical exclusions (CEs) are classes of actions that do not significantly affect the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have been built into the actions themselves (7CFR 372.5(c)).

Environmental Assessment

An environmental assessment (EA) is a public document that succinctly presents information and analysis for the decision maker of the proposed action. An EA can lead to the preparation of an environmental impact statement, a finding of no significant impact (FONSI), or the abandonment of a proposed action.

Environmental Impact Statement

If a major federal action may significantly affect the quality of the human environment (adverse or beneficial) or the proposed action may result in public controversy, then prepare an environmental impact statement (EIS).

Endangered Species Act

The Endangered Species Act (ESA) is a statute requiring that programs consider their potential effects on federally protected species. The ESA requires programs to identify protected species and their habitats in or near program areas and to document how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife

Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

Migratory Bird Treaty Act

The statute requires that programs avoid harm to over 800 endemic bird species, eggs and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

Clean Water Act

The statute requires various permits for work in wetlands and for potential discharge of program chemicals into water, which may require coordination with the Environmental Protection Agency, individual states and the Army Corps of Engineers. Such permits would be needed even if the pesticide label allows for direct application to water.

Tribal Consultation

The executive order requires formal government-to-government communication and interaction if a program might have substantial direct effects on any federally recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed before public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed federal actions intersect with tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

National Historic Preservation Act

The statute requires that programs consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local state historic preservation offices. Documentation under this act involves preparing an inventory of the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may need public involvement and comment before the start of program activities.

Coastal Zone Management Act

The statute requires coordination with states in which programs may impact coastal zone management plans. Federal activities that may affect coastal resources are evaluated through a process called federal consistency. This process affords the public, local governments, tribes and state agencies an opportunity to review the federal action. The federal consistency process is administered individually by states with coastal zone management plans.

Environmental Justice

The executive order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to consider if the actions might impact minority or economically disadvantaged populations and if so, how such impact will be avoided.

Protection of Children

The executive order requires federal agencies to identify, assess and address environmental health and safety risks that may affect children. If such a risk is identified, measures must be described and carried out to minimize such risks.

Glossary

M. fructigena

abiotic. pertaining to the absence of life; diseases not caused by living organisms

acropetal. referring to spore formation in which the most recently formed spore is at the tip of a chain of spores; typically seen as being smaller than the immediate neighbor

actinomycete. Gram-positive filamentous bacteria

acute. less than a 90° angle; pointed

adventitious roots. roots that arise from an atypical place, from a stem rather than as branches of a root

aerobic. requiring free oxygen for respiration

AFLP. Amplified Fragment Length Polymorphism; technique that uses PCR to amplify genomic DNA cleaved by restriction enzymes to generate DNA fingerprints; combines RFLP and arbitrary primer PCR and does not require prior sequence knowledge

amplicon. piece of DNA synthesized using amplification techniques such as PCR

anaerobic. organism that can live without oxygen

anamorph. asexual form of a fungus

annellide. conidiogenous cell elongating during conidiogenesis (progressive) producing blastoconidia in basipetal succession; each conidium is produced through the same opening of the previously formed one and leaves a ring-like band (annellation) at the apex after seceding; the terminal portion, which comprises a series of ring-like scars, is termed the anellated region

antheridium. male sexual organ (male gametangium) found in some fungi

APA. American Phytopathological Society

APHIS. USDA–Animal and Plant Health Inspection Service

apiculus. tiny projection on a spore where it is attached to the sterigma

appressed. pressed close to or lying flat

appressoria. swollen, flattened portion of a fungal filament that adheres to the surface of a higher plant, providing anchorage for invasion by a fungus

approved landfill. state-licensed municipal or private landfill managed under state regulation to prevent leaching of potential pollutants into groundwater

AQAS. Agricultural Quarantine Activity System, a Web database accessible from any USDA–APHIS computer

aerial treatment. application of pesticide to a treatment area via aircraft

ARS. USDA–Agricultural Research Service

a**scoma (plural ascomata).** fruit body containing asci

ascospore. sexually produced spore in an ascus

ascus (plural asci). cell of the ascomycetes in which ascospores are produced after karyogamy and meiosis

aseptate. without a separating wall or membrane

augmentation. intentional addition of natural enemies via mass release in areas in which these enemies are absent, occur too late in the season or pest life cycle or are present in ineffective numbers

autoecious. parasitic fungus that completes the entire life cycle on a single host

barrier. natural or artificial obstacle to movement

basidium. cell of the basidiomycetes which bears on small stalks the basidiospores after karyogamy and meiosis

basidiospore. sexual spore formed externally on the basidium on a sterigma

biological control. development and use of natural means of control through parasites, predators, pathogens and biological tactics to suppress a pest population density below a level that would not occur in their absence, either for a given period or permanently

biological tactics. the use of any natural or derived product or technique utilizing biological applications such as gene transfer, genetic manipulation, pheromone attractants, host substitution or other biological means to suppress a pest population density below a level that would not occur in their absence, either for a

given period or permanently

biometric survey. survey succeeding the delimiting survey in which properties are number and letter coded for survey purposes on a rotational basis

biovar. group of bacterial strains that are distinguishable from other strains of the same species on the basis of their physiological characteristics

biseriate. arranged in two rows

block. units of a detection survey (*e.g.*, 1 square mile) in which all survey activities are conducted

boring dust. brownish, dry, crumbly decay of wood caused by fungi decomposing cellulose and leaving the lignin in a modified state

brachyblasts. short lateral branch

buffer area. survey area that is beyond the core block

bullate. appearing puckered as if blistered

calcareous. composed of, containing or characteristic of calcium carbonate, calcium or limestone; chalky

cambium. meristematic tissue in woody plants that exists between the wood (xylem) and the inner most bark (phloem)

cankers. plant disease characterized (in woody plants) by the death of cambium tissue and the loss and/or malformation of bark, or (in non-woody plants) by the formation of sharply delineated, dry, necrotic localized lesions on the stem; may also be used to refer to the lesion itself, particularly in woody plants

CAPS. Cooperative Agricultural Pest Survey program, partnership between all 50 states and the USDA to detect and monitor exotic pests of economic impact

cast needles. premature drop of needles from a tree

catenulate. arranged in a series of rings or chains

CFR. Code of Federal Regulations

chemical integration. direct application of selected chemicals to the host that are nontoxic or relatively nontoxic to selected parasites or predators

chimeric. composed of parts of different origin

chlamydospore. thick-walled asexual resting spore formed from hyphal cells

(terminal or intercalary) or by transformation of conidial cells that can function as an overwintering stage

chlorosis. yellowing of normally green tissue due to chlorophyll destruction in infected plants

classical biological control. introduction of exotic natural enemies from the region of origin to provide a permanent, self-sustaining suppression of a pest population density below a level that would not occur in their absence

clavate. resembling a club, becoming increasingly wide from the base to the distal end

coenocytic. having multiple nuclei embedded in cytoplasm without cross walls; nonseptate

cold treatment. exposure of a host product to cold temperatures lethal to a target pest; may be used alone or with fumigants

commercial production area. area in which host material is grown for sale

confirmation detection. positive identification of a submitted specimen

conidiogenous. cell that produces conidia

conidioma (plural conidiomata). fruit body containing conidia (*e.g.,* acervulus, pycnidium, sporodochium)

conidiophore. simple or branched hyphae arising from somatic hyphae which bears at its tip or sides, cells which form or become conidia

conidium. nonmotile asexual spore formed on a conidiophore, formed from or as an extension of the hyphal walls; may be single- or multi-celled, simple or complex, round, elongated or spiral in shape; found only in the Ascomycota or Basidiomycota

containment. application of phytosanitary measures in and around an infected area to prevent spread of a pest

control. application of phytosanitary measures in and around an infested area to prevent spread of a pest

conterminous. having a boundary in common

core area. area of 1 square mile surrounding a confirmed detection

coremium. fruiting bodies of certain fungi, consisting of a loosely bound bundle of conidiophores

corm. solid swollen underground bulb-shaped stem or stem base that serves as a reproductive structure

cotyledons. embryonic leaf in seed-bearing plants

CPB. United States Department of Homeland Security—Customs and Border Protection

CPHST. PPQ–Center for Plant Health Science and Technology

crepuscular. active during twilight hours

cross transect survey. survey designed to detect the infestation in the shortest time possible, strung out along the two lines of an axis and run through the most likely host areas; the survey may eventually be replaced by one based on a grid system for improved coverage

crown. portion of a plant, typically at ground level, at which the stem and roots merge

cultural control. intentional use of simple practices or mechanical measures that may be available to control a pest population

cuneate. wedge shaped

DBH. Diameter at Breast Height

decontamination. application of approved chemical or other treatment to contaminated implements, materials or buildings for killing or deactivating a pathogen

delimiting survey. survey conducted in a susceptible area not known to be infested with the target pest

deliquescent. tending to melt or dissolve

dendroid. resembling a tree in form and branching structure

denticulate. having a finely toothed margin

destructive sampling. method of observing signs and symptoms of the presence or absence of a pest by destruction of the living sample unit; for example, removal of bark to look for larvae

detection. process of identifying the presence of something concealed

detection survey. survey conducted in an environmentally favorable area in which the pathogen is not known to occur

developmental thresholds. minimum and/or maximum temperatures that support physiological development of a species

DHS. United Stated Department of Homeland Security

dichotomous. forked, with two symmetrical branches

dieback. death of branches on woody plants, shrubs or trees; typically young shoots, twigs and distal portions of branches dying progressively toward older plant parts

disposal. method used to eliminate diseased plant material or material associated with diseased plant material, usually at an approved landfill

diurnal. active during the day

EAN. Emergency Action Notification

PDEP. PPQ–Pest Detection and Emergency Programs

elicitins. small cysteine-rich lipid-binding proteins

ELISA. Enzyme-Linked Immunosorbent Assay; a serological laboratory technique used to determine the genus and species in a host sampling and testing program; excludes race and biovar

ellipsoid. surface whose plane sections are all ellipses or circles

EM. PPQ–Emergency Management

encysted. to form a cyst or protective covering to lose motility

endophytes. endosymbiont, often a bacterium or fungus, which lives within a plant for at least part of its life without causing apparent disease

entomopathogen. pathogen that induces illness in insects

EPA. United States Environmental Protection Agency

epicenter. initial site of an infestation

epicormic shoot. shoot arising from adventitious or latent buds that form on branches and stems; suckers are produced from the tree base

EPPO. European and Mediterranean Plant Protection Organization

eradication. application of phytosanitary measures to eliminate a pest from an area before it becomes too large in area or numbers for current technology

exotic species. pest species not native to or historically resident in North America

exudate. liquid excreted or discharged from diseased tissues

fascicles. dense cluster or bundle

fastidious phloem-limited. quality of a pathogen that describes its ability to only survive within the phloem capsular system of a plant

FIFRA. Federal Insecticide, Fungicide and Rodenticide Act

flagellum. long tapering process that projects singly or in groups from a cell and is the primary organ of motion of many microorganisms

FONSI. Finding Of No Significant Impact

fructification. the bearing of fruit

fumigation. application of an approved fumigant, such as methyl bromide, as a treatment

funicle. slender stalk or cord; a stalk connecting a seed or ovule with the placenta

fusiform. spindle-shaped; tapering at each end

gametangium (plural gametangia). cell containing gametes or nuclei that act as gametes.

generation. period during which a pest completes all stages of development predicted using biological information

geniculate. bent at a sharp angle

germ tube. hypha that emerges from a resting structure

girdle. encircle and cut through a stem or the bark and outer few rings of wood, disrupting the phloem and xylem

GIS. geographic information systems; a computer system capable of capturing, storing, analyzing and displaying geographically referenced information

globose. spherical or almost spherical

Gram-negative bacteria. class of bacteria that do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation

Gram-positive bacteria. class of bacteria that take up the crystal violet stain used in the Gram staining method of bacterial differentiation

ground spray. using ground spray equipment to apply pesticide to the ground, selected resting places, or host vegetation in a target infested area

gummosis. plant disease in which the lesions exude a sticky liquid

guttule. small oil-like drop inside a fungal spore

haustoria. specialized branch of a parasite formed inside host cells to absorb nutrients

heteroecious. parasitic fungus that develops different stages of the life cycle on different host species

heterothallism. condition in which sexual reproduction can occur only in the presence of genetically different mycelia (see homothallism)

hilum. slightly prominent mark or scar present on a conidium at the point of attachment to a conidiogenous cell

homothallism. condition in which sexual reproduction occurs with a single thallus; self-fertile (see heterothallism)

host. plant which is invaded by a parasite or pathogen and from which it obtains its nutrients

host collecting. collection and retention of infested host material for the purposes of determining characteristics of a pest's use of the host; also known as holding

hot-zone survey. choosing an area, typically residential, on which to concentrate surveys based on known pathway information with ZIP code-based demographic information or other scientific information; also known as a targeted survey or demographic survey

hyaline. transparent or nearly so; translucent; often used in the sense of colorless

hyphae. single, tubular filament of a fungal thallus or mycelium; the basic structural unit of a fungus

ICS. Incident Command System

identification authority. authority to confirm the presence of a particular pest contractible issued by the APHIS-National Identification Services to diagnosticians that have demonstrated proficiency in identification

imbricate. shingle-like; having regularly arranged overlapping edges as in roof tiles

incineration. burning of plants and associated soil or media resulting in their

complete destruction

indigenous. native

infection. establishment of a parasite on or within a host plant

infestation. to overrun or inhabit in numbers or quantities large enough to be harmful, obnoxious or threatening

infested area. area surrounding a single detection site or a group of sites; the standard designated area of 2.5 square miles is used, unless biotic or abiotic factors dictate adjustment of this area.

inoculative augmentation. biological control method of releasing natural enemies seasonally or periodically to reestablish a balance that has not been maintained naturally or has been disrupted by other control methods

inundative augmentation. biological control method that involves the massive production and release of natural enemies to control a pest/pathogen quickly

intercalary. positioned between the apex and base

ISIS. Integrated Survey Information System

isozyme. enzymes that differ in amino acid sequence but catalyze the same chemical reaction

leaf spot. plant disease lesion typically restricted in development in the leaf after reaching a characteristic size

lesion. localized diseased area or wound

limoniform. shaped like a lemon

little leaf. development of abnormally small leaves

macrocyclic. rust fungus with a long life cycle consisting of five stages, each with a characteristic spore

management. application of selected phytosanitary measures in and around an infested area to keep an invading population in check when other means of eradication of the population would fail

MLO. Mycoplasma-Like Organisms

MOA. Mode Of Action

monitoring survey. ongoing survey to verify the characteristics of a pest

population

mononematous. hypha or conidiophore arising singly from the substrate

monophagous. subsisting on only one kind of food

monotrichous. (of bacteria) having a single flagellum at one pole

mottle. disease symptom comprising light and dark areas in an irregular pattern, usually caused by a virus; often used interchangeably with mosaic

mycelium. mass of hyphae constituting the body (thallus) of a fungus

NAPIS. National Agricultural Pest Information System

NASS. National Agricultural Statistics Service

natural enemies. living organisms found in a natural community that kill, weaken or inhibit the biological potential of a pest species

necrosis. death of tissue or cells, usually accompanied by darkening to black or brown

NEPA. National Environmental Policy Act

NIS. PPQ–National Identification Service

nocturnal. active at night

non-migratory. species in which the individuals typically do not move far from the area of their birthplace

non-native. immigrant

NPAG. PPQ–New Pest Advisory Group

NPRG. New Pest Response Guidelines

obclavate. inversely clavate

obligate parasite. organism that can grow only as a parasite in association with its host plant and cannot be grown in artificial culture media

obpyriform. reverse of pear shaped

oogonia. female gametangium of oomycetes, containing one or more gametes

oospore. thick-walled, sexually derived resting spore of oomycetes

ostiole. opening at the top of many fungal fruit bodies (perithecia, pycnidia,

puffball basidiomata) through which spores escape or are expelled

papilla. conic or small rounded elevation, generally translucent, of the wall of gametangia and sporangia, which on breaking serves as the point of exit of planogametes and zoospores

paragynous. having the antheridium contact the oogonium on the side as in many *Pythium* spp.

paraphysis (plural paraphyses). sterile hyphae growing up between the asci in the hymenium of many ascomycetes

parasite. An organism that derives its nourishment from another living organism; not necessarily a pathogen

parasite/predator conservation. conservation of natural enemies through integrated procedures, highly selective predator/parasite friendly insecticides or techniques, biological insecticides or cultural practices favoring parasites/predators

parthenogenesis. development of an unfertilized egg into an adult female; asexual reproduction

PASS. Potentially Actionable Suspect Sample; a presumptive positive sample diagnosed or identified by provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS laboratory due to the nature of the plant sampled and the necessity for federal confirmation

pathogen. organism capable of causing a disease; not necessarily as a parasite

pathway. Means by which plant pests are introduced

PCR. Polymerase Chain Reaction; a laboratory technique that amplifies DNA sequences to determine if a host is infected with a known pathogen

PCR primers. short fragments of single-stranded DNA (15–30 nucleotides long), complementary to DNA sequences that flank the target region of interest; necessary components for the polymerase chain reaction

pedicle. slender stalk or support of spores, sporangia, asci, *etc.*

peduncle. stalk bearing a flower or fruit, or the main stalk of an inflorescence

PERAL. Plant Epidemiology and Risk Analysis Laboratory

percurrent. growing through in the direction of the long axis (*e.g.,* of a

conidiogenous cell proliferating through the tip)

periclinal. relating to cell walls that are parallel to the surface of a plant part, such as a meristem

peridium: wall of fruit-body

perithecium. globose or flask-shaped, ostiolate ascoma

pest. any organisms that damages plants or plant products

PestID. database containing all the information recorded from the PPQ form 309 Pest Interception Record

phenology. the study of seasonal and cyclic natural phenomena, especially in relation to climate and plant and animal life

phialide. conidiogenous cell which produces a basipetal succession of blastic conidia from an open end without any change in the length of the cell

phialidic. describes conidia produced by a phialide

phloem. food-conducting tissue in vascular plants consisting of companion cells and sieve tubes

phyllody. abnormal development of floral parts into leafy structures

plant hardiness zones. geographically defined area in which a specific category of plant life is capable of growing, as defined by climatic conditions, including the ability to withstand the minimum temperatures of the zone

pleomorphic. capable of assuming different shapes; ability of some bacteria to alter their shape or size in response to environmental conditions

plerotic. oospore filling the oogonium

polyphagus. feeding on a wide range of hosts

positive point. single point at which the target species was detected

PPQ. APHIS–Plant Protection and Quarantine

predator. organism that consumes substantial numbers of prey

proliferation. to grow or multiply by producing new parts, tissue, cells

protuberance. something that protrudes such as a bulge, knob or swelling

pseudoparenchymatous. compact mass of tissue, made up of interwoven hyphae or filaments that superficially resembles plant tissue

pycnidium. asexual fruiting body that is hollow and partially lined inside with conidiophores

pyriform. pear shaped

regulated area. area that extends a given distance in any direction from the epicenter of an infestation

regulated articles. all known or suspected hosts of a confirmed infestation of an exotic species including soil and any other suspected product or article

regulatory inspection. visual examination of host material, containers and transport

reniform. kidney shaped

rhizosphere. microenvironment in the soil, immediate around roots

riparian. relating to or located on the banks of a river or stream

rosaceous. like a rose

sanitation. destruction or removal of infected and infested plants or plant parts; decontamination of tools, equipment, containers, work space, hands, *etc.*

saprophyte. organism that obtains nourishment from dead organic material

satellite site. potentially infected property that is beyond a given distance from an infected property

sclerotium (plural sclerotia). firm, frequently rounded, mass of hyphae with or without the addition of host tissue or soil, normally having no spores in or on it

SEL. USDA–ARS–Systematic Entomology Laboratory

septate. with cross walls; having septa; (describing hyphae) partitioned by cross walls known as septa

setae. often pointed, stiff, erect hyphae which protrude from a fruiting body or fertile layer and may have a protective function

setose. covered with bristles or setae

sinuate. having the margin strongly or distinctly wavy, as a leaf

soil treatment. application of an approved pesticide to the soil of nursery stock or

within the drip line of host plants

SPHD. State Plant Health Director

sporangia. saclike fungal structure in which the entire contents are converted into an indefinite number of asexual spores

sporangiophore. hypha that bears a sporangium

sporodochium. cushion-shaped stroma covered by conidiophores

SPRO. State Plant Regulatory Official

steam sterilization. the use of live steam as a treatment on selected regulated items

stellate. star shaped

sterigma. slender, spine-like process arising from the basidium and bearing the basidiospores

stunting. overall reduction on plant height due to shortening of internodes

subglobose. almost round or spherical

subulate. awl shaped; tapering into a sharp point from a broader base

suppression. application of phytosanitary measures in an infected area to reduce pest populations

symbiosis. two different kinds of organisms living together that may, but does not necessarily, benefit each organism

sympodial. pertaining to proliferation of axes in which each successive spore or branch develops behind and to one side of the previous apex at which growth has ceased

symptom. indication of disease by reaction of the host, *e.g.*, canker, leaf spot, wilt

teleomorph. sexual form of a fungus

TESS. Threatened and Endangered Species System

trace-back. to investigate the origin of infested plants through intermediate steps in commercial distribution channels to the origin

trace-forward. to investigate the potential distribution of infected plants from a source through steps in commercial distribution channels

trap survey. determining the presence or absence of a pest by the use of traps placed in a predetermined pattern and serviced on a given schedule

true host. host capable of sustaining reproduction

tuberculate. covered with tubercles (wart-like projections)

TWG. Technical Working Group

tyloses. a balloon like extrusion of the a parenchyma cell into the lumina of a contiguous vessel that partially or completely blocks it

uninucleate. a cell having one nucleus

unitunicate. describes a type of ascus with only one distinct, functional wall layer

USDA. United States Department of Agriculture

USFWS. United States Fish and Wildlife Service

vacuole. generally spherical organelle within a plant cell bound by a membrane and containing dissolved materials such as metabolic precursors, storage materials or waste products

vector. carrier of an infectious agent capable of transmitting infection from one host to another, especially the animal that transfers an infectious agent from one host to another, typically an arthropod

verrucose. covered with warts or projections that resemble warts

viable. the state of being alive; able to germinate as seeds, fungus spores, sclerotia, *etc.*; capable of growth

viresence. development of green color in place of normal flower color

visual survey. examining hosts, substrate or hiding places for eggs, larvae, pupae, adults or visible damage

wilt. drooping of leaves and stems from lack of water (inadequate water supply or excessive transpiration); vascular disease that interrupts normal water uptake

witches' broom. symptom of plant disease that occurs as an abnormal brush-like cluster or dwarfed weak shoots arising at or near the same point; branches and twigs of woody plants may die back

xylem. the woody part of plants; the supporting and water-conducting tissue consisting primarily of tracheids and vessels

yellowing. leaves lose normal green color and become yellow

zonate. pertaining to a target-like development of a tree canker, characterized by successive, perennial rings of callus; referring to any symptom appearing in concentric rings; marked with concentric color bands

zoospores. fungal spore with flagella, capable of locomotion in water

zygote. cell in which two nuclei of opposite mating type have fused