

### ***Lymantria xyli*na (Casuarina tussock moth)**

The *Lymantria xyli*na lure is effective for 180 days. Producing the lure is time-consuming, so using the lure for the full length of effectiveness is strongly encouraged. For negative data reporting, use the lure with the paper delta trap. Check the traps every two weeks to collect samples. Replace the traps once a month (every other trap check), as the sticky surface becomes covered with dust, leaves, etc.

- At the start of the survey season, attach the lure to the trap by stapling the string dispenser to a twist-tie and then stapling the twist-tie to the trap. The twist-tie will reduce repeated handling of the string dispenser when replacing traps throughout the season.
- When replacing the trap (monthly), remove the twist-tie with attached lure from the old trap and staple it to the new trap.

*Lymantria xyli*na AMPS page:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=1533&from=2020>

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### ***Monilinia fructigena* (brown rot; apple brown rot)**

A new approved method for screening is available for *Monilinia fructigena* (brown rot; apple brown rot): Molecular. A conventional polymerase chain reaction (cPCR) assay is approved for screening. This test distinguishes the two exotic species, *Monilinia fructigena* and *M. polystroma*, from the two established species, *M. laxa* and *M. fructicola*. The cPCR assay can be used in combination with morphological identification for species identification.

The work instruction for a real-time PCR (qPCR) assay for species identification is pending approval. This test distinguishes *M. fructigena* from *M. polystroma*.

**To request a copy of diagnostic protocols**, email the S&T Beltsville laboratory at APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov and use the subject line "Diagnostic protocol request".

See the *Monilinia fructigena* AMPS page for more information:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=2031&from=2020>

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### ***Cocadviroid Coconut Cadang Cadang Viroid* (CCCVd)**

A new approved method for screening is available for *Cocadviroid Coconut Cadang Cadang Viroid* (CCCVd): Real-time polymerase chain reaction (qPCR) kits for primary screening are available from Norgen Biotek Corp.: <https://norgenbiotek.com/product/cccvd-detection-kits>. Dr. Rosemarie Hammond tested the commercially available kit for sensitivity and specificity.

New sample submission guidance

Fresh palm samples brown and degrade quickly during transport. To ensure sample quality, dry the palm samples over a drying agent, like calcium chloride or silica gel, prior to shipping to Dr. Hammond's lab. To dry samples we recommend:

1. cutting the leaf tissue into strips,
2. placing the strips into sealable tubes (e.g. 15 ml plastic test tube) containing a small amount of drying agent. Do not overfill tubes to allow for air circulation.
3. sending in entire tube.

If drying is not possible, DO NOT freeze the leaves. Instead, keep the leaves cool by placing the samples into an insulated box with lid and add freezer bags/cold packs. Tape the box shut and package it for shipment.

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See the CCCVd AMPS page for more information:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=1928&from=2020>

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***Phytophthora alni* (alder root and collar rot)**

A new approved method for species confirmation is available for *Phytophthora alni* (alder root and collar rot): Molecular. A conventional polymerase chain reaction (cPCR) assay is approved for species identification. There are three subspecies of *P. alni* (*P. alni* subsp. *alni*, *P. alni* subsp. *uniformis*, and *P. alni* subsp. *multiformis*). Additional testing by the S&T Beltsville laboratory are necessary to confirm the subspecies.

See the *Phytophthora alni* AMPS page for more information:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=1988&from=2020>

**To request a copy of diagnostic protocols**, email the S&T Beltsville laboratory at [PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov](mailto:PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov) and use the subject line "Diagnostic protocol request".

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***Phytophthora austrocedrae* (mal del cipres; cypress mortality)**

A new approved method for species confirmation is available for *Phytophthora austrocedrae* (mal del cipres; cypress mortality): Molecular. A real-time polymerase chain reaction (qPCR) test is approved for species identification.

**Note:** The qPCR protocol uses the Cepheid SmartCycler thermocycler machine. In 2019, the manufacturer discontinued this machine. The S&T Beltsville laboratory is currently adapting the protocol for a different machine. Diagnostic labs may use the protocol for the Cepheid SmartCycler if their machine is in good condition. For the 2020 survey season, please contact the diagnostic laboratory you use for screening to confirm their capacity before you begin collecting samples.

See the *Phytophthora austrocedrae* AMPS page for more information:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=2000&from=2020>

**To request a copy of diagnostic protocols**, email the S&T Beltsville laboratory at APHIS-[PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov](mailto:PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov) and use the subject line "Diagnostic protocol request".

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***Phytophthora kernoviae* (beech bleeding canker)**

A new approved method for species confirmation is available for *Phytophthora kernoviae* (beech bleeding canker): Molecular. A real-time polymerase chain reaction (qPCR) test is approved for species identification.

**Note:** The qPCR protocol uses the Cepheid SmartCycler thermocycler machine. In 2019, the manufacturer discontinued this machine. The S&T Beltsville laboratory is currently adapting the protocol for a different machine. Diagnostic labs may use the protocol for the Cepheid SmartCycler if their machine is in good condition. For the 2020 survey season, please contact the diagnostic laboratory you use for screening to confirm their capacity before you begin collecting samples.

See the *Phytophthora kernoviae* AMPS page for more information:

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=2005&from=2020>

**To request a copy of diagnostic protocols**, email the S&T Beltsville laboratory at APHIS-[PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov](mailto:PPQCPHSTBeltsvilleSampleDiagnostics@usda.gov) and use the subject line "Diagnostic protocol request".

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***Ceratocystis manginecans* & *Raffaelea quercivora* - Sampling and Isolation Protocols**

If you are surveying for *Ceratocystis manginecans* (mango sudden decline) or *Raffaelea quercivora* (Japanese oak wilt), it is important to contact the PPQ National Mycology Specialists ([PPQNISNTMycology@usda.gov](mailto:PPQNISNTMycology@usda.gov)) prior to the start of the survey season. Both require isolation prior to screening. The mycology specialists will provide guidance on how to sample and isolate these fungi and review the laboratory requirements for successful isolation.

See the AMPS pages for more information:

*Ceratocystis manginecans*

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=1754&from=2020>

*Raffaelea quercivora*

<http://pest.ceris.purdue.edu/services/approvedmethods/sheet.php?v=14&from=2020>

If you have questions, please contact CAPS Science Support at [STCAPS@usda.gov](mailto:STCAPS@usda.gov).