INSTRUCTIONS TO QUARANTINE INSPECTORS AT ENTRY POINT FOR IMPORTED APPLE SEEDLINGS FROM ITALY INTO NEPAL

To prevent the introduction of quarantine pests, imported Apple from Italy must undergo **strict inspection** at designated entry points by the EP. The following **symptoms guide** will help quarantine inspectors identify potential infestations or infections caused by pests of concern at the point of entry while executing the Release Order (RO).

Section 1

A. Fungal pathogens

1. Pest name: ***Botryosphaeria dothidea***

Exudation of liquid from a blister

Reddish-brown lesions on bark

Exudation of liquid from a blister in an active canker

Symptoms (detection criteria): Look for the cankers on the grafted saplings. Check if the bark sloughs off easily.

Action: If the presence of pest is suspected in the sample**,** take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

2. Pest name: *Colletotrichum nymphaeae*

Symptoms (detection criteria): Look for cankers on the grafted samplings.

Action: If the presence of pest is suspected in the sample**,** take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

3. Pest name: *Fusarium acuminatum*

Symptoms (detection criteria): Inspect the root portion to check if there are any rotting symptoms. Look for discolorations in vascular bundles.

Action: If the presence of pest is suspected in the sample**,** take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not

release the consignment.

---

4. Pest name: *Pezicula malicortis*

Symptoms (detection criteria): Look for lesions in grafted sampling. Check if the bark sloughs off easily.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

5. Pest name: ***Phaeoacremonium minimum***

Symptoms (detection criteria): Look for discolorations in grafted saplings and check if rots and decayed portions are present. Look for cankers in the bark.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

6. Pest name: ***Phomopsis cotoneastri***

Symptoms (detection criteria): Sunken cankers are present. Inner bark and bark above cambium are split. Check if there are some symptoms.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

7. **Pest name:** ***Phytophthora cambivora***

Symptoms (detection criteria): Infected roots turn red-brown. Some stem lesions show yellow-brown tissue with narrow dark brown, concentric, wavy-margined rings up the stem. Inspect sampling for these symptoms.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

8. Pest name: *Phytophthora megasperma*

Symptoms (detection criteria): Crown rot affects rootstock while collar rot affects bark tissue of scion at or just below soil line. Necrosis of roots

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

9. Pest name: *Phytophthora syringae*

Symptoms (detection criteria: Look for root and collar rot, check if there is characteristic browning and decay of crown and root tissues

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

10. Pest name: *Pythium ultimum*

Symptoms (detection criteria): Look for necrosis of roots. Brown lesions might be present in roots.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

11. Pest name: *Valsa leucostoma*

Symptoms (detection criteria): Cankers are generally not noticeable until they girdle the trunk. Look for ooze form infected tissue.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

B. Bacterial pathogens

1. Pest name: ***Erwinia amylovora***

Symptoms (detection criteria): Look for ooze and discolorations near graft unions.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

2. Pest name: *Phytophthora syringae* pv, *syringae*

Symptoms (detection criteria): Look for the presence of cankers, necrotic spots. Check cankers on base of spurs. Young buds are dead.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

3. Pest name: *Rhizobium rhizogenes*

Symptoms (detection criteria): Look for the presence of excessive proliferation of adventitious roots at or near the region of infection.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

C. Nematode

1. Pest name: ***Paratrichodorous porosus***

Symptoms (detection criteria): Look if the root system is reduced and stubby.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

2. Pest name: ***Pratylenchus vulnus***

Symptoms (detection criteria): Look for necrotic lesions in roots, check if there is cracking of barks.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

D. Phytoplasma

1. Pest name: *Apple rubbery wood phytoplasma*

Figure. Symptoms of rubbery wood phytoplasma

Symptoms (detection criteria): Unusual flexibility, flattened wood and grooves, short internodes. Lack of lignification in xylem. Look for these symptoms. Viral and phytoplasma can be latent in many saplings.

Action: If symptoms are present or the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from

laboratory. Do not release the consignment.

---

2. Pest name: *Candidatus Phytoplasma asteris*

Symptoms (detection criteria): Latent infection may occur in grafted sampling and symptoms generally are not visible.

Action: Viral and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

3. Pest name: *Candidatus Phytoplasma solani*

Symptoms (detection criteria): Latent infection may occur in grafted sampling and symptoms generally are not visible.

Action: Viral and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

4. Pest name: *Phytoplasma aurantiifolia*

Figure: Advanced symptoms of witches' broom disease

Symptoms (detection criteria):

- Affected trees exhibit witches' brooms usually on a single branch initially that may subsequently appear on other branches (El Shereiqi and Gassouma, 1993).

Action: Viral and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

5. Pest name: *Phytoplasma mali*

Symptoms (detection criteria): Latent infection may occur in grafted sampling and symptoms generally are not visible.

Action: Viral and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

E. Virus and Viroids

1. Pest name: Apple stem pitting virus

Figure: Stem pitting on Virginia Crab

Figure: Stem pitting in Virginia Crab above the graft union

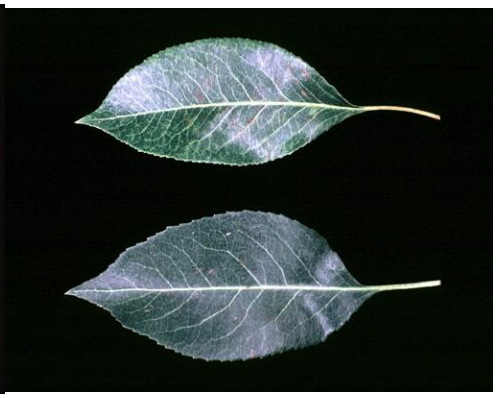


Figure: Vein yellows on a leaf of the pear, healthy control below

Symptoms (detection criteria): Apple varieties remain symptomless after infection with Apple stem pitting virus. However, xylem pitting can be seen on the stem of susceptible apple cultivars. Look for such symptoms if present.

Action: Infection from virus and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

2. Pest name: Cherry leaf roll virus

Symptoms (detection criteria): Latent infection may occur in grafted sampling and symptoms generally are not visible. Look if there is necrotic line in graft union.

Action: Infection from virus and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

3. Pest name: Pear blister canker viroid (PBCVd)

Figure. Bark symptoms induced by PBCVd

Figure. Symptoms induced by PBCVd

Symptoms (detection criteria): In apple, PBCVd are mostly symptomless. There are no characteristic symptoms in either bark, leaves or fruits.

Action: Infection from virus and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

---

4. Pest name: ***Prunus ringspot virus***

Symptoms (detection criteria): Latent infection may occur in grafted sampling and symptoms generally are not visible. Look if internodes are shortened.

Action: Viral and phytoplasma can be latent in many saplings. If the presence of the pathogen is suspected, take the sample and send it to the laboratory of Quarantine Office at the border. Wait for diagnosis from laboratory. Do not release the consignment.

INSTRUCTIONS TO THE QUARANTINE PEST DIAGNOSTIC LABORATORIES AT BORDER OFFICES FOR IMPORTED APPLE FROM ITALY INTO NEPAL

Lab Testing Methods for Quarantine Pests

A. Fungal Pathogens

Pathogen	Testing Method	Detection Criteria	Action
Botryosphaeria dothidea	Visual inspection Standard Blotter test Microscopic examination	Ascospores are unicellular, hyaline, 17-22 µm in length,	If the lab test is positive, instruct the checkpoint to reject

Pathogen	Testing Method	Detection Criteria	Action
	Growth on culture media	fusoid to ovoid with tapered ends. Pycnidia are solitary, globose and covered by mycelium with single ostiole with white to creamy contents. Confirm with molecular identification.	and re-export the shipment.
Colletotrichum nymphaeae	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Colonies on OA are low cottony mycelium with concentric alternating white, salmon and grey rings. Reverse culture is greyish-brown with greyish-salmon centre. Acervuli are colourless. Conidia hyaline, pale orange in mass, 1 celled 11.3* 3.2 µm, cylindrical to ellipsoidal with ends broadly or acutely rounded.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Fusarium acuminatum	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Microconidia are not reliable indicator but macroconidia are long tapering and curved of size 35-58*3.0-5.0 µm. Culture has red pigment.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Pezicula malicortis	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Macroconidia long elliposoid to fusiform, slightly curved, ends are somewhat pointed, aseptate. Microconidia are cylindrical, straight, rounded at apex, truncate at base, hyaline and appear for short time.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

Pathogen	Testing Method	Detection Criteria	Action
		Colonies on Oatmeal agar are white, cottony, with a clear droplet on top. Hazel to olivaceous mycelium.	
Phaeoacremonium minimum	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Colonies on Potato dextrose agar are white to pale yellow, buff, felty, flat. Conidia are elongate or ellipsoid. Phialides are flask shaped.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Phomopsis cotoneastri	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Perithecia are black, globose, subglobose densely clustered in groups. Pycnidia black with elongated nexl. Alpha conidia aseptate, hyaline, smooth, biguttulate. Beta aseptate, hyaline, fusiform	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Phytophthora cambivora	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Culture fluffy, white mycelium. Sporangia are ovate, subglobose and obpyriform to ellipsoidal with well rounded bases. No papillae. Oospores 37-44 µm in diameter.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Phytophthora megasperma	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Sporangia are non-papillate, ovoid to obpyriform, 35-60 x 25-45 µm, persistent on the sporangiophore and proliferate internally. These walled hyphal swellings are commonly observed, and are rounded or angular, and in clusters or chains. Chlamydospores are not produced.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

Pathogen	Testing Method	Detection Criteria	Action
		Oospores are 26-52 µm, averaging 41 µm diameter	
Phytophthora syringae	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Coenocytic hyphae with slender mycelium, rounded hyphal swelling in chains. Ovoid, semipapillate and persistent sporangia.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Pythium ultimum	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	Non- septate coenocytic hyphae, small hyphal swellings are present. Intercalary or terminal spores. Oospores 17-21 µm in diameter.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Valsa leucostoma	Visual inspection Standard Blotter test Microscopic examination Growth on culture media	hyaline, allantoid, aseptate conidia with the size of 4.5–5.5 × 1–1.5 µm. It has black conceptacle, numerous locules.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

B. Bacteria

Pathogen	Testing Method	Detection Criteria	Action
Erwinia amylovora	Isolation on selective medium, biochemical tests, pathogenicity test	*E. amylovora*, according to the methods of Jones and Geider (2001), are: oxidase test (–), oxidative/fermentative (O/F) test (+/+), fluorescent pigment in King's B medium under UV light (–), levan production (+), nitrate reduction (–), citrate utilization (+), gelatine liquefaction (+), urease and indol (–) and colony morphology on CCT medium.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

Pathogen	Testing Method	Detection Criteria	Action
Pseudomonas syringae pv. *syringae*	Isolation on selective media, biochemical tests, pathogenicity test, Toxin based identification	Green fluorescence in King's B medium. Bacterial colonies are circular, milky-white, raised, glistening white by reflected light, slightly iridescent by transmitted light, translucent, the surface smooth, and the margin entire.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Rhizobium rhizogenes	Isolation on selective media, biochemical tests, pathogenicity test	Colonies are circular, entire, domed, glossy, opaque, creamy (white to light beige) and highly mucoid on media rich in carbohydrates. Colony colour varies on selective media from cream to purple or orange on biovar one medium	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

C. Nematode

Pathogen	Testing Method	Detection Criteria	Action
Paratrichodorous porosus	Extraction using Baermann funnel or Sieves and Observe using stereomicroscope as well as compound microscope	Use morphological guides and morphometric confirmation Females: L=420–770 µm, stylet=39–50 µm, V=53–58. Males: L=500–770 µm, stylet=39–48 µm, spicules=33–40 µm. Confirm with molecular test	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Pratylenchus vulnus	Extraction using Baermann funnel, Sieves and observe using stereomicroscope as well as compound microscope	Slender body, straight when fixed, lateral fields with four incisures, cephalic region truncate with three-four annules, heavily sclerotized	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

Pathogen	Testing Method	Detection Criteria	Action
		framework, stylet 14-18 µm with round/cupped basal knobs, vulva at 78-82%, elongate-conoid tail with smooth, subacute/rounded terminus, males abundant, spicules 14-20 µm, gubernaculum 4-6 µm, bursa envelops tail.	

D. Phytoplasma

Pathogen	Testing Method	Detection Criteria	Action
Apple rubbery wood phytoplasma	Molecular diagnosis, woody indexing	PCR positive (amplification), symptoms observation	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Candidatus Phytoplasma asteris	Serological assay Molecular diagnosis	PCR positive (amplification)	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Candidatus Phytoplasma solani	Molecular diagnosis	PCR positive (amplification)	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Phytoplasma aurantifolia	Serological assay Molecular diagnosis	Positive PCR, serology	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Phytoplasma mali	Molecular diagnosis	Positive PCR	Refer to SPS lab. If the lab test is positive,

Pathogen	Testing Method	Detection Criteria	Action
Apple rubbery wood phytoplasma	Molecular diagnosis, woody indexing	PCR positive (amplification), symptoms observation	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
			instruct the checkpoint to reject and re-export the shipment.

E. Virus and Viroids

Pathogen	Testing Method	Detection Criteria	Action
Apple stem pitting virus	Serological assay (ELISA) Molecular diagnosis	Positive results	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Cherry leaf roll virus	Serological assay (ELISA) Molecular diagnosis	Positive results	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Pear blister canker viroid	Molecular diagnosis	Positive results	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Prunus necrotic ringspot virus	Serological assay (ELISA) Molecular diagnosis	Positive results	Refer to SPS lab. If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

---

3. Reporting & Compliance

- **Positive Test:** If a quarantine pest is detected, the consignment must be **quarantined, re-exported, or destroyed**.

- **Negative Test:** If no pests are found, the consignment is cleared for **customs clearance**.
- **Documentation:** Lab results must be submitted to the **Nepal Plant Quarantine Authority** for final approval.

These **testing protocols** ensure that apple imports from Italy comply with Nepal's **phytosanitary standards**, preventing the introduction of quarantine pests.

INSTRUCTIONS TO THE CENTRAL REFERRAL SPS LABORATORY FOR DIAGNOSTICIANS

Molecular Primers for PCR Testing of Quarantine Pests in apple from Italy

The following table lists the **PCR primers** for molecular identification of quarantine pests found in apple. These primers target **species-specific genes** to ensure accurate detection.

---

1. Fungi

Pathogen	Target Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Amplicon Size (bp)	PCR Type	Reference
Botryodiplodia theobromae	Tef1	Bd-F1 (CGCCGAATTTGCCTTATCA)	Bd-R1 (TTAGCATATGGTCGCATAGAC)	NA	qPCR	Romero-Cuadrado et al., 2023
Colletotrichum nymphaeae	GAPDH	NYMGF (GAT AAC ACC AGC TTC GTC GAT ATC)	NYMGR (TCT GTC AGC AAG TTT TGT CTC GGC)	132	qPCR	McHenry & Aćimović, 2024.
Fusarium acuminatum	ITS	ITS1 (TCCGTAGGTGAACCTGCGG)	ITS4 (TCCTCCGCTTATTGATATGC)	-	-	Trabelsi et al., 2017
Pezicula malicortis	β-tubulin	5'-CTT TCT CCG TTG TCC CAT CC-3'	5'-GAA CAT TGC GCA TCT GGT CC-3'	554	Conventional	Cao et al., 2013
Phaeoacremonium minimum	Actin and β-tubulin	ACT 512F (ATGTGCAAGGCCGGTTTCGC) (TTCCCCCGTCTCCACTTCTTCATG)	ACT 783R (TACGAGTCCTTCTGGCCCAT) Bt2b (ACCCTCAGTGTAGTGACCCTTGGC)	300	-	(Ye et al., 2020)
Phomopsis cotoneastri		D_eres3 F: TACTGTTGCCTCGGCGCTAGC	D_eres3 R: TTAACTACTGCTCGGGGTCCT	-	-	Ahn & Thuy, 2023

Pathogen	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Phytophthora cambivora		PC-F3 19 TCTGTCCTAGGTCCACCAT PC-FIP (TGATGGTCTTGCCGTCCAG-TGACCTCCAGGCTGACGTTAT)	PC-B3 (CTACAATTCCGAATAATCACAGTGT) PC-BIP (CCAGATTGTGCGTGCATTCC-GTTAGCTCCATGAAGCACTTTGAA)	-	LAMP	Li et al., 2018
Phytophthora megasperma	Ypt1	Ymeg1F (TCTGCTCTTCCGACTTGGTC)	Ymeg2R (TGGCATTAGTTAGTTTCGTCCA)	196	conventional	Schena et al., 2008
Phytophthora syringae	-	PSF3 (GAGTACTTTTCCTGCTGTGGTG) PSFIP (CCGGAACAACCGTCACTCTAC-GTGAACCGTAGCTGTGTTTG)	PSB3 (TCCAATTGAGATGCCCAC) PS-BIP (GCTGGAGTGACTGTCGAGG-CCGAAGT)	NA	LAMP	Li et al., 2018
Pythium ultimum		K1 (ACGAAGGTTGGTCTGTTG)	K3 (TCTCTACGCAACTAAATGC)	670	Conventional	Kageyama et al., 2003
Valsa leucostoma	ITS	ITS1 (TCCGTAGGTGAACCTGCG)	ITS4 (TCCTCCGCTTATTGATAT)			Zhang et al., 2014

2. Bacteria

Pathogen	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Erwinia amylovora	Chromosomal	G1-F: 5′-CCT GCA TAA ATC ACC GCT GAC AGC TCA ATG-3′	G2-R: 5′-GCT ACC ACT GAT CGC TCG AAT CAA ATC GGC-3′	187		Taylor et al., 2001
Pseudomonas syringae pv. *syringae*		Syr B B1(CTTTCCGTGGTCTTGATGAGG)	B2 (TCGATTTTGCCGTGATGAGTC)	752	conventional	Sorensen et al., 1998
		D1 (AAACCAAGCAAGAGAAGAAGG)	D2 (GGCAATACCGAACAGGAACAC)	446	conventional	Sorensen et al., 1998
Rhizobium rhizogenes		CTG TTG CCT TTC CTC GTC TG	GTC TCA AGT CAT CAT CCG CAT C	226	qPCR	

3. Nematode

Pathogen	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Paratrichodorous porosus	-	SSU18A (AAAGATTAAGCCATGCATG)	SSU26R (CATTCTTGGCAAATGCTTTCG)	1000	Conventional	Floyd 2002
Pratylenchus vulnus	ITS	TGCGGATGTGAGAAGTATGAG	ATGAATTTGGCCATGATTGG	-	qPCR	Hodson et al., 2021

4. Phytoplasma

Pathogen	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Apple rubbery wood phytoplasma	RNA	R16F1 R16F2	R16R1 R16R2	1.2 kb	nested	Bertaccini et al., 1997
Candidatus Phytoplasma asteris	16srRNA	AJ-16Sr-F (5'-CAT AGG GGG CGA GCG TTA TC-3'),	AJ-16Sr-R (5'- CAC ATG GAA TTC CGC TTG CC-3')	150	qPCR	Lamilla et al., 2023
Candidatus Phytoplasma solani	Tuf gene	Tuf-U/f (GATCCAGTGCGTGAAGTTGA)	Tuf-2b (ATTCCACGCAACAAAGCTCC)	242	Nested-qPCR-HMR	Landi et al., 2019
Phytoplasma aurantiifolia	16srRNA	CGGTCTAGTAAGTCAGTG	CATTTTACGCTACA	115	RT PCR	Mazraie et al., 2019
Phytoplasma mali	ACO	qMd-ACO-F (CCA GAA TGT CGA TAG CCT CGT T)	qMd-ACO-R (GGT GCT GGG CTG ATG AAT G)	587	RT PCR	Baric et al., 2011

5. Virus

Pathogen	Target Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Amplicon Size (bp)	PCR Type	Reference
Apple stem pitting virus	RNA	ASP-Ia (5'-AAGAGAAGACATCCAGATTTG-3'	), ASP-IIb (5'-GGAATTTCACAGCACTCCTAACCCTCC-3')	553	RTPCR	Kundu, 2002
Cherry leaf roll virus	UTR region	CLRV 3510F CGGTGGRGWTGCCGGTCCTA	CLRV−3a GTCGGAAAGATTACGTAAAAGG	1200	PCR	Lebas et al., 2016
Pear blister canker viroid		cPBCVd (5'-CCTTCGTCGACGACGAGCCGAG-3'),	hPBCVd (5'-GTCTAGAAGCCTGGGCGCTGG-3')	315	RT PCR	
Prunus necrotic ringspot virus	RNA	PNRSVF (CTTGAAGGACCAACCGAG)	PNRSVR-(ATCTGCTAACGCAGGTAAG)	351	RT PCR	Mekuria et al., 2003

These **molecular primer sets** allow precise **quarantine pest detection** for imports of apple, ensuring compliance with **Nepal's phytosanitary regulation.**

Section-2

1. **Pest name:** ***Anarsia lineatella***

Fig 1: Adult and larva of *Anarsia lineatella*

Symptoms (detection criteria): Look for wilting, drying, and blackening of shoot tips, which may indicate internal larval feeding.
Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for diagnosis from the laboratory. Do not release the consignment.

2. **Pest name:** ***Aspidiotus nerii***

Fig 2*: Aspidiotus nerii*

Symptoms (detection criteria): Inspect stems for the presence of scale insects. Look for small, circular, grayish armored coverings tightly attached to the stem surface. These scale armors are indicative of infestation and may be found clustered along the stems or branches.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

3. **Pest name:** ***Brevipalpus phoenicis***

Fig 3: *Brevipalpus phoenicis*

Symptoms (detection criteria): Check the roots and stem bases carefully for the presence of pests. Look for tiny, reddish-brown, flattened bodies that are often difficult to detect with the naked eye and may require magnification.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

4. **Pest name:** ***Cornu aspersum***

Fig 3: *Cornu aspersum*

Symptoms (detection criteria): Look for the newly hatched juvenile shells that are attached to the stem. They are usually translucent and lack shell bands and flecks. Old individuals are easily recognized because some parts of the periostracum become worn, exposing the underlying calcified shell.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

5. **Pest name:** ***Dysmicoccus brevipes***

Fig 5: *Dysmicoccus brevipes*

Symptoms (detection criteria): Look for black, sooty mold growing on honeydew deposits, white waxy masses on stems**.**

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

6. **Pest name:** ***Eulecanium tiliae***

Fig 6*: Eulecanium tiliae*

Symptoms (detection criteria): Inspect the stem carefully for the presence of adults and second-instar larvae. Look for brown or reddish transverse bands and membranous skin (derma) on the stem.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

7. **Pest name:** ***Harmonia axyridis***

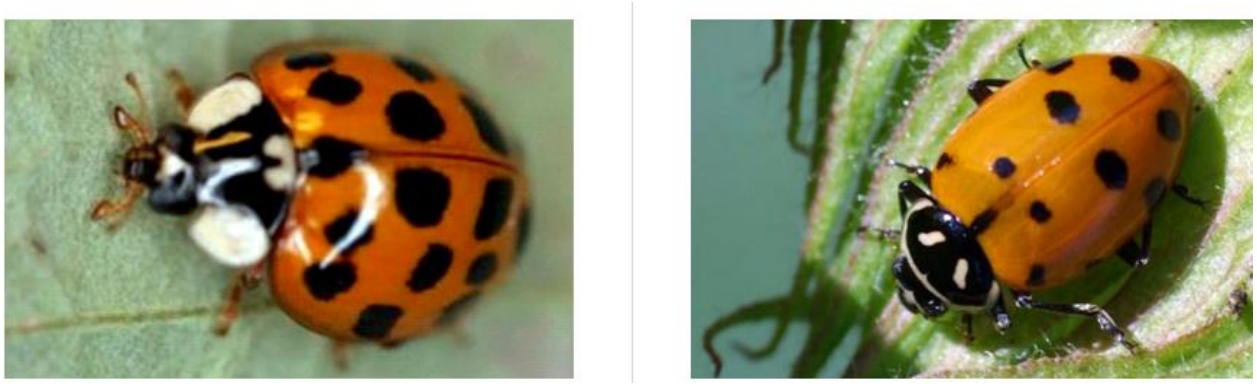


Fig 7: *Harmonia axyridis*

Symptoms (detection criteria): Look for the white "M" or "W" shape on their pronotum (the area behind the head), the oval and convex beetle with orange and red forms may be patterned with anything from 0 to 21 black spots or may display a grid-like black pattern.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

9. **Pest name:** ***Parlatoria oleae***

Fig 9: *Parlatoria oleae*

Symptoms (detection criteria): Look for the Scale armour (Small, grayish-brown s), gumming on bark.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

10. **Pest name: *Parlatoria pergandii***

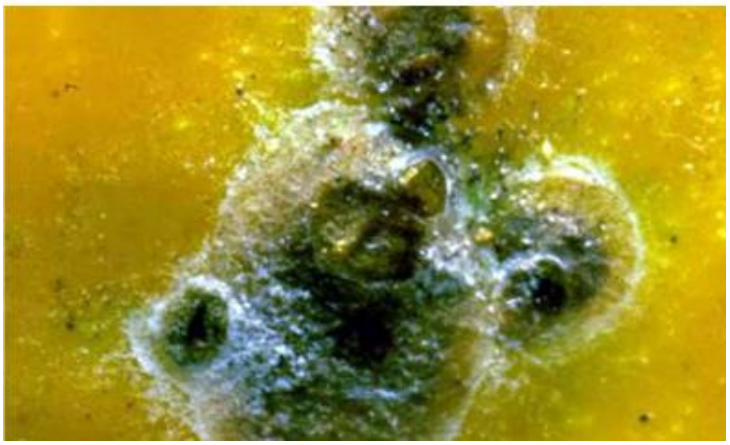


Fig 10: *Parlatoria pergandii* (Chaff Scale)

Symptoms (detection criteria): Look for the scale armour on the stem; they are usually small, grayish brown and also the crawlers are tiny yellowish and mobile. The heavy infested areas show the gummies exudation**.**

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

11. **Pest name:** ***Parthenolecanium corni***

Fig 11: *Parthenolecanium corni* (European Fruit Scale)

Symptoms (detection criteria): Look for Scales that are Brown, convex. Infested areas show symptoms of Honeydew and sooty mold

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

12. **Pest name:** ***Phenacoccus aceris***

Fig 12: Female adult of *Phenacoccus aceris* Fig 13: Nymph of *Phenacoccus aceris*

Symptoms (detection criteria): look for the overwinters as nymphs inside a white cocoon. Examine if the presence of ovisac is also white in color generally seen below the stem.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

13. **Pest name:** ***Pseudococcus calceolariae***

Fig 14: *Pseudococcus calceolariae* (Citrophilus Mealybug)

Symptoms (detection criteria): Examine the steam for Presence of mealybugs or sooty mold. The crawlers are tiny, mobile, and yellowish.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

14. **Pest name: *Pseudococcus comstocki***

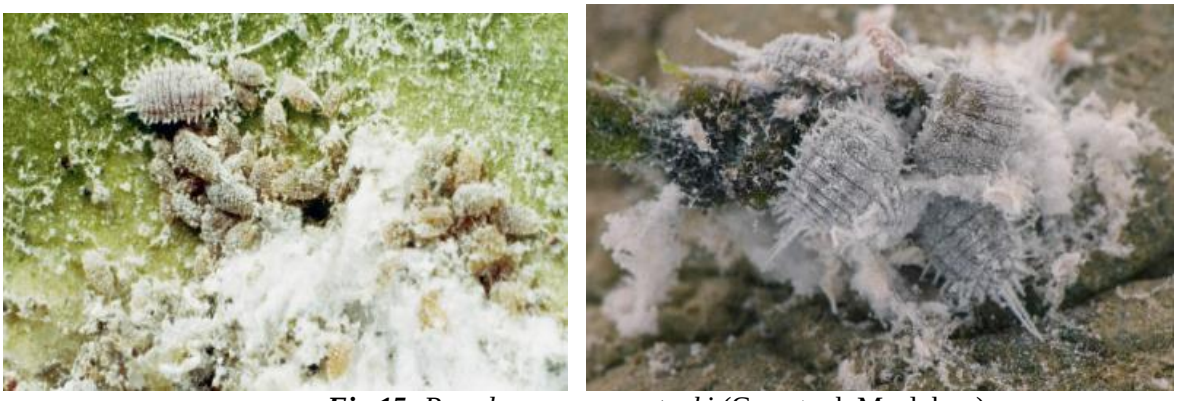


Fig 15: *Pseudococcus comstocki* (Comstock Mealybug)

Symptoms (detection criteria): Look for the Gall-like formations on bark. Check bark cracks for eggs/overwintering females (White, waxy). Look for honeydew or sooty mold.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

15. **Pest name: *Pseudococcus longispinus***

Fig 16: Longtailed Mealybug

Symptoms (detection criteria): Examine bark cracks for white waxy masses.Honeydew and sooty mold on the infected areas.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for diagnosis from the laboratory. Do not release the consignment.

16. **Pest name:** ***Rhopalosiphum oxyacanthae***

Fig 17: *Rhopalosiphum oxyacanthae*

Symptoms (detection criteria): Eggs overwinter in trunk/branch crevices. Look for sooty mold due to honeydew production.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

17. **Pest name:** ***Scolytus rugulosus***

Fig 18: *Scolytus rugulosus* (Shothole Borer)

Symptoms (detection criteria): Look for clear to brownish sap resembling tear drops exuding from 1.5 mm bark holes. Peel bark to reveal numerous dry exit holes from emerging adults.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

18. **Pest name:** ***Synanthedon myopaeformis***

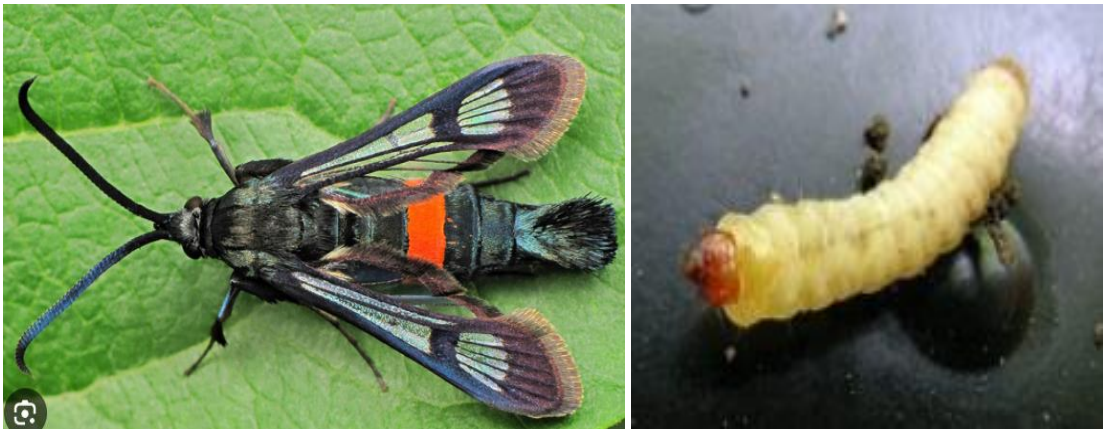


Fig 19: Larva and adult of *Synanthedon myopaeformis* (Clearwing Moth)

Symptoms (detection criteria): Look for the gnawing holes under bark with brownish heaps of wood dust, projecting exuvia. Look for pupal (Brown-yellow, with head tubercles) cases near exit holes.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

19. **Pest name:** ***Takahashia japonica***


Fig 20: *Takahashia japonica*

Symptoms (detection criteria): White, string-like ovisacs, sooty mold from honeydew on the stem.Small, inconspicuous slightly yellow overwintering nymph on the bark are observed.
Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

20. **Pest name:** ***Ceresa alta***

Symptoms (detection criteria): Examine twigs on lower branches for egg slits, eggshells.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

21. **Pest name:** ***Aculus schlechtendali***

Symptoms (detection criteria): Look for the rusting/bronzing bark crevices.

Action: If the presence of a pest is suspected in the sample**,** take the sample and send it to the laboratory of the Quarantine Office at the border. Wait for a diagnosis from the laboratory. Do not release the consignment.

Lab Diagnosis Methods for Quarantine Pests

Insect	**Testing Method**	**Detection Criteria**	**Action**
Anarsia lineatella	Visual inspection Microscopic examination	Larvae: Pupae are smooth, Small, brown, without a cocoon located in cracks and without a cocoon crevice of bark on limbs and trunk Adults: Small moths, grayish-brown	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Aspidiotus nerii	Visual inspection Microscopic examination	Scales: Small, circular, grayish armored coverings. Crawlers: Tiny, mobile, yellowish.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Brevipalpus phoenicis	Visual inspection Microscopic examination	Mites: Tiny, reddish-brown, flattened bodies, requiring magnification.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Cornu aspersum	Visual inspection Microscopic examination	Newly-hatched juvenile shells, which are fragile and translucent, lack any pattern of shell bands and flecks.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.

Insect	Testing Method	Detection Criteria	Action
Dysmicoccus brevipes	Visual inspection Microscopic examination	Mealybugs: White, waxy adults, 3–4 mm, often in clusters.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Eulecanium tiliae	Visual inspection Microscopic examination	Young Females: Oval/circular, light-yellow to cream, brown/reddish bands, membranous derma. Post-Reproductive Females: Convex, pea-sized, yellowish-brown to dark brown, 4.0–6.5 mm long, 3.5–6.0 mm wide, 3.0–5.0 mm high	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Harmonia axyridis	Visual inspection Microscopic examination	5.5–8.5 mm, orange/red with 0–22 black spots or black with red/orange spots. Distinctive Feature: White “M” or “W” on pronotum.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Parlatoria oleae	Visual inspection Microscopic examination	Scales: Small, grayish-brown armoured coverings. Crawlers: Tiny, mobile, yellowish.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Parlatoria pergandii	Visual inspection Microscopic examination	Detection of *Parlatoria pergandii* involves visual inspection for small, grayish-brown armoured scales on stems. gumming on bark, especially in heavy infestations. Crawlers are tiny, mobile, and yellowish, and they settle on plant surfaces, forming the characteristic armoured scales.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Parthenolecanium corni	Visual inspection Microscopic examination	First instar nymphs are typically found on the on stems and branches. The scales are brown, convex, and measure 3–5 mm, and crawlers are tiny, mobile, and yellowish. Additional signs include the presence of honeydew and associated sooty mold on stem	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Phenacoccus aceris	Visual inspection Microscopic examination	Adult female oval, 3 x 4 mm, greenish yellow, covered in white to grey mealy wax, with 18 pairs of short marginal wax filaments. They produce a long, woolly white ovisac, usually on the bark; adults often drop off once the ovisac is fully formed. Scales on the leaves or on the branches, depending on the season.	If the lab confirms the pest, instruct the checkpoint to reject and re-export the shipment.
Pseudococcus calceolariae	Visual inspection	Detection of *Pseudococcus calceolariae* is based on the presence of white, waxy, oval	If the lab confirms the pest,

Insect	Testing Method	Detection Criteria	Action
	Microscopic examination	mealybugs (3–4 mm) often found in clusters, along with tiny, mobile, yellowish crawlers. Signs include sooty mold on leaves and fruits, honeydew accumulation around the stem.	instruct the checkpoint to reject and re-export the shipment.
Pseudococcus comstocki	Visual inspection Microscopic examination	Detection of the pest is based on the presence of white, waxy females with 17 pairs of long filaments, measuring 3–4 mm, and tiny, mobile, yellowish crawlers. Signs include honeydew, sooty mold on leaves and fruits, fruit spotting, altered skin texture, and gall-like formations on bark .	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Pseudococcus longispinus	Visual inspection Microscopic examination	Crawlers settle on stems. where females produce distinctive waxy masses. Females are soft-bodied, oval in shape, white to grayish-white in color, measuring 3–4 mm, and possess two long tail filaments. Males are small, winged.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Rhopalosiphum oxyacanthae	Visual inspection Microscopic examination	Eggs of the pest overwinter in trunk or branch crevices. Apterous females are elongate oval, shiny green to yellow-green (2.1–2.6 mm) with dark green stripes, while alate females are smaller (1.1–2.0 mm), shiny green with a black head and thorax. Siphunculi are short and slightly swollen—yellowish-green in apterous forms and black in alate forms.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Scolytus rugulosus	Visual inspection Microscopic examination	Adults are blackish to dark brown, oblong, and about 2.5 mm long. Larvae and pupae are pale yellowish-white, legless, with a brown head, and measure under 2.5 mm. Eggs are tiny, pale, and concealed within tunnels. The species overwinters as mature larvae (prepupae) beneath the bark. Pupation and adult emergence occur in spring, with multiple generations annually. Females bore into the cambium to lay eggs in galleries; larvae feed under the bark.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Synanthedon myopaeformis	Visual inspection Microscopic examination	Adults have transparent wings (wingspan 18–22 mm) and a red fourth abdominal ring. Caterpillars are light-yellow with a reddish tint and reddish-brown head. Pupae are brown-yellow with head tubercles and abdominal spinules. Eggs hatch and larvae bore under bark, where they overwinter. Caterpillars create twisting tunnels and pupate in silky cocoons.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Takahashia japonica	Visual inspection Microscopic examination	A distinctive identifying characteristic is the formation of conspicuous, white, string-like ovisacs that are unique to this species and easily observable during inspections. Adult females are generally small and inconspicuous prior to ovisac formation, making early detection challenging without close examination.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

Insect	Testing Method	Detection Criteria	Action
Ceresa alta	Visual inspection Microscopic examination	Adults measure 7.7–10.0 mm in length and are robust in form, featuring a cape-like pronotum approximately 2.0–2.5 mm high. Their coloration ranges from pale green to golden yellow, with two distinctive white lines extending from the metapodial horns, black eyes, and transparent forewings. The pronotum is coarsely pitted, covered with hairlike setae, and arched with convergent carinae.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.
Aculus schlechtendali	Visual inspection Microscopic examination	Microscopic mites, requiring magnification for identification, have small, elongated, yellowish bodies. They are often found in concealed parts on bark.	If the lab test is positive, instruct the checkpoint to reject and re-export the shipment.

INSTRUCTIONS TO THE CENTRAL REFERAL SPS LABORATORY FOR DIAGNOSTICIANS

Molecular Primers for PCR Testing of Quarantine Pests in apple from Italy
The following table lists the **PCR primers** for molecular identification of quarantine pests found in apple. These primers target **species-specific genes** to ensure accurate detection.

Insect	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Anarsia lineatella	COXI	LepF1 (5’-ATT CAA CCA ATC ATA AAG ATA TTG G-3’)	LepR1 (5’-TAA ACT TCT GGA TGT CCA AAA AAT CA-3’)	658 bp	DNA barcoding method	Vulchi et al., 2021
Aculus schlechtendali	mtCOI	LCO (5'-GGTCAACAAATCATAAAGATATTGG-3')	HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	~658	Conventional PCR	Folmer et al., 1994
Aspidiotus nerii	GroEL	GroEL225FUZ (5ÐCTAATGACGTAGCTGGAGATGGÐ3)	GroEL1206R (5ÐCTAATGACGTAGCTGGAGATGGÐ3) or GroEL1050RUZ (5ÐCAATACTGCCACTCCACCTGCÐ3)	760-bp	qPCR	(Andersen et al 2014)
Brevipalpus phoenicis	e mitochondrial cytochro	5’-TGATTTTTTGGTCACCCAGAAG-3’	5’-TACAGCTCCTATAGATAAAAC-3’	400 bp	qPCR (MinEluteTM PCR)	Rodrigues et al., 2004

Insect	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
	me oxidase subunit I (Mit-COI) gene					
Ceresa alta	mtCOI	LCO (5'-GGTCAACAAATCATAAAGATATTGG-3')	HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	~658	Conventional PCR	Folmer et al., 1994
Cornu aspersum	Sequence of primers: Ha5, Ha6, Ha8, Ha9, Ha10, and Ha11 (16S rRNA and cyt b mitochondrial genes)	F:GTGTGACACACTGCCCTGGA F:TTATCCGCTTGATATATCCT F:AGTTTGCTGGTTTGTACACTCG F:AGCTAACCCACACTCAGATTT F:GCGTTCAATGTAGTTTATGTGCG F:CGTGTACTACTGGGCAACGT	R:CAATGGCAAACTACTGAAAGCAA R:ACTCGTACATGGTTGAAAAC R:CGTTTTTAGCTCTTGAATACGG R:AGCCAGCTAATATGTTTGGA R:GAGAACATGCATACAAACAAACATG R:ACGGAAAGAGACAGAAAGTGAG	117–207 145–215 152–210 108–172 211–253 175–240	multiplex PCR Kit	(Kougiagka et al., 2022)
Dysmicoccus brevipes	COXI	C1-J-2183 (CAACATTTATTTTGATTTTTTGG)	C1-N-2568 (GCWACWACRTAATAKGTATCATG)	370	qpcr	(Palma-Jiménez et al., 2017)
Eulecanium tiliae	mtCOI	LCO (5'-GGTCAACAAATCATAAAGATATTGG-3')	HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	~658	Conventional PCR	Folmer et al., 1994
Harmonia axyridis	HSP 90	TTGGACCGAAATGGTGAGC	AGGAACAACACGAGGTGCCC	145 bp	real time PCR	Qu et al., 2018
Parlatoria oleae	COI	LCO (5'-GGTCAACAAATCATAAAGATATTGG-3')	HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	~658	Conventional PCR	Folmer et al., 1994
Parlatoria pergandii	COI	LCO (5'-GGTCAACAAATCATAAAGATATTGG-3')	HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')	~658	Conventional PCR	Folmer et al., 1994
Parthenolecanium corni	COI	CAGGAATAATAGGAACATCAATAAGa	ATCAATGTCTAATCCGATAGTAAATAa	550 bp	GeneAmp PCR System	

Insect	Target Gene	Forward Primer (5’ → 3’)	Reverse Primer (5’ → 3’)	Amplicon Size (bp)	PCR Type	Reference
Phenacoccus aceris	28S-18	M-ITS2-F (5′CTCGTGACCAAAGAGTCCTG 3′)	M-ITS2-R (5′ TGCTTAAGTTCAGCGGGTAG 3′)	296 bp	e DNeasy Tissue Kit (QIAGEN, Hilden, Germany)	(Abd-Rabou et al., 2012)
Pseudococcus calceolariae	COX I	5′-CAACATTTATTTTGATTTTTGG-3′.	5′-GCWACWACRTAATAKGTATCATG-3′.	385 bp	DNA barcoding method	Palma-Jiménez & Blanco-Meneses, 2016
Pseudococcus comstocki	COI	MFCO1-ATATCTCAAATTATAAATCA AGAA	MRCO1-ATTACACCTATAGATAAAACATAATG	371bp	Qiagen PCR Purification Kit	(Puig et al., 2021)
Pseudococcus longispinus	COXI	C1-J-2183 CAACATTTATTTTGATTTTTGG	C1-N-2568 GCWACWACRTAATAKGTATCATG	410bp	DNA barcoding method	(Palma-Jiménez et al., 2018)
Rhopalosiphum oxyacanthae	mitochondrial CO1	LepF (ATTCAACCAATCATAAAGATATTGG)	LepR (TAAACTTCTGGATGTCCAAAAAATCA)	700 bp	DNA barcoding method	Foottit
Scolytus rugulosus	COI	1495b 5’-AACAAATCATAAAGATATTGGRAC-3’	rev 750 5’-GAAATTATNCCAATTCCTGG-3’	271 bp	Conventional PCR	Smith & Cognato, 2014
Synanthedon myopaeformis	COXI	5-ATAATYGGRGGATTTGGWAAYTG	3-GTTARTCCNCCYACWGTRAA	~ 700bp	Ex Taq Hot-start PCR Kit	Hansen et al., 2012
Takahashia japonica		**LCO1490**: 5′-GGTCAACAAATCATAAAGATATTGG-3′	**HCO2198**: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	~658 bp	DNA barcoding	Folmer et al., 1994

ENTRY CONDITIONS IN DETAIL FOR IMPORTER'S REFERENCE

1. **Obtain Entry Permit from NPPO-Nepal before entering:** Before entering into any trade agreement, finalizing payment terms (e.g., LC, TT, DAP, DAC, DAA, etc.), or engaging in any customs-related activities, it is mandatory to obtain an Entry Permit from NPPO-Nepal. This is a non-negotiable and critical requirement that must be fulfilled prior to initiating any import procedures for plants and plant-related products including walnut fruit requiring a Phytosanitary Certificate.
2. **Phytosanitary Certificate Requirements:** The Phytosanitary Certificate issued by the NPPO-Italy must strictly adhere to Nepal's regulations and be issued only upon receipt of the Entry Permit from NPPO-Nepal. The Quarantine Authority in Nepal **shall not** put any requests for the release procedure of consignments that arrive at the entry point without meeting the required compliance. Traders are strongly urged to adhere to these regulations to avoid disruptions, penalties, and delays. The original Phytosanitary Certificate issued by NPPO-China must reference the **Approval Reference Number** from the **Entry Permit (EP)** issued by NPPO-Nepal.

Additional Declaration: The Phytosanitary Certificate (PC) must include an additional declaration confirming freedom from following pests, in strict adherence to the Entry Permit (EP) conditions issued by the National Plant Protection Organization of Nepal (NPPO-Nepal).

Quarantine Pests

1. *Aculus schlechtendali*
2. *Anarsia lineatella*
3. *'Candidatus Phytoplasma mali'*
4. *Apple stem pitting virus*
5. *Aspidiotus nerii*
6. *Brevipalpus phoenicis*
7. *'Candidatus Phytoplasma asteris'*
8. *Dysmicoccus brevipes*
9. *Erwinia amylovora*
10. *Harmonia axyridis*
11. *'Candidatus Phytoplasma solani'*
12. *Cherry leaf roll virus*
13. *Parlatoria oleae*
14. *Parlatoria pergandii*
15. *Parthenolecanium corni*
16. *Pear blister canker viroid*
17. *Phytophthora cambivora*
18. *Phytophthora megasperma*
19. *'Candidatus Phytoplasma aurantifolia'*
20. *Prunus necrotic ringspot virus*
21. *Pseudococcus calceolariae*
22. *Pseudococcus comstocki*
23. *Pseudococcus longispinus*
24. *Pseudomonas syringae* pv. *syringae*
25. *Rhizobium rhizogenes*

Potential Quarantine Pests

1. *Ceresa alta*
2. *Eulecanium tiliae*
3. *Phenacoccus aceris*
4. *Rhopalosiphum oxyacanthae*
5. *Scolytus rugulosus*
6. *Synanthedon myopaeformis*
7. *Takahashia japonica*
8. *Botryosphaeria dothidea*
9. *Colletotrichum nymphae*
10. *Pezicula malicorticis*
11. *Phaeoacremonium minimum*
12. *Phomopsis cotoneastri*
13. *Valsa leucostoma*
14. *Fusarium acuminatum*
15. *Cornu aspersum*
16. *Paratrichodorus porosus*
17. *Pratylenchus vulnus*
18. *Phytophthora syringae*
19. *'Candidatus Phytoplasma mali'*

3. Inspection Upon Arrival:

Upon arrival at the designated port of entry in Nepal, all consignments will be subject to a mandatory phytosanitary inspection by the Quarantine Authority of NPPO-Nepal. The inspection will verify compliance with the Entry Permit conditions, Phytosanitary Certificate declarations, and freedom from the listed quarantine pests. Consignments failing to meet these requirements may be detained, treated (if feasible), returned to the country of origin, or destroyed at the importer's expense, as determined by NPPO-Nepal.

4. Packaging & Contamination-Free Requirement:

The apple seedlings must be packed in clean, pest-free, and tamper-proof packaging, if any to prevent any risk of contamination during transit. Packaging materials should comply with international phytosanitary standards and must not contain soil, plant debris, or any unauthorized organic matter. The consignment must be clearly labeled with product details, country of origin, and quarantine treatment information.

5. Approved Ports of Entry:

6. Importer Responsibilities:

Hold importers accountable for ensuring that their consignments comply with Nepal's phytosanitary regulations, including bearing the costs of inspection, post entry confinement in the warehouse or the advanced diagnostic service.

Implementing these measures would help Nepal safeguard its agricultural sector from potential threats posed by quarantine pests associated with Apple seedlings import.

1. □□□□□□□□ □□□□□ **NPPO-**□□□□□□□□ □□□□□□ □□□□□□ **(Entry Permit)** □□□□□□□ □□□□□□□□□

□□□□ □□□ □□□□□□□ □□□□□□□ □□□□□□□□□□ □□□□□, □□□□□□□□ □□□□□□□ (□□□□□, LC, TT, DAP, DAC, DAA □□□) □□ □□□□□ □□□ □□ □□□□□□□□□□□□ □□□□ □□□ □□□□□□□□□□ □□□□□ □□□□ □□□, **NPPO-**□□□□□ □□□ □□□□□□ □□□□□□ □□□□□□□ □□□□□ □□□□□□□□ □□ □□ □□□□□□□□□□ □ □□□-□□□□□□□□□□□□ □□□□ □□, □□□ □□□□□□□ □□□□□□ **(Apple seedlings)** □□□ □□□ □□□□□□□□□ □□□□□□□□□□□ □□□□ □□□□□□□□□□ □□□□□ □□□□□□□□ □□□□ □□□□□□□□□□ □□□□□□

---

2. □□□□□□□□□□□ □□□□□□□□□□ **(Phytosanitary Certificate)** □□ □□□□□□□□□ □□□□□□□□□□□

• **NPPO-Italy** □□□□□□ □□□□ □□□□□□ □□□□□□□□□□□ □□□□□□□□□□ □□□□□□□ □□□□□□□□□□ □□□□□ □□□□□ □□□□□□□□□□□

• Nepal Quarantine Authority □□ □□□□ □□□□□ □□□□□□ □□□□□□□□□□ (EP) □□□□ □□□□ □□□□ □□□ □□□ (consignment) □□ □□□□□□□□□□ □□□□□□□□□ □□□ □□□□□□ □□□□

• Nepal NPPO □□□□□□ □□□□ □□□□□□ EP □□ □□□□□ □□□□□□□ □□□□□ (Approval Reference Number) □□□□□□□□□□□□ □□□□□□□□□□□□ □□□□□□□□ □□□□□□ □□□□□□□□□

□□ □□□□□ **(Additional Declaration):**

□□□□□□□□□□□□ □□□□□□□□□□□□ □□□□□ □□□□□□□□ □□□□□□□□□ □□□□□ □□□□□ □□□□□□□□ □□□□□□□□□:

Quarantine Pests

1. *Aculus schlechtendali*
2. *Anarsia lineatella*
3. *'Candidatus Phytoplasma mali'*
4. *Apple stem pitting virus*
5. *Aspidiotus nerii*
6. *Brevipalpus phoenicis*
7. *'Candidatus Phytoplasma asteris'*
8. *Dysmicoccus brevipes*
9. *Erwinia amylovora*
10. *Harmonia axyridis*
11. *'Candidatus Phytoplasma solani'*
12. *Cherry leaf roll virus*
13. *Parlatoria oleae*
14. *Parlatoria pergandii*

15. *Parthenolecanium corni*
16. *Pear blister canker viroid*
17. *Phytophthora cambivora*
18. *Phytophthora megasperma*
19. *'Candidatus Phytoplasma aurantifolia'*
20. *Prunus necrotic ringspot virus*
21. *Pseudococcus calceolariae*
22. *Pseudococcus comstocki*
23. *Pseudococcus longispinus*
24. *Pseudomonas syringae pv. syringae*
25. *Rhizobium rhizogenes*

Potential Quarantine Pests

1. *Ceresa alta*
2. *Eulecanium tiliae*
3. *Phenacoccus aceris*
4. *Rhopalosiphum oxyacanthae*
5. *Scolytus rugulosus*
6. *Synanthedon myopaeformis*
7. *Takahashia japonica*
8. *Botryosphaeria dothidea*
9. *Colletotrichum nymphae*
10. *Pezicula malicorticis*
11. *Phaeoacremonium minimum*
12. *Phomopsis cotoneastri*
13. *Valsa leucostoma*
14. *Fusarium acuminatum*
15. *Cornu aspersum*
16. *Paratrichodorus porosus*
17. *Pratylenchus vulnus*
18. *Phytophthora syringae*
19. *'Candidatus Phytoplasma mali'*

---

4. □□□□□□□ □□□□□□□□ □□□□□□□□ **(Inspection Upon Arrival)**

• □□□ □□□□□□□□ □□□□□ □□□□□□ □□□□□□ □□□□□□□□ □□□□□ □□□□□□□□□□□□ □□□□□□□□ □□□ □□□□□□□□□□

• □□□□□□□ "□□□□□□ □□□□□□□□□□□□ □□□ □□□□□□□ □□, □□□□" □□ □□□ □□, □□□ □□□□ □ □ □□ □□□□□□ □□□□□□□□□□□□ □□□□□□□□ □ □□□□□ □□□□□ □□□□□□□□□□

• □□□ □□□□□ □□□□□□ □□ □□□□□□□□□ □□□□□□□□ □□□□□□□□:

o □□□□□□ □□□□□□□□□, □□ □□□□□□ □□□□□ □□□ □□□□ □□□□□□□□□□

---

5. □□□□□□□□□ □ □□□□ □□□□□□□ **(Packaging & Contamination-Free Requirement)**

• □□□□□□□ □□□□□□ **(Apple seedlings)** □□□□ □ □□□ □□□□□□□□□□□□ □□□□□□□□□ □□□□□ □□□□□ □□□□□□□□□□
• □□□□□□□□□□□ □□□ □□□□□□ □□□□□□□□□ □ □□□□□ □□□□□ □□□□□□□□□ □□□ □□□□□□□□□□:
o □□□□□, □□□, □□□, □□□□□ □□□□□□, □□□□, □□□□□ □□ □□□□□□□□ □□□□□□□□□□
• □□□□□ □□□□□□ □□□□□□□□□□□ □□□□ □□□□□□□□□□□□ □□□□□□□□ □□□□□□□□□ □□□□□□□□ □□□□□ □□□□□, □□□□□ □□□□□□□□□□ □□□□ □□□□□□□ NNSW □□ □□□□ □□□□□□□□□ □□□□□□□□□□ EP Status □□ **USED** □□□□□□□□□□□

---

6. □□□□□□□□ □□□□□□ □□□□□□□□□ **(Approved Ports of Entry)**
(□□□□□□□□ □□□□□□ □□□□□□**)**
(□□□□ □□□□□□□□ □□□□□, □□□ □□□□□□ □□ □□□□ □□□□□□ □□□□□□□□□□)

---

7. □□□□□□□□□□□ □□□□□□□□□□ **(Importer Responsibilities)**
• □□□□□□□□□□□ □□□□□ □□□□□□ □□□□□□□ □□□□□□□□□□□□ □□□□□□□□□□ □□□□□ □□□□□ □□□ □□□□ □□□□□□□□□ □□□□□□□□□□
• □□□□□ □□□□□□ □□□□□□□□□□□ □□□□ □□□□□□□□□□□□ □□□□□□□□ □□□□□□□□□ □□□□□□□□ □□□□□ □□□□□, □□□□□ □□□□□□□□□□ □□□□ □□□□□□□ NNSW □□ □□□□ □□□□□□□□□ □□□□□□□□□□ EP status □□ **USED** □□□□□□□□□□□
• □□□□□□□□, □□□□□□□ (post-entry confinement), □□ □□□□ □□□□□□□□ □□□□□□□ □□□□□□ □□□□□□□ □□□□□□ □□□□□□□□□□□□□
• □□ □□□ □□□□□□□□□ □□□□□ □□□□□ □□□□□ □□□□□□□ □□□□□□ **(Apple seedlings)** □□□□ □□□□□□□□□□□ □□□□ □□□□□, □□□□ □□ □□□□□ □□□□□□

□□□□□□□□
□□□□□□□ **Apple seedlings (**□□□□□□□ □□□□□□**)** □□□□ □□□□□ □□□□□□□□ Quarantine Pest □□□□□□ □□□□ □□□□□□□□□□ □□□□□□□□ □□□□ □□ □□□□□□□□□ □□□□□□□□□□□ □□□□□□ □□

References:

Abd-Rabou, S., Shalaby, H., Germain, J. F., Ris, N., Kreiter, P., & Malausa, T. (2012). Identification of mealybug pest species (Hemiptera: Pseudococcidae) in Egypt and France, using a DNA barcoding approach. *Bulletin of Entomological Research, 102*(5), 515–523. https://doi.org/10.1017/S0007485312000092

Ahn, S., & Thuy, N. T. D. (2023). Development of Molecular Markers to Detect *Diaporthe* spp. from Decayed Soybean Seeds. *Mycobiology*, *51*(6), 463–467. https://doi.org/10.1080/12298093.2023.2281725

Aigoun-Mouhous, W., Mahamedi, A. E., León, M., Chaouia, C., Zitouni, A., Barankova, K., ... & Berraf-Tebbal, A. (2021). *Cadophora sabaouae* sp. nov. and *Phaeoacremonium* species associated with Petri disease on grapevine propagation material and young grapevines in Algeria. *Plant Disease*, *105*(11), 3657-3668.

Andersen, J. C., Gwiazdowski, R. A., & Gruwell, M. E. (2014). Molecular evolution of sexual and parthenogenetic lineages of the armored scale insect *Aspidiotus nerii* (Hemiptera: Diaspididae) and its primary bacterial endosymbiont, *Uzinura diaspidicola*. *Annals of the Entomological Society of America, 107*(5), 954–960. https://doi.org/10.1603/AN14052

Bangels, E., Peusens, G., Bylemans, D., & Beliën, T. (2014). Biology and control of the apple mealybug *Phenacoccus aceris* (Signoret) in Belgium. *Communications in Agricultural and Applied Biological Sciences, 79*, 239–244.

Baric, S., Berger, J., Cainelli, C., Kerschbamer, C., Letschka, T., & Dalla Via, J. (2011). Seasonal colonisation of apple trees by '*Candidatus Phytoplasma mali'revealed* by a new quantitative TaqMan real-time PCR approach. *European Journal of Plant Pathology*, *129*(3), 455-467.

Bertaccini, A., Vibio, M., Franova-Honetslegrova, J., & Janeckova, M. (1997). Molecular detection of phytoplasmas in apple with rubbery wood symptoms. In *XVII International Symposium Virus and Virus-Like Diseases of Temperate Fruit Crops 472* (pp. 693-700).

Cao, D. , Li, X. , Cao, J. and Wang, W. (2013). PCR Detection of the Three *Neofabraea* Pathogenic Species Responsible for Apple Bull's Eye Rot. *Advances in Microbiology*, **3**, 61-64. doi: 10.4236/aim.2013.31009

Chen, C., Verkley, G. J., Sun, G., Groenewald, J. Z., & Crous, P. W. (2016). Redefining common endophytes and plant pathogens in *Neofabraea*, *Pezicula*, and related genera. *Fungal Biology*, *120*(11), 1291-1322.

Cowie, R. (2022). *Cornu aspersum* (common garden snail). CABI Compendium. https://doi.org/10.1079/cabicompendium.26821

Fekih Hassen, I., Roussel, S., Kummert, J., Fakhfakh, H., Marrakchi, M., & Jijakli, M. H. (2006). Development of a Rapid RT-PCR Test for the Detection of *Peach Latent Mosaic Viroid*, *Pear Blister Canker Viroid*, *Hop Stunt Viroid* and *Apple Scar Skin Viroid* in Fruit Trees from Tunisia. *Journal of Phytopathology*, *154*(4), 217-223.

Floyd, R., Abebe, E., Papert, A., & Blaxter, M. (2002). Molecular barcodes for soil nematode identification. *Molecular ecology*, *11*(4), 839-850.

Foottit, R. G., Maw, H. E. L., von Dohlen, C. D., & Hebert, P. D. N. (2008). Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources*, *8*(6), 1189–1201. https://doi.org/10.1111/j.1755-0998.2008.02297.x

Ghosh, D., Bhose, S., Manimekalai, R., & Gowda, S. (2013). Molecular detection of *Candidatus* Phytoplasma spp. causing witches' broom disease of acid lime (Citrus aurantifolia) in India. *Journal of Plant Biochemistry and Biotechnology*, *22*, 343-347.

Hansen, J. A., Klingeman, W. E., Moulton, J. K., Oliver, J. B., Windham, M. T., Zhang, A., & Trigiano, R. N. (2012). Molecular identification of Synanthedonini members (Lepidoptera: Sesiidae) using cytochrome oxidase I. *Annals of the Entomological Society of America*, *105*(4), 520–528. https://doi.org/10.1603/AN11169

Hodson, A. K., Cicchetto, A., & Fierro, F. A. (2021). Real time PCR assays to detect and quantify the nematodes *Pratylenchus vulnus* and *Mesocriconema xenoplax*. *Crop Protection*, *145*, 105617.

Jones, L. S. (1935). Observations of the habits and seasonal life history of *Anarsia lineatella* in California.

Kageyama, K., Komatsu, T., & Suga, H. (2003). Refined PCR protocol for detection of plant pathogens in soil. *Journal of General Plant Pathology*, *69*, 153-160.

Kageyama, K., Ohyama, A., & Hyakumachi, M. (1997). Detection of *Pythium ultimum* Using Polymerase Chain Reaction with Species-Specific Primers. *Plant disease*, *81*(10), 1155–1160. https://doi.org/10.1094/PDIS.1997.81.10.1155

Kougiagka, E., Gkafas, G. A., Exadactylos, A., & Hatziioannou, M. (2022). Morphology and genetic structure profile of farmed snails *Cornu aspersum aspersum* and *Cornu aspersum maximum* in Greece. *Sustainability*, *14*(23), 15965. https://doi.org/10.3390/su142315965

Kundu, J. K. (2002). The application of RT-PCR assay for the detection of Apple stem pitting virus and Apple stem grooving virus in four apple cultivars. *PLANT PROTECTION SCIENCE-PRAGUE-*, *38*(1), 13-17.

Lamilla, J., Galvez, A., & Franco-Lara, L. (2023). Simultaneous detection and quantification by multiplex qPCR of'*Candidatus* Phytoplasma *asteris*' and'*Candidatus* Phytoplasma f*raxini*'in a plant host and insect vectors. *Tropical Plant Pathology*, *48*(5), 564-574.

Landi, L., Murolo, S., & Romanazzi, G. (2019). Detection of '*Candidatus* Phytoplasma *solani'in* roots from Bois noir symptomatic and recovered grapevines. *Scientific Reports*, *9*(1), 2013.

Lebas, B. S. M., Veerakone, S., Liefting, L. W., Tang, J., Perez-Egusquiza, Z., von Bargen, S., & Ward, L. (2016). Comparison of diagnostic techniques for the detection and differentiation of Cherry leaf roll virus strains for quarantine purposes. *Journal of virological methods*, *234*, 142-151.

Li, Guan-Rong; Huang, Guo-Ming; Zhu, Lin-Hui; Lv, Dajin; Cao, Baohong; Liao, Fang; Luo, Jia-Feng . (2018). Loop-mediated isothermal amplification (LAMP) detection of *Phytophthora hibernalis, P. syringae and P. cambivora*. *Journal of Plant Pathology, ()*, –. doi:10.1007/s42161-018-0136-5

Marsberg, A., Kemler, M., Jami, F., Nagel, J. H., Postma-Smidt, A., Naidoo, S., ... & Slippers, B. (2017). *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular plant pathology*, *18*(4), 477-488.

Mazraie, M. A., Izadpanah, K., Hamzehzarghani, H., Salehi, M., & Faghihi, M. M. (2019). Spread and colonization pattern of '*Candidatus* Phytoplasma *aurantifolia'in* lime plants [Citrus aurantifolia (Christm.) Swingle] as revealed by real-time PCR assay. *Journal of Plant Pathology*, *101*, 629-637.

McHenry, D. J., & Aćimović, S. G. (2024). New Species-Specific Real-Time PCR Assays for *Colletotrichum* Species Causing Bitter Rot of Apple. *Microorganisms*, *12*(5), 878. https://doi.org/10.3390/microorganisms12050878

Mekuria, G., Ramesh, S. A., Alberts, E., Bertozzi, T., Wirthensohn, M., Collins, G., & Sedgley, M. (2003). Comparison of ELISA and RT-PCR for the detection of Prunus necrotic ring spot virus and prune dwarf virus in almond (Prunus dulcis). *Journal of virological methods*, *114*(1), 65-69.

Mounde, L. G., Ateka, E. M., Kihurani, A. W., & Wasilwa, L. (2012). Morphological characterization and identification of *Phytophthora* species causing citrus gummosis in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, *12*(7).

Palma-Jiménez, M., & Blanco-Meneses, M. (2016). First record of morphological and molecular identification of mealybug *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) in Costa Rica. *Universal Journal of Agricultural Research, 4*(4), 125–133. https://doi.org/10.13189/ujar.2016.040403

Palma-Jiménez, M., & Blanco-Meneses, M. (2017). Morphological and molecular identification of *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) in Costa Rica. *Journal of Entomology and Zoology Studies, 5*(2), 1211–1218.

Palma-Jiménez, M., Blanco-Meneses, M., & Sánchez, C. G. (2018). Identification of *Pseudococcus viburni* and *Pseudococcus longispinus* (Hemiptera: Pseudococcidae) in *Musa* sp. *Trends in Entomology*, *14*, 33–43.

Puig, A. S., Wurzel, S., Suarez, S., Niogret, J., & Marelli, J. P. (2021, July). Molecular identification of mealybug species (Hemiptera: Pseudococcidae) affecting *Theobroma cacao* for improved pest management. In *Proceedings of the 1st International Electronic Conference on Entomology* (pp. 1–15).

Rodrigues, J. C. V., Gallo-Meagher, M., Ochoa, R., Childers, C. C., & Adams, B. J. (2004). Mitochondrial DNA and RAPD polymorphisms in the haploid mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae). *Experimental & Applied Acarology, 34*, 275–290. https://doi.org/10.1023/B:APPA.0000021771.92696.7e

Romero-Cuadrado, L., López-Herrera, C. J., Aguado, A., & Capote, N. (2023). Duplex Real-Time PCR Assays for the Simultaneous Detection and Quantification of Botryosphaeriaceae Species Causing Canker Diseases in Woody Crops. Plants (Basel, Switzerland), 12(11), 2205. https://doi.org/10.3390/plants12112205

Schena, L., Duncan, J. M., & Cooke, D. E. L. (2008). Development and application of a PCR-based 'molecular tool box'for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant pathology*, *57*(1), 64-75.

Schoch C.L., et al. (2020).NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). baaa062. PubMed: 32761142 PMC: PMC7408187.

Smith, S., & Cognato, A. (2014). A taxonomic monograph of Nearctic *Scolytus* Geoffroy (Coleoptera, Curculionidae, Scolytinae). *ZooKeys*, *450*, 1–182. https://doi.org/10.3897/zookeys.450.7452

Sorensen, K. N., Kim, K. H., & Takemoto, J. Y. (1998). PCR Detection of Cyclic Lipodepsinonapeptide-Producing *Pseudomonas syringae* pv. *syringae* and Similarity of Strains. *Applied and environmental microbiology*, *64*(1), 226–230. https://doi.org/10.1128/AEM.64.1.226-230.1998

Taylor, R.K., Guilford, P.J., Clark, R.G., Hale, C.N. & Forster, R.L.S. 2001. Detection of *Erwinia amylovora* in plant material using novel polymerase chain reaction (PCR) primers. New Zealand. Journal of Crop and Horticultural Science, 29: 35–43.

Trabelsi, R., Sellami, H., Gharbi, Y., Krid, S., Cheffi, M., Kammoun, S., Dammak, M., Mseddi, A., Gdoura, R., & Triki, M. A. (2017). Morphological and molecular characterization of *Fusarium* spp. associated with olive trees dieback in Tunisia. *3 Biotech*, *7*(1), 28. https://doi.org/10.1007/s13205-016-0587-3

Vulchi, R., Daane, K. M., & Wenger, J. A. (2021). Development of DNA Melt Curve Analysis for the Identification of Lepidopteran Pests in Almonds and Pistachios. *Insects*, *12*(6), 553. https://doi.org/10.3390/insects12060553

Wünsch, R., Reichmuth, A., Neumayer, S., Marx, A., & Kolvenbach, B. A Quantitative Qpcr Method for the Quantification of Rhizobium Rhizogenes in Aqueous Samples. *Available at SSRN 4735454*.

Wynns, A. A., Jensen, A. B., Eilenberg, J., & Delalibera, I. (2019). *Colletotrichum nymphaeae* var. *entomophilum* var. nov. a natural enemy of the citrus scale insect, *Praelongorthezia praelonga* (Hemiptera: Ortheziidae). *Scientia agricola*, *77*(5), e20180269.

Ye, Q. T., Manawasinghe, I. S., Zhang, W., Mugnai, L., Hyde, K. D., Li, X. H., & Yan, J. Y. (2020). First report of *Phaeoacremonium minimum* associated with grapevine trunk diseases in China. *Plant Dis*, *104*(1259), 10-1094.

Zhang, M. X., Zhai, L. F., Xu, W. X., Hong, N., & Wang, G. P. (2014). First Report of *Valsa leucostoma* Causing Valsa Canker of Pyrus communis (cv. Duchess de' Angouleme) in China. *Plant disease*, *98*(3), 422. https://doi.org/10.1094/PDIS-07-13-0704-PDN

Survey Guidelines for Quarantine and Potential Quarantine Insect Pest of Apple

Potential Quarantine Pests

1. **Ceresa alta (Buffalo Treehopper)**

Common Name: Buffalo Treehopper

Time of Detection: April to October

Sites Affected: Lower branches (up to 2 m), twigs, ground cover plants (e.g., weeds)

Symptoms:

- Paired, parallel longitudinal egg slits on upper twig surfaces, often with protruding whitish eggshells.
- Twig dieback or breakage beyond oviposition sites.
- Leaf symptoms: downward rolling, thickening, bright upper surfaces, yellowing, or reddening.

Pest Diagnostic Characters:

- Adults 7.7–10.0 mm, robust, capelike pronotum 2.0–2.5 mm high.

- Pale-green to golden-yellow, two white lines from metopidial horns, black eyes, transparent forewings.
- Pronotum is coarsely pitted with hairlike setae, arched with convergent carinae.
- Nymphs are Smaller, less conspicuous, found on ground cover.

Detection and Inspection Method:

- Examine twigs on lower branches for egg slits, eggshells, or dieback.
- Check leaves for rolling, thickening, or discoloration.
- Inspect ground cover near tree bases for nymphs (April–June)

- **References**:

CABI. (2022). *Ceresa alta* (buffalo treehopper). CABI International. https://doi.org/10.1079/cabicompendium.51411

2. *Eulecanium tiliae* (Nut Scale)

Figure: *Eulecanium tiliae*

Common Name: Nut Scale
Stage of Host Associated: Young to mature trees, with greater infestation in young trees
Symptoms:

- Branch/twig death from cambium feeding, worsened by toxic saliva.
- Leaf symptoms: premature yellowing, smaller/thicker leaves, chloroplast degeneration.
- Honeydew production causing sooty mold, reducing photosynthesis.
- Greater damage in young trees or those with small crowns.

Pest Diagnostic Characters:

- Young Females: Oval/circular, light-yellow to cream, brown/reddish bands, membranous derma.
- Post-Reproductive Females: Convex, pea-sized, yellowish-brown to dark brown, 4.0–6.5 mm long, 3.5–6.0 mm wide, 3.0–5.0 mm high.

Detection and Inspection Method:

- Survey twigs/branches for adults and second-instar larvae (late autumn–spring).
- Check lower leaf surfaces, especially mid-veins, for nymphs (May–August).
- Look for yellowing leaves, sooty mold, or twig dieback.

References

CABI Compendium. (2022). *Eulecanium tiliae* (nut scale). CABI International. https://doi.org/10.1079/cabicompendium.30152

3. ***Phenacoccus aceris*** (Mealybug)

Female adult of ***Phenacoccus aceris***

First instar nymph of ***Phenacoccus aceris***

Parts of Plant Affected: Leaves, branches, bark
Timefor Detection: March to November
Likely Sites Affected: Underside of leaves (near veins), bark crevices, branches near fruit clusters

Detection Method

Based on symptoms:

- White, woolly ovisacs on bark or leaf undersides.
- Reduced vigor, leaf yellowing, or premature leaf drop from feeding.
- Honeydew and sooty mold on infested areas.

Based on pest life cycle:

- Look for the overwintering structure nymphs in white cocoons under bark/crevices
- Females form ovisacs on branches/leaf undersides, laying hundreds of eggs.
- Inspect the ovisac which is long, woolly, white, typically on bark

Pest Diagnostic Characters:

- Adult Female: Oval, 3 x 4 mm, greenish-yellow, covered in white/gray mealy wax, 18 pairs of short wax filaments.
- Ovisac: Long, woolly, white, typically on bark

References:

CABI. (2022). *Phenacoccus aceris* (apple mealybug). CABI International. https://doi.org/10.1079/cabicompendium.40167

4. *Rhopalosiphum oxyacanthae* (Aphid)

Figure: *Rhopalosiphum oxyacanthae*

- Timefor Detection: March to June
- Likely Sites Affected: Buds, young foliage, trunk crevices, older branches

Symptoms:
o Feeding on buds/young leaves causes curling, stunting, or reduced vigor.
o Honeydew production leading to sooty mold.

o Presence of ants attending aphid colonies.

Pest Life Cycle:
o Eggs overwinter in trunk/branch crevices, in diapause until mid-January.
o Fundatrix hatch March–April, feed on buds/young leaves.
o Two apterous generations on leaves, followed by alatae migrating to Poaceae (May–June).
o Up to 12 generations on secondary hosts (grasses/cereals).

Pest Diagnostic Characters:
o Apterous Females (Apple): Elongate oval, shiny green to yellow-green, 2.1–2.6 mm, dark green stripes.
o Alate Females (Apple): Shiny green, black head/thorax, 1.1–2.0 mm.
o Siphunculi: Short, yellowish-green (apterous) or black (alate), slightly swollen.

References

Jovicic, I. (2022). *Rhopalosiphum oxyacanthae* (apple-grass aphid). CABI Compendium. CABI International. https://doi.org/10.1079/cabicompendium.47316

Detection and Inspection Method:

Visual Inspection:

- Examine buds/young leaves for apterous females (March–April).
- Check trunk/branch crevices for overwintering eggs (winter–early spring).
- Look for curled leaves, sooty mold, or ant activity.

***Scolytus rugulosus* (Shothole Borer)**

Figure: ***Scolytus rugulosus*** **(Shothole Borer)**

Appropriate time for detection: Spring to fall
Likely sites affected: Trunk, branches

Symptoms:
o Clear to brownish sap resembling tear drops exuding from 1.5 mm bark holes (initial attack).
o Numerous dry exit holes from emerging adults.
o Winding tunnels under bark with brown frass. The female constructs an egg gallery about 2.5 cm long under the bark, parallel with the grain. Larvae are present year-round inside branches. When the insects are abundant, fruit clusters become wilted, and associated leaves become brown (D), resembling a fire blight infection.

Pest life cycle:
o Overwinters as mature larvae (prepupae) under bark.
o Pupation and adult emergence in spring; multiple generations per year.
o Females bore into cambium, lay eggs along tunnels; larvae feed under bark.

Pest diagnostic characters:
o Adults: Blackish to dark brown, oblong, 2.5 mm long.
o Larvae/Pupae: Pale yellowish-white, legless grubs, brown head, <2.5 mm.
o Eggs: Tiny, pale, hidden in tunnels.
• Detection and Inspection Method:
o Visual Inspection:

- Check trunk/branches for sap-exuding holes or dry exit holes.
- Peel bark to reveal tunnels, larvae, or frass.
- Look for the symptomatic trees.

References

https://www.canr.msu.edu/ipm/diseases/shothole_borer

CABI Compendium. (2022). *Scolytus rugulosus* (shothole borer). CABI International. https://doi.org/10.1079/cabicompendium.49215

Synanthedon myopaeformis (Clearwing Moth)

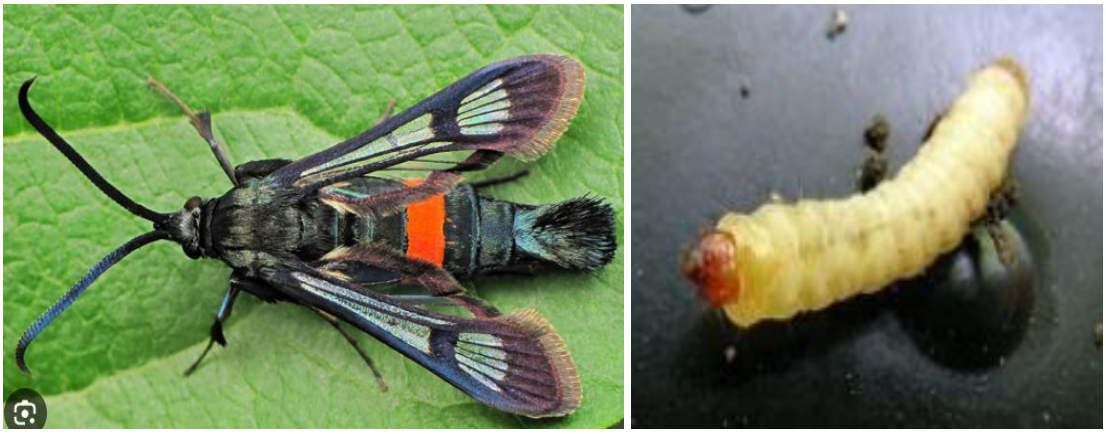


Figure: Larva and adult of ***Synanthedon myopaeformis*** (Clearwing Moth)

Time of detection: Spring to summer

Likely Sites Affected: Trunk, thick branches

Symptoms:

- Gnawing holes under bark with brownish heaps of wood dust, excrement, and plant juice.
- Bark scaling, loss of vigor, or tree death.
- Projecting exuvia on affected trees.

Pest life cycle:

- Caterpillars overwinter under bark (1st/2nd years).
- Spring: Caterpillars gnaw twisting holes, pupate in silky cocoons.
- Pupation lasts ~2 weeks; adults emerge, females lay 200–250 eggs in bark cracks.
- Eggs hatch, caterpillars bore under bark.

Pest Diagnostic Characters:

- Adults: Transparent wings (18–22 mm wingspan), fourth abdominal ring is red.
- Caterpillars: Light-yellow with reddish tint, reddish-brown head.
- Pupa: Brown-yellow, with head tubercles and abdominal spinules.

Detection and Inspection Method:
o Visual Inspection:
♣ Examine trunk/thick branches for gnawing holes, brownish heaps, or exuvia.

♣ Check for bark scaling or tree vigor loss.
♣ Look for pupal cases near exit holes.

References

CABI. (2022). *Scolytus rugulosus* (shothole borer). CABI International. https://doi.org/10.1079/cabicompendium.49215

***Takahashia japonica* (Scale Insect)**

Figure: reproductive females with ovisacs

Figure: Overwintering nymph of ***T. japonica***

Timefor Detection: March to June
Likely Sites Affected: Lower leaf surfaces, branches

Symptoms:
o White, string-like ovisacs on branches or leaf undersides.
o Reduced vigor, leaf yellowing, or sooty mold from honeydew.

Pest Life Cycle:
o Parthenogenetic, univoltine; females produce 4000–5000 eggs (April–May).
o Eggs hatch May–June; crawlers feed on lower leaf surfaces (summer).
o Second-instar nymphs overwinter on branches, moult to adults in March.

Pest Diagnostic Characters:
o Crawlers: Small, inconspicuous, main dispersal stage.
o Ovisacs: Conspicuous, white, string-like loops, unique to this species.
o Females: Small, inconspicuous before ovisac formation.

Detection and Inspection Method:
Visual Inspection:
♣ Check lower leaf surfaces for crawlers (May–June).
♣ Inspect branches for white, string-like ovisacs (spring–summer).
♣ Look for sooty mold or leaf yellowing.

References

https://planthealthportal.defra.gov.uk/assets/factsheets/Takahashia_japonica_Factsheet_2024.pdf

B. Quarantine Pests

1. *Aculus schlechtendali* (Apple Rust Mite)


Timefor Detection: Growing Time(spring–summer)
Likely Sites Affected: Lower leaf surfaces, fruit surfaces

Symptoms:
o Rusting, bronzing, premature drying, or senescence of foliage.
o Increased transpiration, reduced photosynthesis.
o Russeting on developing pomes.
o Feeds on young leaves and developing fruit throughout the growing season.

o Overwinters in bark crevices or bud scales.

Pest Diagnostic Characters:
o Microscopic mites, requiring magnification for identification.
o Small, elongated, yellowish bodies.

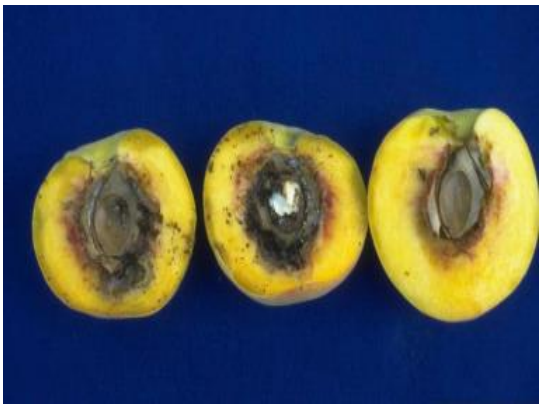
Detection and Inspection Method:
o Visual Inspection:
♣ Inspect lower leaf surfaces for rusting/bronzing.
♣ Check fruit for russeting.
♣ Use sticky tape to collect mites from leaves.

References

https://treefruit.wsu.edu/crop-protection/opm/apple-rust-mite/

Anarsia lineatella (Peach Twig Borer)



• Common Name: Peach Twig Borer

• Timefor Detection: Spring
• Likely Sites Affected: Newly formed shoots, fruits

Symptoms:
o Wilting, drying, and blackening of shoot tips.
o Fruit destruction by larvae
o Overwintering larvae bore into shoots in spring.
o Larvae feed on shoots and fruits, pupate, and emerge as adults.

Pest Diagnostic Characters:
o Larvae: Small, brown, feeding in shoots/fruits.
o Adults: Small moths, grayish-brown.

Detection and Inspection Method:
o Visual Inspection:
♣ Check newly formed shoots for wilting/blackening.
♣ Inspect fruits for larval entry holes or gumming.
♣ Look for frass or webbing on shoots.

References

CABI Compendium. (2022). *Anarsia lineatella* (peach twig borer). CABI International. https://doi.org/10.1079/cabicompendium.5154

Aspidiotus nerii (Oleander Scale)


Common Name: Oleander Scale
• Timefor Detection: Year-round
• Likely Sites Affected: Lower branches, stems, leaves, fruits

Symptoms:
o Scale armour on stems, leaves, fruits.
o Wilting, yellowing, or reduced photosynthesis.
o Green spots on fruits (e.g., olives), fruit deformity.
o Heavy infestations cause leaf/shoot malformation or plant death.

Pest Life Cycle:
o Continuous reproduction; multiple generations per year.
o Crawlers settle on plant surfaces, forming armoured scales.

Pest Diagnostic Characters:
o Scales: Small, circular, grayish armoured coverings.
o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:
o Visual Inspection:
♣ Check lower branches, stems, and leaves for scale armour.
♣ Inspect fruits for green spots or deformity.
♣ Remove armour to confirm live insects.

References:

cabicompendium.7418, CABI Compendium, doi:10.1079/cabicompendium.7418, CABI International, Aspidiotus nerii (Oleander scale), (2022)

***Brevipalpus phoenicis* (False Spider Mite)**


Common Name: False Spider Mite
Likely Sites Affected: Leaf undersides, midribs, borders

Symptoms:
o Necrotic brown spots along leaf midribs and borders.
o Entire leaf underside brown, leading to defoliation.
o Transmits Citrus leprosis virus C.

Pest Life Cycle:
o Continuous reproduction during warm conditions.
o Eggs laid on leaf undersides, developing into nymphs and adults.

Pest Diagnostic Characters:
o Mites: Tiny, reddish-brown, flattened bodies, requiring magnification.

Detection and Inspection Method:
o Visual Inspection:
♣ Check leaf undersides for brown spots or discoloration.
♣ Look for defoliation or stressed trees.
♣ Collect mites after leaf washing.

Dysmicoccus brevipes (Pineapple Mealybug)

• Common Name: Pineapple Mealybug
• Likely Sites Affected: Roots, stem bases, leaf undersides, fruits

Symptoms:
o Reddening to pink leaves, inward leaf margin reflexing, wilting.
o Reduced vigor, yellow spotting, premature leaf drop.
o Honeydew and sooty mold on leaves/fruits.
o Black spot from fungal infection in fruit cavities.

Pest life cycle:
o Continuous reproduction; multiple generations per year.
o Crawlers spread to roots, stems, leaves, and fruits.

Pest diagnostic characters:
o Mealybugs: White, waxy adults, 3–4 mm, often in clusters.

Detection and inspection method:
Visual inspection:
♣ Check roots, stem bases, leaf undersides, and fruits for white waxy masses.
♣ Look for honeydew, sooty mold, or wilting symptoms.
♣ Inspect fruit cavities for black spot.

References
CABI Compendium. (2022). *Dysmicoccus brevipes* (pineapple mealybug). CABI International. https://doi.org/10.1079/cabicompendium.20248

Ectomyelois ceratoniae (Carob Moth)

Adult

Adult

Early instar larva

Late instar larva

• Common Name: Carob Moth
• Stage of Host Associated: Fruiting trees
• Likely Sites Affected: Fruits

Symptoms:
o Larval feeding causes fruit damage, webbing, and frass.
o Fruit rot or premature drop.

Pest Life Cycle:
o Eggs laid on fruits; larvae bore into fruit, pupate inside.
o Multiple generations per year.

Pest Diagnostic Characters:
o Early Instar Larvae: Small, white, with dark head.
o Late Instar Larvae: Larger, pinkish or greenish, with dark spots.
o Adults: Small, grayish-brown moths.

Detection and Inspection Method:

o Visual Inspection:

♣ Check fruits for entry holes, webbing, or frass.

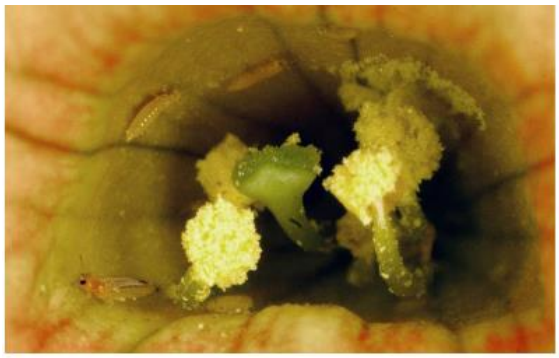
♣ Look for damaged or dropped fruits.

♣ Cut fruits to find larvae.

References

Dueñas-López, M. A. (2022). *Ectomyelois ceratoniae* (carob moth). CABI Compendium. CABI International. https://doi.org/10.1079/cabicompendium.35348

Frankiniella occidentalis (Western Flower Thrips)



Feeding in flower

Nymph

Leaf damaged by western flower thrips

Common Name: Western Flower Thrips

• Stage of Host Associated: All stages

• Likely Sites Affected: Buds, flowers, leaf undersides, fruit surfaces

Symptoms:

o White streaking on dark flowers, silvering on leaves.

o Bud/fruit deformation, spotting on fruit skin.
o Transmission of tospoviruses (e.g., TSWV, INSV).

Pest Life Cycle:
o Eggs laid in plant tissues; larvae and adults feed on plant surfaces.
o Multiple generations per year.

Pest Diagnostic Characters:
o Adults: Tiny (1–2 mm), yellowish to brown, winged.
o Larvae: Smaller, wingless, pale.

Detection and Inspection Method:
o Visual Inspection:
♣ Check buds, flowers, and leaf undersides for thrips or damage.
♣ Look for silvering, spotting, or deformed fruits.
♣ Use beating to dislodge thrips from plants.

References

Reitz, S. (2022). *Frankliniella occidentalis* (western flower thrips). CABI Compendium. CABI International. https://doi.org/10.1079/cabicompendium.24426

Harmonia axyridis **(Harlequin Ladybird)**

Distinctive feature is the white “M” or “W” shape on their pronotum (the area behind the head),

Adult of ***Harmonia axyridis***

• Common Name: Harlequin Ladybird
• Parts of Plant Affected: None (predator, not pest)
• Host Associated: Aphids
• Timefor Detection: Early spring

Symptoms:
o Presence of ladybirds on plants, no direct plant damage.

Life Cycle:
o Adults live ~1 year, reproduce without dormancy, two generations per year.
o Larvae and adults are predatory.

Diagnostic Characters:
o Adults: 5.5–8.5 mm, orange/red with 0–22 black spots or black with red/orange spots.
o Distinctive Feature: White “M” or “W” on pronotum.

Detection and Inspection Method:
o Visual Inspection:
♣ Check leaves/branches for ladybirds.
♣ Look for “M” or “W” on pronotum.
♣ Observe for predatory behavior on aphids.

References

Roy, H. (2022). *Harmonia axyridis* (harlequin ladybird). CABI Compendium. CABI International. https://doi.org/10.1079/cabicompendium.26515

***Parlatoria oleae* (Olive Scale)**


• Common Name: Olive Scale

• Timefor Detection: Year-round
• Likely Sites Affected: Inner canopy, trunks, branches, leaf midribs, fruit rinds

Symptoms:
o Scale armour on stems, leaves, fruits.
o Green spots on fruits, wilting, yellowing.
o Heavy infestations cause bark gumming, branch death.

Pest Life Cycle:
o Continuous reproduction; multiple generations per year.
o Crawlers settle on plant surfaces, forming armoured scales.

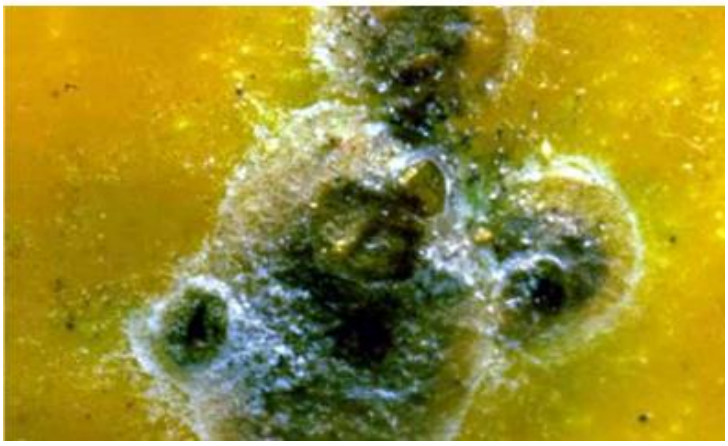
Pest Diagnostic Characters:
o Scales: Small, grayish-brown armoured coverings.
o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:
o Visual Inspection:
♣ Inspect inner canopy, trunks, branches for scale armour.
♣ Check leaf midribs and fruit rinds for scales.
♣ Look for green spots on fruits, gumming on bark.

References

pwkb.species.38906, PlantwisePlus Knowledge Bank, doi:10.1079/pwkb.species.38906, CABI International, Parlatoria oleae (olive scale), (2022)

***Parlatoria pergandii* (Chaff Scale)**



Parlatoria pergandii (Chaff Scale)

Common Name: Chaff Scale
Timefor Detection: Year-round
Likely Sites Affected: Inner canopy, trunks, branches, leaf midribs, fruit rinds

Symptoms:
o Scale armour on stems, leaves, fruits.
o Green spots on fruits, wilting, yellowing.
o Heavy infestations cause bark gumming, branch death.

Pest Life Cycle:
o Continuous reproduction; multiple generations per year.
o Crawlers settle on plant surfaces, forming armoured scales.

Pest Diagnostic Characters:
o Scales: Small, grayish-brown armoured coverings.
o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:
o Visual Inspection:
♣ Inspect inner canopy, trunks, branches for scale armour.
♣ Check leaf midribs and fruit rinds for scales.
♣ Look for green spots on fruits, gumming on bark.

References

CABI Compendium. (2022). *Parlatoria pergandii* (chaff scale). CABI International. https://doi.org/10.1079/cabicompendium.38907

***Parthenolecanium corni* (European Fruit Scale)**


Parthenolecanium corni (European Fruit Scale)

• Common Name: European Fruit Scale
• Likely Sites Affected: Leaf undersides, stems, branches

Symptoms:
o Chlorotic spotting, premature leaf shedding, wilting.
o Honeydew and sooty mold reducing photosynthesis.
o Reduced vigor and market value.

Pest Life Cycle:
o First instars on leaf undersides; later stages on stems/branches.
o Continuous reproduction; multiple generations per year.

Pest Diagnostic Characters:
o Scales: Brown, convex, 3–5 mm.
o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:
o Visual Inspection:
♣ Check leaf undersides for first instars.

♣ Inspect stems/branches for later stages.
♣ Look for honeydew and sooty mold.

References

CABI Compendium. (2022). *Parthenolecanium corni* (European fruit lecanium). CABI International. https://doi.org/10.1079/cabicompendium.45556

***Pseudococcus calceolariae* (Citrophilus Mealybug)**

Figure: *Pseudococcus calceolariae* (Citrophilus Mealybug)

• Common Name: Citrophilus Mealybug
• Likely Sites Affected: Leaf undersides, fruit calyx, stem axils

Symptoms:
o Presence of mealybugs or sooty mold on leaves and fruits.
o Honeydew around fruit calyx or sepals, indicating hidden mealybugs.
o Reduced vigor in heavy infestations.

Pest Life Cycle:
o Continuous reproduction in warm, temperate climates; multiple generations per year.
o Crawlers settle in sheltered areas (e.g., fruit calyx, leaf axils).

Pest Diagnostic Characters:
o Mealybugs: White, waxy, oval, 3–4 mm, often in clusters.
o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:

o Visual Inspection:

♣ Examine leaf undersides, fruit calyx, and stem axils for white waxy mealybugs.

♣ Check for sooty mold or sticky honeydew on foliage and fruits.

References:

CABI Compendium. (2022). *Pseudococcus calceolariae* (scarlet mealybug). CABI International. https://doi.org/10.1079/cabicompendium.45086

Pseudococcus comstocki (Comstock Mealybug)

Figure: ***Pseudococcus comstocki*** (Comstock Mealybug)

Common Name: Comstock Mealybug
Time for Detection: Year-round
Likely Sites Affected: Bark cracks, leaf undersides, fruit calyx

Symptoms:

o Honeydew and sooty mold on leaves and fruits.

o Fruit spotting and altered skin texture.

o Gall-like formations on bark and near leaf veins.

Pest Life Cycle:

o Overwintering as eggs or females in bark cracks.

o Multiple generations per year; crawlers spread to leaves and fruits.

Pest Diagnostic Characters:

o Females: White, waxy, with 17 pairs of long filaments, 3–4 mm.

o Crawlers: Tiny, mobile, yellowish.

Detection and Inspection Method:

o Visual Inspection:

♣ Check bark cracks for eggs/overwintering females (early season).

♣ Inspect leaf undersides, fruit calyx, and green tissues (spring).

♣ Examine southern side of trees for summer/autumn populations.

♣ Look for honeydew or sooty mold.

References

CABI Compendium. (2022). *Pseudococcus comstocki* (Comstock mealybug). CABI International. https://doi.org/10.1079/cabicompendium.45084

Pseudococcus longispinus (Longtailed Mealybug)

• Common Name: Longtailed Mealybug

• Likely Sites Affected: Leaf undersides, young stems, bark cracks, fruits

Symptoms:

o Leaf yellowing, curling, and drop.

o Stunted growth, cosmetic fruit damage.

o Honeydew and sooty mold; may attract ants.

o Potential vector of plant viruses.

Pest Life Cycle:

o Continuous reproduction; multiple generations per year.

o Crawlers settle on leaves, stems, and fruits; females produce waxy masses.

Pest Diagnostic Characters:
o Females: Soft, oval, white/grayish-white, 3–4 mm, with two long tail filaments.
o Males: Small, winged, short-lived.

Detection and Inspection Method:
o Visual Inspection:
♣ Examine leaf undersides, young stems, and bark cracks for white waxy masses.
♣ Check fruits for mealybugs or sooty mold.
♣ Look for ant activity near infested areas.

References

CABI Compendium. (2022). *Pseudococcus longispinus* (long-tailed mealybug). CABI International. https://doi.org/10.1079/cabicompendium.45079

Survey Guidelines for Quarantine and Potential Quarantine pathogens of Apple

Apple rubbery wood phytoplasma

Other scientific names: *Apple rubbery wood disease, Apple rubbery wood MLO, Apple rubbery wood virus, Pyrusvirus molliens, Quince bark necrosis virus, Quince yellow blotch agent, Quince yellow blotch virus*

Hosts: *Malus domestica*, *Pyrus communis, Prunus avium, P. cerasus*, and *Cydonia oblonga* (Nemeth, 1986).

Transmission: The disease is spread through root grafts and propagating material derived from infected plants or young nursery stock grown from diseased mother plants.

Symptoms to look for in post- entry quarantine:

- Central branch of the tree stops growing and vigorous shoots develop from the base, and in Golden Delicious trees, a characteristic swelling appears on the main trunk (Pollini et al., 1995). Internodes are shortened and annual growth is reduced so that infected trees are frequently small.
- No fruit or leaf symptoms can be observed.
- Reduction or lack of lignification and the histological changes within the xylem occurs primarily in second year growth or older, and were most notable in wood formed during summer months.

Figure. Symptoms of rubbery wood phytoplasma

Figure. Symptoms of rubbery wood phytoplasma

Apple stem pitting virus

Hosts: Apple, Pear

Transmission: The virus is transmitted by grafting and propagating material. The are no known vector.

Symptoms to look for in post- entry quarantine:

- Apple varieties remain symptomless after infection with ASPV, but growth and crop yield can be reduced. However, xylem pitting can be seen on the stem of susceptible apple cultivars.
- On pear, chlorotic banding of the leaf veins, particularly of the secondary and tertiary veins can be seen. Red mottling of leaves can also be observed in the summer (Cameron, 1989).

Figure: Stem pitting on Virginia Crab

Figure: Stem pitting in Virginia Crab above the graft union

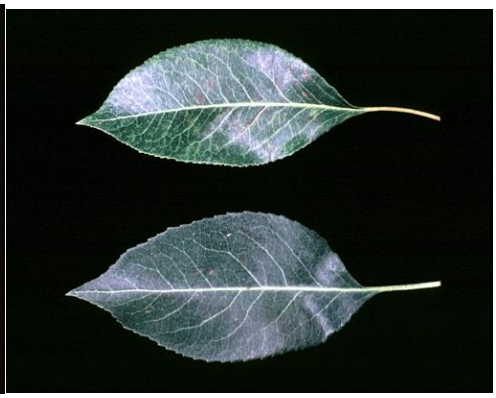


Figure: Vein yellows on a leaf of the pear, healthy control below

Botryosphaeria dothidea

Other scientific names: *Botryosphaeria berengeriana, Dothiorella mali*

Hosts: Apple, Kiwi

Symptoms to look for in post- entry quarantine:

- Small circular blisters on twigs
- Sunken necrotic lesions
- Band- like cankers on the trunk
- Cankers girdle the trunk
- Older cankers often have papery bark that sloughs off sometimes appearing slimy

Exudation of liquid from a blister

Reddish-brown lesions on bark

Exudation of liquid from a blister in an active canker

Colletotrichum nymphaeae

Other scientific names: *Ascochyta nymphaeae*, *Phyllosticta nymphaeae*

Hosts: Apple, plum, walnut, pecan

Symptoms to look for in post- entry quarantine:

- In leaves, red to brown irregular lesions are found.
- Chlorotic halos may be present.
- Concentric rings on the spots.
- Sporulations on the spots

Erwinia amylovora

Other scientific names: *Bacillus amylovorus* (Burrill) Trevisan, 1889, *Bacterium amylovorum* Chester, 1901, *Erwinia amylovora* f.sp. *rubi* Starr et al., 1951, *Micrococcus amylovorus* Burrill, 1882

Hosts: *E. amylovora* is a pathogen of plants in the family Rosaceae; most of the natural hosts are in the subfamily Maloideae.

Wild *Pyrus* (*P. amygdaliformis*, *P. syriaca*) in southern Europe and in the Mediterranean area, *Crataegus* (*C. oxyacantha* [*C. laevigata*], *C. monogyna*) in northern and central Europe, and ornamentals (*Pyracantha*, *Cotoneaster*, *Sorbus*) throughout Europe are important sources of inoculum for apple and pear orchards.

Transmission: Local spread of the disease within and between trees is a consequence of the exudates (bacterial cells of the pathogen embedded in exopolysaccharides) produced by infected trees, which is easily transported by birds, insects, wind or rain (McManus and Jones, 1994). Dispersal of the pathogen into new regions and countries may occur from the shipping of infected plant material.

Symptoms to look for in post- entry quarantine:

- Fire blight's basic symptom is necrosis or death of tissues.
- Infected blossoms initially become water-soaked and of a darker green. Within 5-30 plus days (commonly 5-10 days), the spurs begin to collapse, turning brown to black.
- Infected shoots turn brown to black from the tip; shoots often bend near the tip to form a so-called 'shepherd-crook' shape. Shoots invaded from their base exhibit necrosis of basal leaves and the stem.
- Discoloration in leaves and fruits followed by collapse of the leaves and fruit.
- During wet, humid weather, infected leaves and particularly the fruit often exude a milky, sticky liquid, or ooze containing bacteria.
- Infected bark on branches, scaffold limbs, trunk and rootstock turn darker than normal. When the outer bark is peeled away, the inner tissues are water-soaked often with reddish streaks when first invaded; later the tissues are dark brown to black. As disease progression slows, lesions become sunken and sometimes cracked at the margins, forming a canker.
- Trees with rootstock blight may exhibit liquid bleeding from the crown at or just below the graft union in early summer.

Figure: Milky ooze containing fire blight bacteria exuding from an infected fruit.

Figure Fire blight on apple; twig with blossom and shoot blight

Figure: Shoot blight on apple (early stage)

Figure: Oozing apple fruitlets

Figure: Fire blight lesion on Apple rootstock

Figure: Blossom infections on a cider apple cultivar

Figure: Exudate on the trunk of a pear tree

Fusarium acuminatum

Other scientific names: *Giberella acuminata*, *Fusarium scirpi*,

Hosts: wide

Transmission: Transmission occurs through spores that land on the fruit surface, possibly entering through lenticels or wounds, leading to localized black spot lesions.

Symptoms to look for in post- entry quarantine:

- Root rot
- Localized lesions in fruits
- Small black spots on fruits

***Candidatus* Phytoplasma asteris**

Other scientific names: *Aconitum proliferation, Aconitum virescence, Cyclamen virescence, Diplotaxis virescence, Echinacea phyllody, Limonium proliferation, Salix proliferation, Aster yellows phytoplasmas* (AY)

Figure 1: Worldwide distribution map of *Candidatus* Phytoplasma asteris

Hosts: Apple, pear, cherry, mango, maize, onion, gladiolus, oat, wheat, broccoli, cabbage, marigold

Latent period: Up to 2 years (symptom development) (Hogenhout et al., 2008), 7 to 80 days (in vector)

Transmission: The pathogen is not seed transmissible. The use of infected vegetative propagating material is responsible for long-distance movement of the pathogen and intentional introduction into new areas. In addition, the pathogen is also transmitted by a wide range of leafhopper, among which *Marcosteles fascifrons* is a major vector.

Symptoms to look for in post- entry quarantine:

- In herbaceous plant, yellowing of the leaves, stunting, proliferation of auxiliary shoots resulting in a witch's broom appearance, bunchy appearance of growth at the ends of stems, virescence of flowers and sterility, phyllody, shortening of internodes, elongation and etiolation of internodes, small and deformed leaves.
- Yellowing, decline, sparse foliage and dieback are predominant in woody plant hosts.

Candidatus* Phytoplasma *solani

Other scientific names: *Phytoplasma solani*

Hosts: Tomato, potato, tobacco, pepper, celery, carrot, parsley, garden bean, grape and maize.

Latent period: Up to 2 months (in vector)

Transmission: The pathogen is transmitted by vegetative propagation of infected host plants. The pathogen can also be transmitted by the parasitic plant dodder. The planthopper *Hyalesthes obsoletus* and *Reptalus panzeri are* vectors of the pathogen.

Symptoms to look for in post- entry quarantine:

Symptoms of the '*Ca*. Phytoplasma solani'-associated diseases affecting major crops are described in the following paragraphs. Diseases caused in other plants include yellowing, reddening, decline, dwarfism, leaf malformation and degeneration diseases.

Bois noir (BN): produces typical grapevine yellows (GY) symptoms, desiccation of inflorescences, berry shrivel, leaf discoloration, reduction of growth and irregular ripening of wood (Belli et al., 2010).

Tomato stolbur: Symptoms are best observed during summer. Internodes near to the plant apex are shorter. The leaves are discolored and/or show yellowing/purpling. Adventitious roots sometimes appear on the stem. Plants infected early are bushy. The flowers are abnormally straight, sterile and they show different morphological changes

Potato stolbur: Plants grown from infected tubers give rise to normal or spindly sprouts (hair-sprouting. Top leaf rolling and purplish, shortened internodes, aerial tubers, early senescence and death. This is followed by production of aerial stolons and tubers in different parts of the stems close to the axils (Mitrovic et al., 2016).

Maize redness: Symptoms are best observed from July to September. Midrib reddening, followed by reddening of leaves and stalks and then whole-plant desiccation. Abnormal ear development and reduced seed numbers.

Figure: Grapevine yellows symptoms on Vitis vinifera.

Figure: Grapevine yellows symptoms on Vitis vinifera.

Figure: Grapevine yellows symptoms on Vitis vinifera.

Figure: Presence of aerial tubers

Figure: Bushy appearance of tomato plant

Figure: Symptoms of maize redness.

Figure: Stolbur symptoms on tuber

Figure: Stolbur phytoplasma symptoms

Pear blister canker viroid

Hosts: Pear, apple

Latent period: 2-3 years

Transmission: Transmission of PBCVd is through mechanical inoculation from pruning tools and grafting/budding to infective propagative materials and is a potential pathway for spread. No animal vector is known and it is not known to be seed transmitted (Flores et al., 2011).

Symptoms to look for in post- entry quarantine:

- Generally symptomless in apple.
- Petiole and leaf necrosis, bark pustules, bark scaling, bark splitting and tree death (Desvignes et al., 1999).
- Characteristic symptoms are seen in bark.

Figure. Bark symptoms induced by PBCVd

Figure. Symptoms induced by PBCVd

Phytoplasma aurantifolia

Other scientific names: *Candidatus* Phytoplasma *aurantifolia* Zreik et al 1995

Hosts: Lime, trifoliate orange, grapefruit, lemon, citron, apple, tomato.

Latent period: 2-6 weeks (in vector),

Transmission: The pathogen is graft transmissible. *Hishimonus phycitis* is the insect vector.

Symptoms to look for in post- entry quarantine:

- Production of small-sized leaves on proliferating shoots that tend to be yellowish-green, and reduced flower and fruit production.
- Affected trees exhibit witches' brooms usually on a single branch initially that may subsequently appear on other branches.
- Infected twigs fail to bear flowers and fruits, and trees may die within 4-5 years after the first appearance of symptoms (El Shereiqi and Gassouma, 1993).

Figure: Advanced symptoms of witches' broom disease

Cherry leaf roll virus (Walnut ringspot)

Other scientific names: *Cherry leaf roll nepovirus*

Hosts: Sweet cherry, walnut, grape, American elm, raspberry, blackberry

Latent period: NA

Transmission: CLRV is often dispersed by human commerce with infected seeds or plants, and by pollen and seeds. CLRV is readily transmitted by water (Bandte et al., 2007) and root conation (Büttner et al., 2011). It is also transmitted by graft inoculation.

Symptoms to look for in post- entry quarantine:

- Variety of chlorosis such as yellow vein netting, chlorotic ring spots and mottling as well as malformations such as leaf roll, to dieback of branches or whole trees (Bandte and Büttner, 2001). Chlorotic leaf areas often turn necrotic later in the vegetation period and CLRV-affected trees develop smaller leaves leading to a scattered appearance of the canopy in mature trees.
- In walnut, infection is associated with chlorotic mosaic, line-patterns, black line disease and decline.
- In cherry, infection is associated with leaf rolling and plant death (Schimanski et al., 1975).
- In raspberry, infection is associated with severe stunting, fruiting canes with small, distorted leaves, some with line-pattern symptoms, severe chlorotic mottling or ringspots (Jones and Wood, 1978).
- Routine tests ensure early detection in gene banks, nurseries, orchards and public greens, that must be followed by seed/plant eradication and other sanitary selection procedures, finally to ensure certified virus-free seeds and planting material and prevent the spread of CLRV.

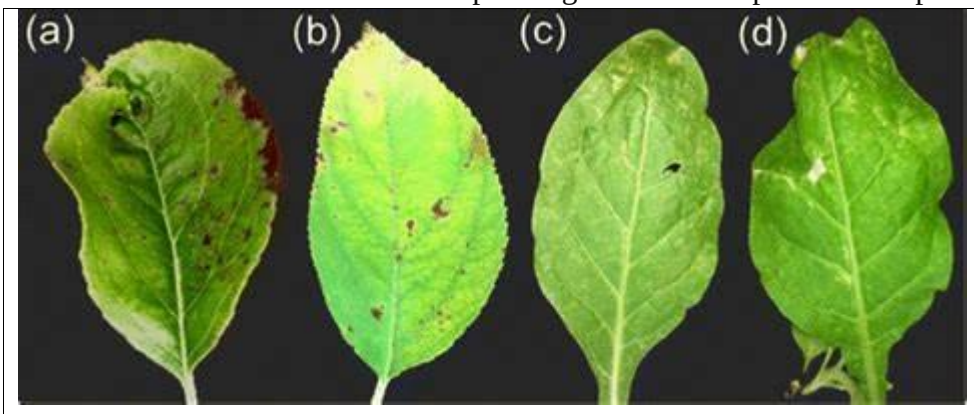


Figure. Symptoms observed in apple leaves: a Leaf tip deformation; b Necrotic spots and mottling. Symptom development following inoculation of Cherry leaf roll virus in Nicotiana occidentalis: c Localized symptom of yellow mottling; d Systemic symptoms of lamina deformation, necrosis and yellow mottling

Paratrichodorous porosus

Other scientific names: *Atlantadorus porosus, Paratrichodorus (Atlantadorus) porosus Trichodorus bucrius Trichodorus porosus*

Hosts: Apple, Onion, cabbage, Chinese cabbage, camellia, tea, rough lemon, lemon, mandarin, sweet orange, grapefruit, yam, walnut, wild tomato, banana, plantain, apricot, peach, European pear, loganberry, sugarcane, sorghum, Mexican marigold, French marigold, grapevine, corn, maize.

Transmission: It transmits tobacco rattle tobravirus.

Symptoms to look for in post- entry quarantine:

- They are ectoparasite of roots.
- Root system is reduced.
- Plants showing viral symptoms such as necrotic spots and lines on the leaves, leaf petioles and stems.

Pezicula malicortis

Other scientific names: *Cryptosporiopsis curvispora, Cryptosporiopsis malicorticis, Cryptosporiopsis perennans, Gloeosporium malicorticis, Gloeosporium perennans, Macrophoma curvispora, Neofabraea malicorticis, Neofabraea perennans*

Hosts: Apple and pear

Transmission: Local spread of the disease within and between trees is a consequence of the conidia. Dispersal of the pathogen into new regions and countries may occur from the shipping of infected plant material.

Symptoms to look for in post- entry quarantine:

- Bull's-eye rot in fruits
- Lesions in woods
- Branch lesions as small, circular spots that are purple or red when wet.
- On older cankers, bark sloughs off leaving only the last fibers behind.
- Cracked barks

Anthracnose cankers (source: Washington State University)

Distinct margins between healthy and diseased tissues (source: Michigan State University)

Phytoplasma mali

Other scientific names: Apple proliferation phytoplasma, *Candidatus* Phytoplasma *mali*

Hosts: Apple, plum

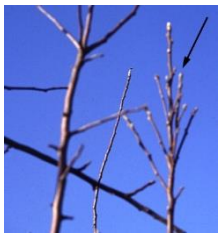
Transmission: *Cacopsylla melanoneura, Cacopsylla picta, Fieberiella florii*

Symptoms to look for in post- entry quarantine:

- Leaves roll downward and become brittle.
- Leaves are irregularly serrated.
- Summer leaves are chlorotic
- Early defoliation.
- Rosette of terminal leaves.
- Numerous petals on flower.
- Fruits are reduced in size.
- Angle between secondary shoots and main shoots is abnormally narrow.

Symptoms on cv. Florina (proliferation symptoms)

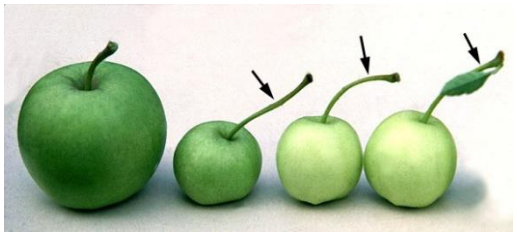
Symptoms on cv. Golden Delicious (pale leaves)



Witches broom of secondary twigs

Chlorotic leaves with enlarged stipules

Enlarged flowers with numerous petals



Small fruit with elongated, narrow petioles

Pythium ultimum

Other scientific names: NA

Hosts: Wide (Apple, strawberry, rice, maize, vegetables)

Transmission: Overwatering and soil saturation

Symptoms to look for in post- entry quarantine:

- Root rot and necrosis: rapid necrosis of root, brown lesions
- Distinct margins between healthy and necrotic root sections
- Wilting of plants
- Collar rot

Figure. *Pythium* infected roots

Pratylenchus vulnus

Other scientific names: Walnut root lesion nematode

Hosts: Apple, kiwi, walnut, pecan, orange, grapefruit, coffee, carrot, strawberry, etc.

Symptoms to look for in post- entry quarantine:

- Dark colored lesion on roots
- Small cracks in bark
- Large necrotic lesions
- General decline

Figure. Necrosed roots

Pseudomonas syringae* pv. *syringae

Other scientific names: *Bacillus cerasi, Bacillus gummis, Bacillus matthiolae, Bacillus spongiosus, Bacterium cerasi, Bacterium cerasi* var. *prunicola, Bacterium citrarefaciens, Bacterium citriputeale, Bacterium gummis, Bacterium hibisci, Bacterium holci, Bacterium matthiolae, Bacterium nectarophilum, Bacterium prunicola, Bacterium rimaefaciens, Bacterium spongiosum, Bacterium syringae, Bacterium trifoliorum, Bacterium utiformica, Bacterium vignae, Bacterium vignae* var. *leguminophilum, Bacterium viridifaciens, Chlorobacter syringae, Phytomonas cerasi, Phytomonas cerasi* var. *prunicola, Phytomonas citrarefaciens, Phytomonas citriputealis, Phytomonas hibisci, Phytomonas holci, Phytomonas matthiolae, Phytomonas nectarophila, Phytomonas prunicola, Phytomonas rimaefaciens, Phytomonas spongiosa, Phytomonas syringae, Phytomonas trifoliorum, Phytomonas utiformica, Phytomonas vignae, Phytomonas vignae* var. *leguminophila, Phytomonas viridifaciens, Pseudomonas cerasi, Pseudomonas cerasi* f. sp. *pyri, Pseudomonas cerasi* var. *prunicola, Pseudomonas cerasi var. pyri, Pseudomonas citrarefaciens, Pseudomonas citriputealis, Pseudomonas hibisci, Pseudomonas holci, Pseudomonas japonica, Pseudomonas matthiolae, Pseudomonas medicaginis, Pseudomonas nectarophila, Pseudomonas oryzicola, Pseudomonas prunicola, Pseudomonas spongiosa, Pseudomonas striafaciens var. japonica, Pseudomonas syringae, Pseudomonas syringae* f.sp. *prunicola, Pseudomonas syringae* pv. *japonica, Pseudomonas trifoliorum, Pseudomonas utiformica, Pseudomonas vignae, Pseudomonas vignae* var. *leguminophila, Pseudomonas viridifaciens*

Hosts: Wide (Apple, Kiwi, coffee, onion, vegetables, etc.)

Transmission: It is transmitted and disseminated through living plants and vegetative propagation material and by wind-driven rain.

Symptoms to look for in post- entry quarantine:

- Discolored or necrotic spots on shoots
- No halo
- Dieback of shoots
- Cankers on older twigs
- Gumming cankers
- Cankers on base of spurs
- Brown blister like appearance in spring
- Leaves are curled inward
- Blast of dormant buds
- Death of buds
- Water-soaked spots in flowers

Discoloration beneath bark

Death of spur

Phytophthora megasperma

Apples are most susceptible to infection between the pink phonological stage and shoot elongation. *P. syringae*, *P. megasperma*, and *P. cryptogea* are capable of extensively infecting apple during the dormancy period.

Other scientific names: *Pythiomorpha miyabeana*

Hosts: Wide **(**Apple, almond, avocado, chestnut, citrus, cinnamon, kiwi, macadamia nut, pear, pecan, persimmon, pineapple, pines, tomato, walnut, watermelon**)**

Symptoms to look for in post- entry quarantine:

- Crown rot affects rootstock while collar rot affects bark tissue of scion at or just below soil line.
- Necrosis of roots
- Foliar symptoms appear as chlorosis with reddening or purpling of leaves with normal bloom.
- General decline and wilting of trees
- Cankers are present at the soil line.
- Reddish-brown to orange discoloration of inner bark below soil line
- Rotted tissues and healthy tissue have distinct margins
- Developing fruits after infection are small.

Fig. Apple root rot (left), and crown rot (right)

Prunus necrotic ringspot virus

Other scientific names: Cherry line pattern virus, European plum line pattern virus, Hop B virus, Hop C virus, North American plum line pattern virus, Peach ringspot virus, Plum (North American) line pattern virus, Plum line pattern virus (in part), Prunus necrotic ringspot ilarvirus, Prunus ringspot virus, Rose chlorotic mottle virus, Rose line pattern virus, Rose vein banding virus, Rose yellow mosaic virus, Rose yellow vein mosaic virus, Sour cherry necrotic ringspot virus

Hosts: Wide (Apple, peach, plum, rose, cucumber, almond etc.)

Transmission: Graft transmission

Symptoms to look for in post- entry quarantine:

- Mosaic patterns and yellow vein banding on leaves.
- Chlorotic spots, lines, or rings as leaves emerge.
- In severe cases, chlorotic areas may become necrotic and fall out, creating "shot-holed" or tattered leaves.
- Stunting of growth and reduced vigor.
- Possible delayed fruit maturity and marked fruit.
- Some trees may show symptoms one year and remain symptomless in subsequent years (known as "shock" symptoms)

Figure. Shot- holed and tattered leaves

Rhizobium rhizogenes

Other scientific names: *Agrobacterium rhizogenes*

Hosts: Wide (Apple, chestnut, asparagus, citrus, tea, carrot, crucifers, etc.)

Transmission: Infection likely occurs via contaminated propagation material and soil; the bacteria survive in soil and spread through rain, wind, human activity, and contaminated tools.

Symptoms to look for in post- entry quarantine:

- The infection causes *hairy root disease*, characterized by excessive proliferation of adventitious roots at or near the root region of infection, often called "hairy roots".
- Plants develop galls (tumor-like swellings) on roots, stems below ground, or at the crown; these galls start white and later turn brown or tannish-brown and become necrotic with age.
- Galls may also be found on lateral roots and above ground on stems and canes; initially, galls resemble wound callus but develop more rapidly and have a soft, spongy texture early on, becoming hard as they mature.
- Infected apple plants may show poorly developed root systems and stunted growth, making them more susceptible to environmental stresses like winter injury or wind damage.
- The hairy root symptom is sometimes called non-infectious hairy root because isolating the pathogenic Rhizobium from hairy root tissue is difficult; roots may appear normal after several years despite initial symptoms.
- The disease can clog irrigation drippers in greenhouse conditions due to excessive root growth.

Figure. Hairy root symptoms

Phaeoacremonium minimum:

Other scientific names: *Phaeoacremonium aleophilum*, *Togninia minima*

Hosts: Grapevines, Apple

Transmission: Pruning wounds are major entry points for conidia, rain-splashes transmit the spores.

Symptoms to look for in post- entry quarantine:

- Wood discoloration and decay, particularly in the stems and lower parts of the trunk, often accompanied by extensive cankers.
- On leaves, symptoms can manifest as light-green or chlorotic spots between the veins, which gradually spread over time to the distal parts of shoots.
- A general decline in tree health, including dieback symptoms, may be observed due to the pathogen's effect on vascular tissues.

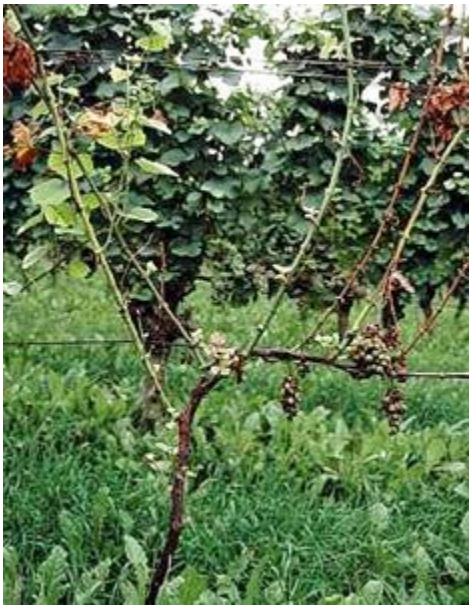


Figure. Symptoms of *P. minimum*

Valsa leucostoma

Other scientific names: *Valsa cincta*, *Cytospora leucostoma*, *Leucostoma persoonia*,

Hosts: Peach, plum, prune, cherry, apple

Transmission: Conidia and ascospores transmit this pathogen.

Symptoms to look for in post- entry quarantine:

- Initially appear as sunken discolored area with light and dark concentric circles of dead tissue.
- Cankers, not sunken and generally not noticeable until they girdle the trunk.
- Nodal infections 2 to 4 weeks after bud break.
- Amber gum ooze from infected tissue.
- Branch cankers
- Flagging of dead scaffold branches
- Leaves turn yellow, droop, may wilt.
- Dead twigs have minute pin-head-sized black sporulating structures.

Figure. *Leucostoma* canker

Figure. *Leucostoma* canker (dark circles of dead tissue)

Figure**.** Nodal infection

Figure**.** Dead twigs

Phomopsis cotoneastri

Other scientific names: *Diaporthe eres*

Hosts: Wide (apple, persimmon, apricot, almond, plum etc.)

Transmission: Infected planting materials and wind or rain-splashed spores help transmit the pathogen.

Symptoms to look for in post- entry quarantine:

- Sunken cankers in trunk
- Diebacks
- Leaf spots
- Streak lesions in leaves
- Black pycnidia on dead tissues
- Inner bark and bark above infected cambium on branches are sunken and split.

Figure. *Diaporthe eres* symptoms

Figure. *Diaporthe eres* symptoms

***Phytophthora cambivora*:**

Other scientific names:

Hosts: Wide **(**Apple, almond, avocado, chestnut, citrus, cinnamon, kiwi, macadamia nut, pear, pecan, persimmon, pineapple, pines, tomato, walnut, watermelon**)**

Symptoms to look for in post- entry quarantine:

- Sunken brown-black lesions on shoots
- Wilting or rust-brown/purplish-bronzed leaves.
- Infected roots turn red-brown.
- Some stem lesions show yellow-brown tissue with narrow dark brown, concentric, wavy-margined rings up the stem.
- Thinning of the crown, chlorotic (yellowed) leaves, and reduced leaf size in adult trees.
- Cankers may develop at the base of the trunk, and the disease can kill young trees just as they begin to bear fruit.
- Brown watery exudates are seen.

***Phytophthora syringae*:**

Active in low temperatures. Apples are most susceptible to infection between the pink phonological stage and shoot elongation. *P. syringae*, *P. megasperma*, and *P. cryptogea* are capable of extensively infecting apple during the dormancy period. apple varieties grown on vigorous rootstock are more susceptible than those on less vigorous root stock.

Other scientific names:

Hosts: Wide **(**Apple, almond, avocado, chestnut, citrus, cinnamon, kiwi, macadamia nut, pear, pecan, persimmon, pineapple, pines, tomato, walnut, watermelon**)**

Symptoms to look for in post- entry quarantine:

- Root and collar rot, with characteristic browning and decay of crown and root tissues
- Above-ground symptoms such as stunted, yellow leaves, cessation of shoot growth, wilting, and dieback
- Fruit rot is more common in cool, wet conditions during harvest.
- Lesions are less extensive than those caused by *P. cambivora* or *P. cactorum*