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## Assay of stored soils for presence of *Phytophthora agathidicida*

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June 2015

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**Confidential report for:**  
The Ministry for Primary Industries

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## CONTENTS

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|                                       |          |
|---------------------------------------|----------|
| <b>Executive summary</b> .....        | <b>1</b> |
| <b>1 Introduction</b> .....           | <b>3</b> |
| <b>2 Methods</b> .....                | <b>3</b> |
| <b>3 Results and discussion</b> ..... | <b>5</b> |
| <b>4 Acknowledgments</b> .....        | <b>8</b> |



## EXECUTIVE SUMMARY

### **Assay of stored soils for the presence of *Phytophthora agathidicida***

Horner IJ, Hough EG  
Plant & Food Research, Havelock North

**June 2015**

Kauri soils that previously tested positive for *Phytophthora agathidicida* were stored for up to 9 years and then retested using standard soil baiting protocols. *P. agathidicida* was readily re-isolated from 17 out of 22 soils tested, indicating that the pathogen is capable of long-term survival even in the absence of fresh host material. Storage conditions varied, but for the most part soils were stored moist at 10°C. Over 80% of soils stored for 4 – 6 years still yielded viable *P. agathidicida*.

Results suggest that soils collected and stored appropriately can be tested or re-checked for *P. agathidicida* presence after a period of months or even years without significantly compromising isolation of the pathogen.

The long-term survival of *P. agathidicida* in soil demonstrated in this study also has ecological significance. Although the spores in the stored soils would not have been exposed to all the micro- and macro-fauna interactions that may occur in the forest, the soils were stored moist and mostly at temperatures that would have allowed plenty of microbial activity. From this study we can speculate that *P. agathidicida* could potentially survive in the soil for many years, even in the absence of a suitable host.

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## 1 INTRODUCTION

Over the past 9 years, over 1200 soils have been sent from various parties to the Plant & Food Research (PFR) laboratory in Havelock North, for testing the presence of *Phytophthora agathidicida* (= *P. taxon* Agathis; PTA). After taking a suitable sample for testing, remaining soil had been stored. The current work was to determine whether the *P. agathidicida* had survived the storage and whether the pathogen could still be extracted from samples using standard baiting procedures. This was expected to give some insight into pathogen survival, and also into required handling and storage of the soils.

## 2 METHODS

In May 2015, a selection of soils that had been stored for 2 to 9 years in sealed plastic bags was selected for the study. Soils were originally from a range of kauri forest sites in the Auckland and Northland regions. In selecting soils for the current study, emphasis was placed on soils that had been stored for the longest period; the majority of soils had been stored for 4 to 6 years. Twenty-two soils that had previously tested positive for *P. agathidicida* and five soils that had tested negative were chosen.

Storage conditions for the oldest samples varied. Some of the earlier soils were stored at ambient room temperature, then in a 1 – 2°C coolstore. Since late 2010, all soils have been stored at 10°C after an initial period of 1 – 5 weeks at room temperature.

After removal from storage, soil samples were subjected to the standard extended baiting protocol for isolating *P. agathidicida* from soil (Table 1). Soil fresh weight and air-dried weight were measured, to give an indication of the moisture content in the stored sample. Following the first baiting test, any samples where *P. agathidicida* could not be detected were retested by the same process, using a fresh portion of the remaining sample from the 10°C coolstore. Following the bait-plating step (day 10) of the second test, flooded samples were re-baited with fresh cedar needles for a further 4 days, then baits were plated onto *Phytophthora*-selective P<sub>5</sub>ARPH agar (corn-meal-agar amended with antibiotics).

Depending on soil availability, the fresh weight of soil re-tested ranged from 12 to 180 g, with most samples exceeding 50 g.

**Table 1. Extended soil baiting protocols used in the current work for isolating *Phytophthora agathidicida* from stored soils.**

| Day     | Procedure   |
|---------|---|
| 1       | Thoroughly mix soil samples in their bags and break up lumps.<br>Spread soil sample onto paper towels in trays on the lab bench.<br>Air-dry on lab bench for 2 - 3 days.<br>Check soil daily and crumble clods.<br>Avoid cross-contamination between samples.   |
| 4       | After air-drying, moisten soil samples in baiting containers with distilled water.<br>Add sufficient water to moisten, but not saturate the soil.<br>Check after 1 h, add more water to dry spots, apply lid.<br>Incubate in light for 4 days at room temperature (20–22°C).  |
| 8       | After 4 days, flood the soil with distilled water, to a depth of water of 5-10 cm above the soil surface.<br>Minimise soil disturbance and water turbulence.<br>Float five Himalayan cedar ( <i>Cedrus deodara</i> ) needles on water surface.<br>Add three freshly germinated, intact lupin radicles ('NZ Blue') to the water surface (suspended on Parafilm®).<br>Incubate at 20°C in light for 2 days. |
| 10      | After 2 days, remove the bait tissues and wash in a single rinse of sterile water.<br>Soak in 50% ethanol (ETOH) for 30 s.<br>Remove from ETOH, rinse in sterile water.<br>Blot dry on paper towels.<br>Place leaflets/lupins onto P5ARPH agar plates.<br>Label and seal plates, incubate in the dark at 18–20°C.   |
| 12 - 17 | Check plates after two days of incubation, and isolate <i>Phytophthora</i> -like cultures to V8® juice agar.<br>Recheck bait plates periodically for up to 7 days, subbing cultures to V8.  |
| 19 - 25 | Microscopic examination and identification of cultures.   |

### 3 RESULTS AND DISCUSSION

The soils tested, their origins, collection dates, storage conditions, original *P. agathidicida* status and recent isolation of *P. agathidicida* are presented in Table 2.

Of the five soils tested where the historical test had failed to detect *P. agathidicida*, one soil tested positive in the current round of retesting. This sample (Soil\_84) was collected from a site where other samples had tested positive when first collected, so the recent detection is not surprising. The historical test was presumably a false negative. *P. agathidicida* could not be detected in the remaining four soils that initially tested negative for *P. agathidicida*.

Of the 22 soils that initially tested positive for *P. agathidicida* when received in the PFR laboratory, 16 tested positive in the first re-test, and a further one tested positive in the second re-test. *P. agathidicida* could not be isolated from five of the twenty-two soils. Two of these soils had been stored for 9 years at temperatures ranging from 1°C, to 10°C and 20°C. The remaining three soils where *P. agathidicida* could not be re-isolated had been stored for about 5 years at 10°C after the initial period at room temperature. One of these soils (Soil\_103) was very dry when it came out of storage.

*P. agathidicida* could not be isolated from the two oldest samples, collected 9 years ago. These samples were relatively small, and had undergone a range of storage temperatures, including about three years at 1 – 2°C. While the current study cannot prove that these storage conditions are detrimental to *P. agathidicida* survival, other studies (Horner, unpublished) have shown that *P. agathidicida* was very difficult to extract from inoculated kauri roots stored for one month at 1°C.

The fact that *P. agathidicida* could still be extracted from soil after many years of storage indicates that the pathogen is capable of long-term survival, even in the absence of fresh host material for colonisation. While the 10°C storage conditions are cooler than would normally be encountered in the field, and spores would be protected from much of the macro- and micro-fauna that would be present in the field, the storage conditions would still have allowed plenty of biological activity. Most soils had moderate to high soil moisture content during storage (Table 2). This potential for long-term survival in the absence of fresh host material has ecological significance, and we can speculate that *P. agathidicida* could potentially survive in forest soil for many years, even in the absence of a host.

The results indicate that delays in processing soils following collection should not substantially compromise subsequent extraction of *P. agathidicida*, provided soils are stored appropriately. This work does not provide enough information to determine the optimum temperature for storage, although it does suggest that 10°C is an appropriate temperature for long-term storage. The fact that *P. agathidicida* was also extracted from soils that were held for a year at 20°C before being stored at 10°C for a further 4 years indicates that storage temperature may not be critical for long-term survival.

With regard to future collecting and testing of soils, results suggest that provided soils are stored in an appropriate way (in the absence of further information, 10°C is recommended), there is no urgency for processing the samples, at least in terms of test integrity. It should be possible to store soils for validation or checking of results without significantly compromising the integrity of the *P. agathidicida*.

**Table 2. Soil collection and storage notes, plus *Phytophthora agathidicida* (PTA) test results before and after storage. Soils are listed in order of collection date.**

| PFR Lab Soil ID # | Collection Location/ Notes                           | Soil collection date | Date received in PFR lab | Storage conditions <sup>1</sup>          | Fresh weight tested (g) | Soil water content in storage % <sup>2</sup> | Original Result <sup>3</sup> | 2015 re-test #1 | 2015 re-test #2 | 2015 re-test #3 |
|-------------------|--|----------------------|--------------------------|--|-------------------------|--|------------------------------|-----------------|-----------------|-----------------|
| TS1/MAF           | TS1 soil core. MAF Northland                         | 10/04/2006           | Apr. 2006                | 1 y at 20°C, 3 y at 1-2°C, 4.5 y at 10°C | 33                      | 156  | PTA                          | nil             | nil             | nil             |
| T2.1/MAF          | T2.1 soil core. MAF Northland                        | 10/04/2006           | Apr. 2006                | 1 y at 20°C, 3 y at 1-2°C, 4.5 y at 10°C | 12                      | 142  | PTA                          | nil             | nil             | nil             |
| Hui083            | Hui083   | Oct. 2010            | Dec. 2010                | 4.5 y at 10°C                            | 124                     | 63   | PTA                          | PTA             | - <sup>4</sup>  | -               |
| Huia bulk         | Huia Bulk - sent Dec 2009                            | 2009                 | Dec. 2009                | 1 y at 20°C, 4.5 y at 10°C               | 86                      | 69   | PTA                          | PTA             | -               | -               |
| Soil_23           | Great Barrier Tree 1                                 | 29/05/2009           | 21/07/2010               | 1 y at 10°C, 4 mo at 20°C, 4.5 y at 10°C | 52                      | 40   | PTA                          | PTA & Phyt. sp  | -               | -               |
| Soil_26           | Great Barrier Tree 5                                 | 29/05/2009           | 21/07/2010               | 1 y at 10°C, 4 mo at 20°C, 4.5 y at 10°C | 63                      | 45   | PTA                          | PTA             | -               | -               |
| Soil_24           | Great Barrier Tree 2                                 | 29/05/2009           | 21/07/2010               | 1 y at 10°C, 4 mo at 20°C, 4.5 y at 10°C | 45                      | 77   | nil                          | Phyt. sp        | nil             | nil             |
| Soil_103          | Awhitu 165.1   | mid 2009?            | 25/10/2010               | 18 mo at 20°C, 4.5 y at 10°C             | 88                      | 5  | PTA                          | nil             | nil             | nil             |
| Soil_20           | Raetea plantation                                    | Mar. 2010            | 21/07/2010               | 4 mo at 20°C, 4.5 y at 10°C              | 79                      | 87   | PTA                          | PTA             | -               | -               |
| Soil_28           | Phy131 Tree 1-3 bet. dead & infected tree (Trounson) | Mar. 2010            | 21/07/2010               | 4 mo at 20°C, 4.5 y at 10°C              | 68                      | 140  | PTA                          | PTA             | -               | -               |
| Soil_38           | Site 277, Oratia.                                    | 5/08/2010            | 12/08/2010               | 3 mo at 20°C, 4.5 y at 10°C              | 138                     | 84   | PTA                          | nil             | nil             | nil             |
| Soil_46           | Colin McCahon House #57.2                            | 24/08/2010           | 31/08/2010               | 3 mo at 20°C, 4.5 y at 10°C              | 145                     | 76   | PTA                          | nil             | PTA             | -               |
| Soil_43           | Hunua1 Tr1, Mangatangi Hill                          | 27/08/2010           |                          | 3 mo at 20°C, 4.5 y at 10°C              | 95                      | 102  | nil                          | nil             | nil             | nil             |
| Soil_79           | (#13.2) 3 Armour Rd, Parau                           |                      | 29/10/2010               | 4.5 y at 10°C                            | 63                      | 187  | PTA                          | PTA             | -               | -               |
| Soil_87           | (#30.2) 47 Whatipu Rd, Little Huia                   |                      | 29/10/2010               | 4.5 y at 10°C                            | 79                      | 82   | PTA                          | PTA             | -               | -               |
| Soil_83           | (27.1) Kauri Loop Rd, Oratia                         |                      | 29/10/2010               | 4.5 y at 10°C                            | 49                      | 83   | PTA                          | PTA             | -               | -               |
| Soil_84           | (27.2) Kauri Loop Rd, Oratia                         |                      | 29/10/2010               | 4.5 y at 10°C                            | 138                     | 33   | nil                          | PTA             | -               | -               |
| Soil_96           | (55.1) Lopdell House, Titirangi                      |                      | 29/10/2010               | 4.5 y at 10°C                            | 87                      | 35   | PTA                          | PTA             | -               | -               |
| Soil_82           | (22.3) Muriwai Rd, Muriwai                           |                      | 29/10/2010               | 4.5 y at 10°C                            | 70                      | 29   | PTA                          | nil             | nil             | nil             |
| Soil_189          | (NEW#43) E1747574 N5909754 MAHOE(EAST)               | 17/02/2011           | 23/2/2011                | 4 y at 10°C                              | 118                     | 49   | PTA                          | PTA             | -               | -               |

**Table 2. Continued...**

| PFR Lab Soil ID # | Collection Location/ Notes                         | Soil collection date | Date received in PFR lab | Storage conditions <sup>1</sup> | Fresh weight tested (g) | Soil water content in storage % <sup>2</sup> | Original Result <sup>3</sup> | 2015 re-test #1 | 2015 re-test #2 | 2015 re-test #3 |
|-------------------|--|----------------------|--------------------------|---------------------------------|-------------------------|--|------------------------------|-----------------|-----------------|-----------------|
| Soil_283          | T7. McCahon House                                  | 21/06/11             | 1/07/2011                | 4 y at 10°C                     | 180                     | 51   | PTA                          | PTA             | -               | -               |
| Soil_428          | Huia Dam trial #5                                  | 9/02/12              | 12/02/12                 | 3 y at 10°C                     | 98                      | 104  | PTA                          | PTA             | -               | -               |
| Soil_433          | Whatipu trial #3                                   | 9/02/12              | 12/02/12                 | 3 y at 10°C                     | 138                     | 63   | nil                          | Phyt sp         | nil             | nil             |
| Soil_559          | PTS1. Parau Track - E1739682 N5904942              | 10/10/12             | 1/11/2012                | 2 y 7 mo at 10°C                | 126                     | 88   | PTA & Phyt sp                | PTA & Phyt sp   | -               | -               |
| Soil_560          | PTS2. Parau Track - E1739722 N5904775              | 10/10/12             | 1/11/12                  | 2 y 7 mo at 10°C                | 180                     | 48   | nil                          | nil             | nil             | nil             |
| Soil_653          | MCH17. McCahon House E1748158 N5909686             | 23/5/13              | 6/6/13                   | 2 y at 10°C                     | 70                      | 109  | PTA                          | PTA             | -               | -               |
| MPI_865           | WP38-AH03. Puketotara Peninsula E1717028; N5986083 | 31/3/14              | 3/4/14                   | 1 y 2 mo at 10°C                | 163                     | 24   | PTA                          | PTA             | -               | -               |

<sup>1</sup>Storage times and conditions are approximate. Most soils were held at laboratory temperature (approx. 20°C) for at least 1 month before being transferred to storage units.

<sup>2</sup>Soil water content in storage was calculated by weight of water in soil divided by the dry weight of the soil.

<sup>3</sup>'Original result' refers to the result from the standard *P. agathidicida* (PTA) baiting test carried out when the sample was first received in the PFR laboratory.

<sup>4</sup>"-" = not tested, as the previous test was positive for *P. agathidicida*.

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