INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0148/2022/27–5–361–370
DOI: 10.17957/IJAB/15.1936
http://www.fspublishers.org

*Full Length Article*

# Study on Soil Properties and Species Conformity of *Phytophthora* Species in a Pineapple Field

**Supriyono Loekito[1*], Afandi[2], Auliana Afandi[3], Naomasa Nishimura[4], Hiroyuki Koyama[4] and Masateru Senge[5]**
[1]*Research and Development, PT Great Giant Pineapple, Jl. Raya Terbanggi Km 77, Central Lampung, Lampung Indonesia, 34163*
[2]*Department of Soil Science, Faculty of Agriculture, Lampung University, J. Sumantri Brojonegoro 1, Bandar Lampung, Lampung, Indonesia 35145*
[3]*National Research and Innovation Agency, Gedung B.J. Habibie, Jalan M.H. Thamrin No. 8, Jakarta Pusat, Indonesia 10340*
[4]*Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu, Japan*
[5]*Gifu University Laboratory, Ltd. Union, 1-1 Yanagido, Gifu, Japan*
[*]For correspondence: Supriyono.Loekito@ggpc.co.id
*Received 04 April 2022; Accepted 30 April 2022; Published 26 May 2022*

## Abstract

Phytophthora heart rot is the most important disease associated with Phytophthora pathogen losses pineapples in the most pineapple farm in Indonesia. This work was carried out to study soil properties around pineapples, confirm *Phytophthora* species, and examine the soil-applied sulfur in 'Smooth Cayenne' MD2 pineapple. The species conformity was done molecularly by sequencing cytochrome c oxidase 1 (COX 1) region at Oomycete Research Laboratory, Gifu University, Japan. The physical and chemical soil properties around the healthy pineapples (healthy soil) and infected pineapples (infested soil) were observed at Great Giant Pineapple (GGP) Laboratory. To find the effects of sulfur in lowering soil pH, about 15 kg of soil was treated with sulfur at doses of 0, 7.5, 15 and 22.5, 30 g. The species of genus *Phytophthora* which attacked pineapples at GGP was identified as *P. nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) Tucker. The basic local alignment search tool (BLAST) search result, showing 98% similarities to the COX 1 gene of *P. nicotianae*. The infested soil contained significantly more clay and fewer sand particles, considerably higher soil compactness and average soil pH than healthy soil. The soil-applied sulfur, equaling a dose of 500 kg $ha^{-1}$, was enough to lower soil pH from 6.3 to 5.1–5.4 when it needed. The result showed that a strategy to control heart rot disease would be more effective when it could modify soil environment to be unfavorable for pathogens to grow and infect the plants. Further work is needed to improve integrated disease management. © 2022 Friends Science Publishers

**Keywords:** Heart rot; *Phytophthora* spp.; Smooth cayenne MD2; Soil properties

## Introduction

Pineapple (*Ananas comosus* L. Merr.) is one of the most popular tropical fruits in the world. In 2015, pineapple cultivar MD2 was planted for the fresh market at Great Giant Pineapple Company (GGP) plantation in Lampung, Sumatra, about 45 meters above sea level, and also grown in other areas of Indonesia. Most of Lampung has a humid tropical climate characterized by high rainfall (2,500 mm per year), air temperatures between 21° and 33°C, relative humidity around 83%, duration of effective sunshine of 4.6 h a day, and a standard evaporation rate of 3.6 mm a day. The year-round temperatures, heavy rainfall, and high humidity are unique to the humid tropics and cause the organic material in the soil to decompose at a high rate, resulting in low chemical fertility, a high clay content, and low soil pH. Commonly pineapple was planted in a raised bed double row (Fig. 1A); erosion problem usually was come when the rainfall was high, which caused damaged bed shape and disturbed roots and plants during pineapple growth. The pineapple was planted in lowered beds single rows in this research to get a better setting plant (Fig. 1B). Farming under such soil conditions involves many obstacles. One of the major weaknesses of this variety was its high susceptibility to diseases caused by the pathogen Phytophthora in particular (Anderson *et al.* 2012). The problem of soil-borne diseases cannot be avoided (Silva *et al.* 2019). The incidence may severer when MD2 pineapple was produced economically and planted in a single lowered bed.

Phytophthora is a soil-borne pathogen that cause diseases in many crops worldwide (Green and Nelson 2015). The inoculums can survive in the soil for several years by making a resistant body called *Chlamydospore* (Joy and

Sindhu 2012). There are many species of genus *Phytophthora* all over the earth, and some species can cause disease to more than 100 different plant species (Drenth and Guest 2004). Pineapple heart rot is caused by *Phytophthora nicotianae, P. cinnamomi*, and *Pythium arrhenomanes* (Bartholomew *et al.* 2003) but the most common in the tropical region is *P. nicotianae* and *P. cinnamomi*. *P. nicotianae* is known to cause heart rot disease only, while *P. cinnamomi* can produces heart and root rot in pineapple (Kennet 1993; Anderson *et al.* 2012). As a soil-borne pathogen, *P. nicotianae* remains the most destructive plant pathogen with a broad range of hosts and habitats (Panabières *et al.* 2016), that can be found indigenously in many kind environments, such as forest soil (Jung *et al.* 2000), even mountainous areas (Vettraino *et al.* 2009).

Most of the areas planted with pineapples at GGP have been continuously planted in rotation with bananas. The pineapple-banana rotation has been effectively suppressed the incidence of Panama disease *(Fusarium oxysporum* f. spp. *cubense)* in bananas (Wang *et al.* 2015). The optimum soil pH for growing pineapples is 4.7 to 5.5 (Uchida and Hue 2000), while the growing of bananas is in the range of 5.0 to 7.5 (Weinert and Simpson 2016). The soil must be limed with dolomite to raise the soil pH and increase the soil calcium and magnesium contents to meet the needs of the banana plants. However, when the soil pH is greater than 5.5 for pineapple, it must be reduced to minimize disease risk, and soil-applied sulfur becomes an alternative. Soil property will determine what strategy is most appropriate to control the disease integrally. For example, when the soil organic content (C-organic) was low, applied compost is necessary to stabilized soil structure. Organic amendments reduce the disease incidence of Phytophthora due to a decrease in soil microbial activity and functional diversity (Zofio *et al.* 2010).

The control of soil-borne pathogens is usually carried out through chemical disinfestations (Mihajlovic *et al.* 2017; Panth *et al.* 2020). To suppress the pathogen, farmers usually applied fungicide by dipping the seed materials in a fungicide suspension before planting (Radmer *et al.* 2017). However, the heavy use of chemical disinfestations has hazardous effects on the environment and human health when used for long periods (Aktar *et al.* 2009). Recent studies also reported the emergence of fungicide-resistant *P. nicotianae* among the natural population (Panabières *et al.* 2016). Therefore, a strategy needs to be developed to minimize it by exploring disease, pathogen, and soil properties typically found in humid tropical climates. The species of Phytophthora must be identified correctly in order to be controlled effectively because different species may be inactive and thrive in different soil environmental conditions. For example, *P. nicotianae* is relatively inactive in soils below pH 4.7, while *P. cinnamomi* is inactive in soil below pH 4.0.

The incidence of the disease depends on the susceptibility of the pineapple (host plant), soil environment, and the number and virulence of Phytophthora as a pathogen (Pagán and Garcia-Arenal 2018; Velásquez *et al.* 2018). Nevertheless, no studies have been conducted to identify the species of genus *Phytophthora* which attacked MD2 pineapples in ultisol soil at the GGP plantation and Indonesia commonly.

The objectives of this study were (1) to confirm the species of *Phytophthora* which attacked pineapple in the GGP field, (2) to identify the soil properties related to the disease incidence, and (3) to examine the soil-applied sulfur in decreasing soil pH. The hypothesis of this experiment was *P. nicotianae* is more dominant in the soil, soil properties related to the disease incidence closely, and certain dose of sulfur possibly to decrease soil pH when it needed to suppress the pathogen.

The conformity of the species pathogen, the relation of soil properties to the disease incidence, and the examination of soil-applied sulfur to decrease soil pH, as they relate to the disease, are reported in the present study. It is believed that this important data will hopefully contribute to the development of integrated disease management not only for GGP but for all farmers, especially for pineapple planted in low soil fertility of Ultisol soil under humid tropical climates all over the earth.

## Materials and Methods

To utilized the GGP plantation, 32,000 ha of the field was divided into blocks (10–15 ha), consisting of several plots (0.7–1.2 ha). Smooth Cayenne cultivar MD2 was planted in a single-row lowered bed at a density of 66,668 plants $ha^{-1}$. The width between the rows was 60 cm, and the distance between the plants in each row was 25 cm. Secondary ditches of 1.2 m in width and 0.6 m in depth were excavated along the plot area, and tertiary ditches of 0.4 m in width and 0.3 m in depth were excavated across the plot area to manage the soil drainage.

### Soil properties

**Soil physical properties of infested and healthy soils:** The soil properties of both from the non-infected plants (healthy soil) and infected plants (infested soil) were observed at eight different block locations (409 G, 409 G1, 411 G, 411 K2, 415 V, 419 G, 419 H and 420 M1), five sample points for each of the blocks were measured from June–July 2020. The soil texture was determined with the hydrometer method. The soil compactness was measured employing the method of Yergeau and Obropta (2013), with a Dickey John penetrometer. The bulk density (*BD)* was measured by the core method implemented by Al-Shammary *et al.* (2018). A pycnometer analyzed the particle density (PD). The soil porosity (f) was calculated based on the bulk density and particle density data ($f = (1 - \frac{BD}{PD}) \times 100\%$) and a mini disk infiltrometer measured the soil infiltration rate.

**Soil chemical properties of infested and healthy soils:**

The soil pH in the water was measured by a metler toledo pH meter. The Walkey and Black method determined the C-organic content, using a similar procedure with Jha *et al.* (2014). Finally, Ca and Mg in the soil were extracted with neutralized 1N acetic acid at pH 7 and analyzed by atomic absorption spectroscopy (AAS)-Gray Bartlett Charlton (GBC), similar to the method implemented by Cano-Reinoso *et al.* (2022).

**Sulfur treatment incubation test**

An incubation test was done to determine the effect of soil-applied sulfur in lowering soil pH in the open area by mixing soil with sulfur in black color polyethylene bag (polybag). The test was carried out from June 2020-August 2020 (with monthly rainfall were 294.5, 110.6 and 82.3 mm respectively for June, July and August). About 1,200 kg of soil was collected from the infested soil, mixed, and then homogenized before measuring the initial soil pH (pH 6.28). Amount of 15 kg soil was treated with coarse powder sulfur by mixing it in six treatment doses of 0, 7.5, 11.25, 15.0, 18.75 and 22.5 g equal to 0, 500, 750, 1000, 1250 and 1500 kg $ha^{-1}$; the samples were then placed in 14 poly bags, 20 cm x 40 cm in size, per treatment, in the open area. The soil pH was measured before treatments were applied, then at 4, 5, 6, 7 and 8 weeks after treatment. The soil pH was measured five times per poly bag for every observation time.

The soil physical and chemical properties (soil texture, infiltration rate, bulk density, particle density, porosity, soil pH, Ca content, Mg content, and C-organic) were observed at GGP Laboratory. Statistical examinations were conducted using Minitab 16 software. The collected data were analyzed using the Two-Sample T-test of difference at $P < 0.05$.

**Conformity of *Phytophthora* spp.**

The isolates suspected to be associated with heart rot disease in pineapple were collected from the GGP plantation. The Phytophthora was isolated from infected plant tissues on selective NARM media (Morita and Tojo 2007). The media contained two antibiotics, namely Ampicillin and Rifampicin, to suppress the bacteria contamination and two antifungals, namely Nystatin and Miconazole, to prevent the growth of yeast and other unwanted fungi. The isolates suspected of heart rot disease in pineapple were collected from the GGP plantation. The infected plant tissue was first surface sterilized using alcohol and directly put onto NARM media. After four days, single mycelia were then transferred to corn meal agar (CMA) for purification. The pure culture then grown in the V8 agar to enhance mycelial growth. For DNA extraction, a small loopful of mycelia into 100 $\mu$L PrepMan Ultra Reagent (Applied Biosystem) and follow the procedure from the manufacturer.

To obtain the identity of the species, the DNA was identified at molecular level by sequencing of the cytochrome c oxidase 1 (COX1) gene (Robideau *et al.* 2011). The COI genes were amplified by PCR using primers OomCoxl-Levup (5'-TCAWCMWGATGGCTTTTTTCAAC-3') and Fm85mod (5'-RRHWACKTGACTDATRATACCAAA-3') modified from Martin and Tooley (2003). The 25-$\mu$ reaction mixtures contained 1 $\mu$ DNA, 2 $\mu$ of each primer, 0.4 mg $mL^{-1}$ BSA, 0.4 m*M* dNTPs, 0.125 U of TaKaRa Taq DNA polymerase (Takara Bio, Kusatsu, Japan), and PCR buffer (10 m*M* Tris-HCl, pH 8.3, 50 m*M* KCl and 1.5 m*M* $MgCl_2$). The PCR reactions were carried out in a T100 DNA Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The amplification condition were: 94°C for 2 min followed by 35 cycles of 94°C for 1 min, 55°C for 30 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. All PCR products were checked for successful amplification by electrophoresis in 2% (w/v) agarose gels (TAKARA L03 agarose, Takara Bio). The PCR products were purified using the ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific). Sequencing was performed using the BigDye Terminator v. 3.1 cycle sequencing kit (Thermo Fisher Scientific) and the manufacturer's instructions. The sequencing products were purified by ethanol precipitation and analyzed using an ABI 3100 DNA sequencer (Thermo Fisher Scientific). The sequences were then edited using Bioedit. Similar to the method implemented by Afandi *et al.* (2021). The obtained sequences were checked for similarity to other nucleotide sequences deposited in the NCBI database (www.ncbi.nlm.nih.gov) using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The DNA extraction was done at Biotechnology Research Center, Universitas Gadjah Mada, Indonesia, whereas the microsatellite amplification was done at Oomycete Research Laboratory, River Basin Research Center, Gifu University, Japan.

## Results

**Soil properties**

**Soil physical properties of infested and healthy soils:** The result shows that the soil textures in the pineapple field were sandy clay loam in the infested soil, with an average composition of 64.9 ± 4.22% sand, 12.8 ± 3.80% silt, and 22.4 ± 3.35% clay; and sandy loam in the healthy soil, with an average composition of 68.2 ± 5.71% sand, 11.9 ± 3.07% silt, and 19.9 ± 5.08% clay. There was consistently no difference in soil textures between the infested and healthy soils in the same block area, but the infested soil contained significantly more clay and fewer sand particles than the healthy soil (Table 2).

The penetrograph profile of each location is shown in

**Table 1:** Result of sequence similarity check using BLAST

| Description | Ident | Accession |
|---|---|---|
| *Phytophthora nicotianae* voucher P6915 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 98% | HQ261377.1 |
| *Phytophthora nicotianae* voucher P10381 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 98% | HQ261378.1 |
| *Phytophthora nicotianae* voucher P10297 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial | 98% | HQ261379.1 |

**Table 2:** Soil physical properties (texture, compactness and infiltration rate) of infested soil compared to healthy soil

| Treatments | Soil texture | | | Soil compactness | | | Infiltration rate (cm hour$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | Sand (%) | Silt (%) | Clay (%) | 0 -20 cm (kPa) | 20-40 cm (kPa) | 40-60 cm (kPa) | |
| Infested soil | 64.87 ± 4.22 a | 12.76 ± 3.80 a | 22.37 ± 3.35 a | 556.8 ± 68.1 a | 1097.0 ± 174.0 a | 1207.2 ± 16.6 a | 6.22 ± 3.91 a |
| Healthy soil | 68.23 ± 5.71b | 11.92 ± 3.07 a | 19.85 ± 5.68 b | 446.2 ± 66.2 b | 991.0 ± 107.0 b | 1132.0 ± 16.5 b | 8.81 ± 5.46 a |
| *P*-value | 0.04 | 0.28 | 0.02 | 0.00 | 0.03 | 0.00 | 0.09 |

Mean of 8 blocks, 5 spots measurement per block. Values within a column followed by the same letter are not significantly different ($P < 0.05$) according to T-test of difference

**Table 3:** Soil physical properties (bulk density, particle density and porosity) of infested soil compared to healthy soil

| Treatments | Bulk density (g cm$^3$) | Particle density (g cm$^3$) | Porosity (%) |
|---|---|---|---|
| Infested soil | 1410 ± 0.15 a | 2283 + 0.08 a | 38.24 + 6.41 a |
| Healthy soil | 1364 ± 0.17 a | 2271 + 0.05 a | 39.94 + 7.23 a |
| *P*-value | 0.24 | 0.43 | 0.27 |

Mean of 8 blocks, 5 spots measurement per block. Values within a column followed by the same letter are not significantly different ($P < 0.05$) according to T-test of difference

**Table 4:** Soil chemical properties (pH, Ca, Mg and C-organic) of infested soil compared to healthy soil

| Treatment | Soil pH | Ca (ppm) | Mg (ppm) | C-organic (%) |
|---|---|---|---|---|
| Infested soil | 6.6 ± 0.7 a | 824 ± 391 a | 271 ± 131 a | 1.01 ± 0.36 a |
| Healthy soil | 6.0 ± 1.1 b | 734 ± 440 a | 210 ±143 b | 0.95 ± 0.14 a |
| *P*-value | 0.00 | 0.34 | 0.05 | 0.31 |

Mean of 8 blocks, 5 spots measurement per block. Values within a column followed by the same letter are not significantly different ($P < 0.05$) according to T-test of difference

Fig. 4. The soil compactness at depths of 0–20 cm, 20–40 cm, and 40–60 cm was significantly different between the infested soil and healthy soil, as shown in Table 2. The values of compactness for the infested soil were higher than those for the healthy soil at most depths; the average difference between the two areas was 111 kPa at a depth of 0–20 cm, 104 kPa at a depth of 20–40 cm, and 75 kPa at a depth of 40–60 cm. The water infiltration rate into the infested soil was 6.22 cm h$^{-1}$ ± 3.91 cm h$^{-1}$ not significantly lower than that in healthy soil 8.81 cm h$^{-1}$ ± 5.46 cm h$^{-1}$ (Table 2).

These observations showed that the average bulk density (BD) in the infested soil was 1.41 g cm$^{-3}$ ± 0.15 g cm$^{-3}$, not significantly higher than the BD in the healthy soil 1.36 g cm$^{-3}$ ± 0.17 g cm$^{-3}$. The value of soil particle density (PD) in the infested soil (2.28 g cm$^{-3}$ ± 0.08 g cm$^{-3}$) and healthy soil (2.27 g cm$^{-3}$ ± 0.05 g cm$^{-3}$) were relatively low compared to normal PD, which was around 2.65 (Table 3).

**Soil chemical properties of infested and healthy soils:** The study shows that the average soil pH in the infested soil, where *P. nicotianae* heart rot disease symptoms were found was 6.6 ± 0.7, higher and significantly different from the soil pH value of 6.0 ± 0.1 in the healthy soil (Table 4). The average soil calcium (Ca) content in the infested soil was 824 ppm ± 391 ppm, not significantly different compare to 734 ppm ± 440 ppm of healthy soil. There was a significant difference between the soil Mg content in the infested soil (271 ppm ± 131 ppm) and healthy soil (210 ppm ± 143 ppm). C-organic content in the infested soil 1.01 ± 0.36% does not significantly differ from C-organic content in healthy soil (0.95 ± 0.14%).

**Incubation test of sulfur**

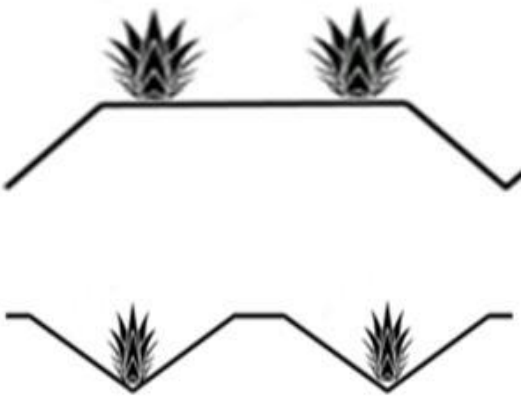
The incubation test result indicated the changes in soil pH value after sulfur application at various doses (Fig. 5). Soil applied sulfur equal to a dose of 500 kg ha$^{-1}$ was enough to lower the soil pH from 6.3 to 5.1–5.4; more than 750 kg ha$^{-1}$ such a soil pH is too low and not ideal for pineapple growth and production. Also, in the graphic it is possible to observe that all the sulfur doses administrated decrease the soil pH value drastically to below 4 in the week 4 before increase back to stable soil pH value. The soil pH showed continuous declines to below 4 at week 8 when the soil was treated by 1250 – 1500 kg ha$^{-1}$.

**Isolation of *Phytophthora spp.***

The isolates were collected from diseased pineapple tissue (Fig. 2). The pure culture was observed morphologically under inverted microscope for it sexual and asexual structure (Fig. 3). The DNA was extracted from the mycelia tissue and amplified the CO1 gene prior to sequencing. Identification by BLAST search again worldwide database showed identity with *Phytophthora nicotianae* reference sequence (Table 1).

## Discussion

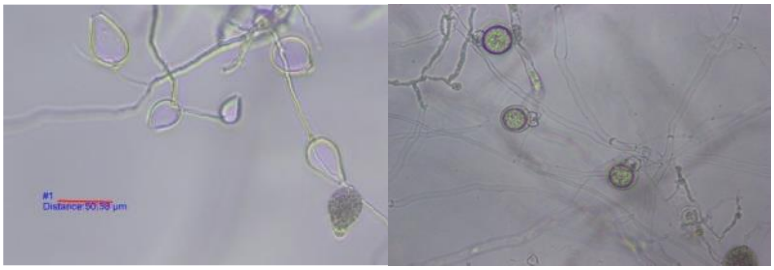
The growth of pineapples is determined, among others, by the soil environment's physical state. Meanwhile, the



**A**

B

**Fig. 1: A**. Double-row raised bed with a bed width of 1.2 m; **B**. Single-row lowered bed with a bed width of 0.6 m



A B

**Fig. 2: A**. Sporangia (asexual) of *Phytophthora nicotianae*; **B**. Oogonia and antheridia (sexual) of *Phytophthora nicotianae* observed under inverted microscope with 40x magnification

A B

**Fig. 3:** Different views of a pineapple heart rot infection (*Phytophthora nicotianae*) symptoms in the field. **A**. Wide view; **B**. Closest view. The symptoms are described as soft rotting of the basal white tissues of the youngest leaves

virulence and survival of pathogens are also determined by the soil environment. It is shown in this study that the healthy soil contained more sand and fewer clay particles and less compactness than the infested soil significantly. Soil compactness in the infested soil was higher than healthy soil significantly at all soil depths observed (Table. 2). *Phytophthora* species could be found over different soil textures of sandy loam, loamy silty, or clayey soils (Jung *et al.* 2000; Jönsson *et al.* 2005; Chepsergon *et al.* 2020). Other studies on flooded soils showed that *P. megasperma* could move upward through 65 mm of sandy loam soil but rarely move more than 24 mm upward through silt loam soil (Pfender 1977; Hansen 2015).

The density and porosity of soil determine the possibility of water binding, air movement, penetration of plant roots, etc. Infiltration rate, porosity, and bulk density were not significantly different between healthy and infested soil, but the infiltration rate in the healthy soil is 8.81 ± 5.46 cm $h^{-1}$ higher than 6.22 ± 3.91 cm $h^{-1}$ of infested soil. Soil porosity in the infested soil was 38.24 ± 6.41% lower than 39.94 ± 7.23% of healthy soil. More clays particle accumulation, higher soil compactness in the infested soil causes water infiltration through the eluviations horizon to be slow. It can cause temporary water logging during a heavy rainfall season. *Phytophthora* pathogens are soil inhabitants and require water for spore production and infection (Joy and

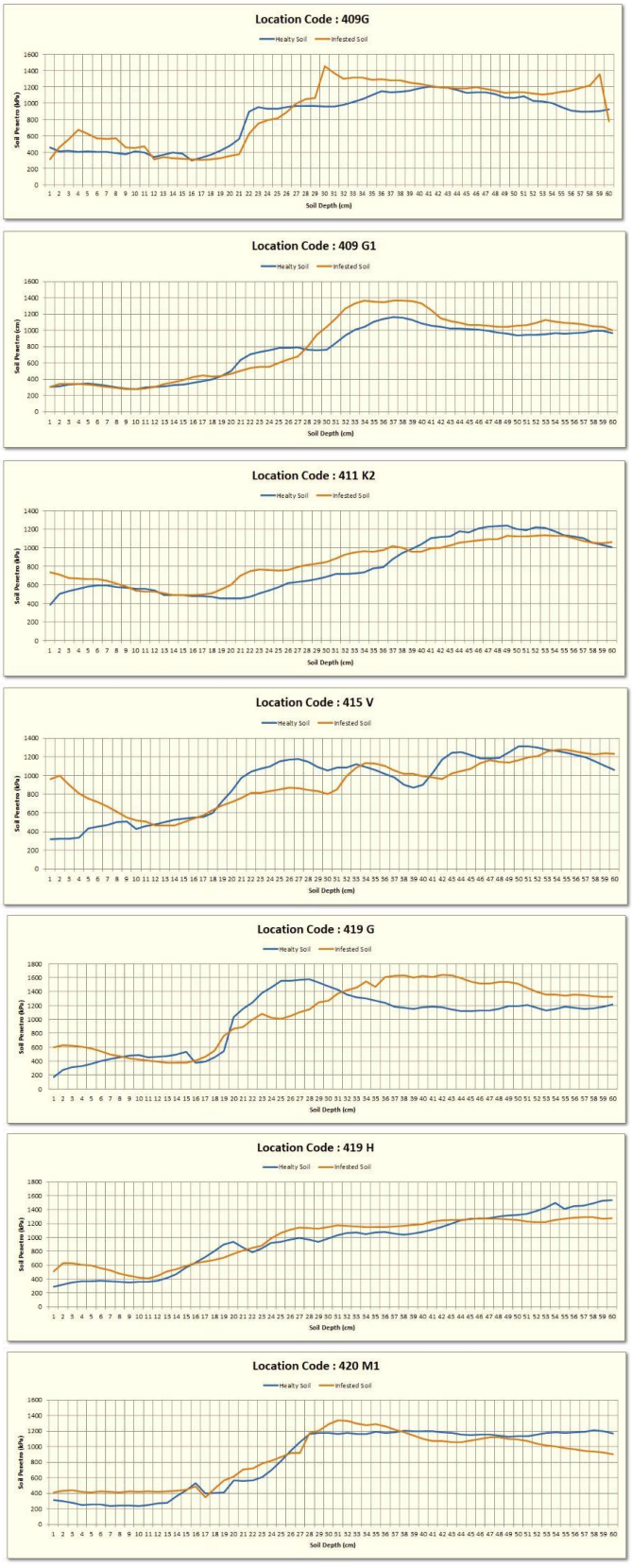



**Fig. 4:** Penetrograph of healthy soil and infested soil at different depths and locations

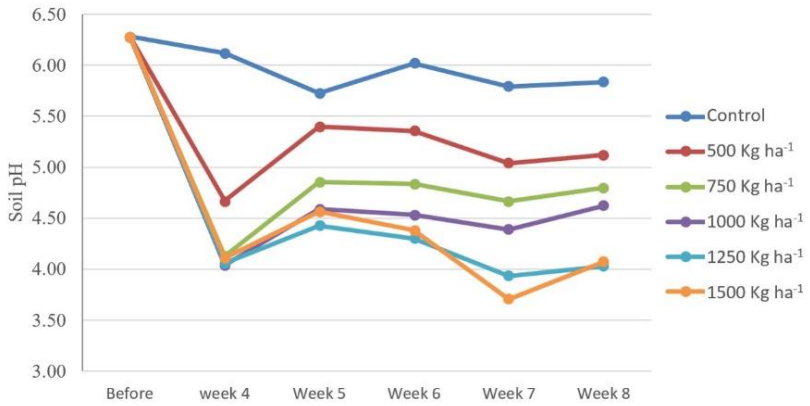



**Fig. 5:** The changes of soil pH values after sulfur application during the weeks of the experiment. Treatments: Control, 500, 750, 1000, 1250 and 1500 kg ha$^{-1}$

Sindhu 2012; Chepsergon *et al.* 2020). Sporangia are produced only in soil with water below field capacity (Reeves 1975; Sarker *et al.* 2015). *P. cinnamomi* thrives in water-saturated and cool soils and in poorly drained soils, while *P. nicotianae* is less dependent on free water to produce spores and infect pineapple (Green and Nelson 2015).

As regards changes in chemical properties of soil by infestation with fungus, pineapple heart rot and root rot diseases can occur when the soil pH rises above 5.5 (Frossard 1976; Sinclair *et al.* 1993; Green and Nelson 2015). The result showed that the average soil pH in invested soil was 6.6 ± 0.7, significantly higher than 6.0 ± 1.1 of healthy soil. *P. nicotianae* is relatively inactive in soils below pH 5.0, while *P cinnamomi* is inactive in soil below pH 4.0 (Kennet 1993; Chepsergon *et al.* 2020). When the soil pH reaches 5.5 and above, application of acidic fertilizer such as ammonium sulfate is considered instead of urea fertilizer. Even though pineapple growing stronger and healthier in soil with optimum soil pH and *Phytophthora* suppressed in low soil pH, but lowering soil pH at too low level is also not the right choice. Mineral $Al^{+3}$ can be dissolved and become toxic to plant growth (Chen and Lin 2010; Chen *et al.* 2020).

Soil Ca content is very high both in healthy soil and infested soil since the requirement standard for pineapple is 100 mg kg$^{-1}$, and Ca deficiency will appear in pineapple plants when the Ca content in the soil falls to below 25 mg kg$^{-1}$ (Malézieux and Bartholomew 2003; Vásquéz-Jiménez and Bartholomew 2018). The high Ca content in the soil was affected by applying a high dose of dolomite (7 ton ha$^{-1}$) massively during banana cultivation before pineapple. Unfortunately, soil amendment with dolomite lime, hydrated lime, and lime did not show inhibition activity against the sporangium formation of *P. parasitica* that could be done by gypsum (Tsao *et al.* 1986; Yeo *et al.* 2017). Ca ions may stimulate a compound known to be implicated in the defense mechanisms of plants, called a phytoalexin, as a result of fungal attacks (Zook *et al.* 1987; Edel *et al.* 2017).

There was a significant difference in this study between the soil Mg content in the infested soil and that in the healthy soil. All the soils had a high Mg content of more than 50 mg kg$^{-1}$ standard (Kelly 1993; Sembrayram *et al.* 2015) affected by the application of a massive high dose of dolomite (7 ton ha$^{-1}$) during banana cultivation before pineapple. The availability of Mg may vary depending on the environmental condition (especially soil pH), the previous crop, microbial activity in the rhizosphere, herbicide program for weed control, and ratios with other mineral nutrients, especially Ca, K, and Mn (Huber and Jones 2013). Mg ions induce the sporangia of *P. parasitica* to become nonfunctional or prevent the release of zoospores (Tsao *et al.* 1986; Huber and Jones 2013), suppressing *Phytophthora* by influencing how pathogens invade and colonize plant tissue (Nome *et al.* 2009; Huber and Jones 2013). When the Mg nutrient is sufficient during plant growth, the structural integrity of the middle lamella and the production of energy necessary for defense functions and the inactivation of pathogen metabolites will increase (Huber and Jones 2013).

This study showed that there was no significant difference in the soil C-organic contents. The level of C-organic contents both in the healthy and infested soils was low. Although pineapples tolerate low soil fertility, a high content of organic matter in the soil is desirable to obtain a high yield. Compost has also the potential to release inhibitors to suppress soil phytophatogenic agents and reduce the incidence of diseases (Reisinger *et al.* 1992). A negative relationship was found between increasing the decomposition level of the organic matter (compost age) and the population development of both the pathogen and the other microorganisms, as well as the incidence of disease (Chung *et al.* 1988; Blaya *et al.* 2016). The disease incidence was dominantly affected by the higher soil pH level rather than the C-organic content itself. The GGP produces compost mostly from their own cattle's dung mixed with other organic waste, with pH values of the manure compost product lying in the range of around 7–8.

The soil pH provides insight for increased yields of specific crops through nutrient recycling and availability, enhancing crop growth (Neina 2019). A pH level in the range of 4.7–5.5 must be maintained in the soil, as this level is better for growing pineapple with a lower risk of *Phytophthora* than higher soil pH levels.

Sulfur is generally used in problematic soils to use as soil regulator and decrease the soil pH. In soils, sulfur occurs in organic and inorganic forms, while organic sulfur compounds are largely immobile. Inorganic sulfur is more mobile and sulfate ($SO_4^{2-}$) is the most mobile (Scherer 2009). The incubation test result indicated that sulfur application equal to a dose of 500 kg $ha^{-1}$ was enough to lower the soil pH from 6.3 to 5.1–5.4, in the range optimum soil pH for growing pineapple 4.7–5.5 (Uchida and Howe 2000). Applying more than 750 kg $ha^{-1}$ led to a soil pH that is too low and not ideal for pineapple growth and production. The soil pH showed continuous decrease dropped to below 4.0 when sulfur was applied of 1250–1500 kg $ha^{-1}$. Lowering the soil pH is effective for controlling *Phytophthora*, but not ideal for pineapple growth and production.

The element sulfur must be oxidized to $SO_4^{2-}$ and $H^+$ by microbial action of autotrophic *Thiobacillus* spp. to acidify the soil and to provide available sulfur to plants (Turan *et al.* 2013). The microbial activity and microbial oxidation is dependent on many factors such as soil type, soil moisture and aeration, temperature, particle size, etc. The required dose of sulfur is very dependent on the soil texture. Sandy soil needs relatively little sulfur, whereas soil with a high clay content or organic matter requires much more sulfur. Previous research reported that the element sulfur required to lower the soil pH from 6.0 to 4.5 is 0.6 ton $ha^{-1}$ for sand, 1.7 ton $ha^{-1}$ for loam and 2.5 ton $ha^{-1}$ for clay (Hanson and Handcock 2011).

In tropical countries such as Vietnam and Thailand, the disease is majorly caused by *P. nicotianae* rather than *P. cinnamomi* (Sangchote *et al.* 2004; Drenth and Guest 2004; Thanh *et al.* 2004). The symptoms of the infection caused by *P. nicotianae* and *P. cinnamomi* are the same. The symptoms of the infection include soft rotting of the basal white tissues of the youngest leaves at the heart of the apical meristem. The infected leaves are easily pulled from the plant, and as the disease progress, sufficiently the plant die (Fig. 3). When the pineapple varieties more susceptible, the infection can move up through the peduncle and rot the fruit (Green and Nelson 2015).

It was confirmed that the pathogen found in the GGP plantation was suspected to be *Phytophthora nicotianae*, with a similarity rate of 98% (Fig. 2). *P. nicotianae* have arachnoid branching mycelium and non-cadoucus sporangia (Bush *et al.* 2006), amphigynous antheridia, oval or spherical and 9–10 × 10–12 $\mu$m in size, smooth and spherical oogonia with a diameter of 15–64 $\mu$m, 1–2 $\mu$m thick wall, and aplerotic oospores 13–35 $\mu$m in diameter (Waterhouse and Waterson 1964a; Waterhouse and Waterson 1964b).

## Conclusion

The species of *Phytophthora* which attacked pineapples at the GGP plantation was only *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur). Strategy to control these pathogens will be more effective when soil environments could be modified to be unfavorable for pathogens to grow and infect the pineapple. Soil with more clay, less sand particle, compact, low infiltration rate, high soil pH should be maintained to minimize pathogen growth. It should be mentioned that high Ca and Mg contents in the soil have the potential effect of minimizing the disease, as long as the soil pH level does not increase.

## Acknowledgments

The authors would like to express their gratitude to the United Graduate School of Agricultural Science (UGSAS), Gifu University, Japan for the support and funding provided for this research through the Ronpaku Program. Sincere thanks are also extended to the Management of PT. Great Giant Pineapple, for permitting, funding and supporting this study. Thanks are also extended to the R&D PT. GGP colleagues, Mr. Ziaurrahman and Mr. Diego.

## Conflicts of Interest

The author declares no conflicts of interest.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Funding Source

This research was funded by PT.Great Giant Pineapple (GGP) Lampung, Indonesia and a part of the research was supported by RONPAKU-Program of UGSAS-Gifu University, Japan.

## References

Afandi A, S Subandiyah, A Wibowo, A Hieno, Afandi, S Loekito, H Suga, K Kageyama (2021). Population genetics analysis of *Phytophthora nicotianae* associated with heart rot in pineapple revealed geneflow between population. *Biodivers J Biol Divers* 22:3342–3348

Al-Shammary AAG, AZ Kouzani, A Kaynak, SY Khoo, M Norton, W Gates (2018). Soil bulk density estimation method: A Review. *Pedosphere* 28:581–596

Aktar W, D Sengupta, A Chowdhury (2009). Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip Toxicol* 2:1–12

Anderson JM, KG Pegg, C Scott, A Drenth (2012). Phosphonate applied as a pre-plant dip controls *Phytophthora cinnamomi* root and heart rot in susceptible pineapple hybrids. *Aust Plant Pathol* 41:59–68

Bartholomew DP, RE Paull, KG Rohrbach (2003). *The Pineapple: Botany, Production, and Uses*. Department of Tropical Plant and Soil Science, CTAHR, University of Hawaii, USA

Blaya J, FC Marhuenda, JA Pascual, M Ros (2016). Microbiota characteristization of compost using omics approaches opens new perspectives for *Phytophthora* root rot control. *PLoS One* 11:1–19

Bush EA, EL Stromberg, C Hong, PA Richardson, P Kong (2006). Illustration of key morphological characteristics of *Phytophthora* species identified in Virginia nursery irrigation water. *Plant Health Progr* 7:1–11

Cano-Reinoso DM, K Kharisun, L Soesanto, C Wibowo (2022). Effect of calcium and silicon fertilization after flowering on pineapple mineral status and flesh translucency. *Plant Physiol Rep* 27:96–108

Chen J, J Gong, M Xu (2020). Implication of continuous and rotational cropping practices on soil bacterial communities in pineapple cultivation. *Eur J Soil Biol* 97:1–9

Chen J, Y Lin (2010). Effect of aluminium on variations in the proteins in pineapple roots. *Soil Sci Plant Nutr* 56:438–444

Chepsergon J, TE Motaung, D Bellieny-Rabelo, LN Moleleki (2020). Organic, don't agonize: Strategic success of *Phytophthora* species. *Microorganism* 8:1–21

Chung YR, HAJ Hoiting, WA Dick, LJ Herr (1988). Effects of organic matter decomposition level and cellulose amendment on the Inoculum potential of *Rhizoctonia solani* in hardwood bark media. *Phytopathology* 78:836–840

Drenth A, DI Guest (2004). Principles of *Phytophthora* disease management. *In: Diversity and Management of Phytophthora in Southeast Asia*, pp:154–160. Drent A, DI Guest (Eds) ACIAR, Melbourne, Australia

Edel KI, E Marchandier, C Brawnlee, J Kudla, AM Hetherington (2017). The evolution of Ca-based signaling in plants. *Curr Biol* 27:1–13

Frossard P (1976). Study of hearth rot of pineapple caused by *Phytophthora*, pH, calcium and fungicide treatments in the field. *Fruits* 31:617–621

Green J, S Nelson (2015). Heart and root rots of pineapple. *Plant Dis* 106:1–7

Hansen EM (2015). *Phytophthora* species emerging as pathogens of forest trees. *Curr For Rep* 1:1–12

Hanson E, J Hancock (2011). *Managing the Nutrition of Highbush Blueberries*, Michigan State University Extension. USA

Huber DM, JB Jones (2013). The role of magnesium in plant disease. *Plant Soil* 368:73–85

Jha P, AK Biswas, BL Lakaria, R Saha, M Singh, AS Rao (2014). Predicting total organic carbon content of soils from Walkley and Black analysis. *Commun Soil Sci Plant Anal* 45:713–725

Jönsson U, T Jung, K Sonesson, U Rosengren (2005). Relationships between health of *Quercus robur*, Ccurrence of *Phytophthora* species and site conditions in southern Sweden. *Plant Pathol* 54:502–511

Joy PP, G Sindhu (2012). *Diseases of Pineapple (Ananas comosus): Pathogen, Symptoms, Infections, Spread and Management*. Kerala Agricultural University, India

Jung T, H Blaschke, W Obwald (2000). Involvement of soilborne *Phytophthora* species in central European oak decline and the effect of site factors on the disease. *Plant Pathol* 49:706–716

Kelly DS (1993). Nutritional Disorder. *In: Pineapple Pest and Disorders*. Boradley RH, RC Wassman, ER Sinclair (Eds). Departement of Primary Industries, Brisbane

Kennet GP (1993). Diseases. *In: Pineapple Pest and Disorders*, pp:11–12. Boradley R, RC Wassman, ER Sinclair (Eds). Department of Primary Industries, Brisbane

Malézieux E, DP Bartholomew (2003). Plant nutrition. *In: The Pineapple: Botany, Production and Uses*, pp:143–166. Bartholomew DP, RE Paull, KG Rohbarch (Eds). CABI, Wallingford

Martin FN, PW Tooley (2003). Phylogenic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* 95:269–284

Mihajlovic M, E Rekanovic, J Hrustic, M Grahovac, B Tanovic (2017). Methods for management of soil-borne plant pathogens. *Pest Phytomed* 32:9–24

Morita Y, M Tojo (2007). Modifications of PARP medium using fluazinam, miconazole, and nystatin for detection of *Pythium* spp. in soil. *Plant Dis* 91:1591–1599

Neina D (2019). The role of soil pH in plant nutrition and soil remediation. *Appl Environ Soil Sci* 2019:1–9

Nome C, PC Magalhães, E Oliveira, S Nome (2009). Differences in intracellular localization of corn stunt spiroplasmas in magnesium treated maize. *Biocell* 33:133–136

Pagán I, F García-Arenal (2018). Tolerance to plant pathogens: Theory and experimental evidence. *Intl J Mol Sci* 19:810–826

Panabières F, S Gul, GS Ali, MB Allagni, JD Dalio, N Gudmested, ML Kuhn, SG Roy, L Schena, A Zambounis (2016). *Phytophthora nicotianae* disease worldwide: New knowledge of a long-recognised pathogen. *Phytopathol Mediterr* 55:20–40

Panth M, SC Hassler, F Baysal-Gurel (2020). Methods for management of soilborne diseases in crop production. *Agriculture* 10:1–16

Pfender WP (1977). Production of sporangia and release of zoospores by *Phytophthora megasperma* in Soil. *Phytopathology* 67:657–663

Radmer L, G Anderson, DM Malvick, JE Kurle, A Rendahl, A Malik (2017). *Pythium*, *Phytophthora*, and *Phytopythium* spp. Associated with soybean in Minnesota, their relative aggressiveness on soybean and corn, and their sensitivity to seed treatment fungicides. *Plant Dis* 101:62–72

Reeves RJ (1975). Behaviour of *Phytophthora cinnamomi* Rands in different soils and water regimes. *Soil Biol Biochem* 7:19–24

Reisinger O, S Durecu, F Toutain (1992). Effect of natural substances on plants: Biological control of telluric phytopathogenic fungi by an antifungal compost. *In: Humus, Its Structure and Role in Agriculture and Environment*, pp:145–153. Kubat J (Ed). Amsterdam, Netherland

Robideau GP, AWAMD Cock, MD Coffey, CA Lévacque (2011). DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Mol Ecol Res* 11:1002–1011

Sangchote S, S Poonlpolgul, S Poonpolgul, R Sdaodee, M Kanjanamaneesathian, T Baothong, P Lumyong (2004). *Phytophthora* diseases in Thailand. *In: Diversity and Management of Phytophthora in Southeast Asia*, pp:77–82. Drenth A, DI Guest (Eds). Australian Centre for International Agricultural Research, Melbourne, Australia

Sarker SR, J McComb, TI Burgess, GESJ Hardy (2021). Timing and abundance of sporangia production and zoospore release influences the recovery of different *Phytophthora* species by baiting. *Fung Biol* 125:477–484

Scherer HW (2009). Sulfur in soils. *J Plant Nutr Soil Sci* 172:326–359

Sembrayram M, A Gransee, V Wahle, H Thiel (2015). Role of magnesium fertilizers in agriculture: Plant-soil continnum. *Crop Pasture Sci* 66:1219–1229

Silva JMD, CCND Cristo, YC Montaldo, CDS Silva, EDOA Sena, RB Vigoderis, G Barroso, JSB Neto, JULD Oliveira, TMCD Santos (2019). Microbial activity and population of a red-yellow podzolic soil under organic and conventional cultivation systems of *Citrus sinensis* (L.) Osbeck. *Rev Ciênc Agrár* 42:340–346

Sinclair ER, RC Wassman III, RH Broadley, DES Kelly, KG Pegg, GK Waite, GR Stirling, DP Bartholomew (1993). Pineapple pests and disorders – A book for pineapple farmers. *In: Proceedings of the 1st International Pineapple Symposium*, pp:455–458, Honolulu, Hawaii, USA

Thanh DVT, NV Vien, A Drenth (2004). *Phytophthora* diseases in Vietnam *In: Diversity and Management of Phytophthora in Southeast Asia*, pp:83–89. Drenth A, DI Guest (Eds). Australian Centre for International Agricultural Research

Tsao PH, GC Draft, A Sztejnberg, Y Miyata (1986). Potting mixes for control *Phytophthora* root rot. University of California, Riverside. Schoolar.lib.vt.edu.ejournal.JARS

Turan MA, S Taban, AV Katkat, Z Kucukyumuk (2013). The evaluation of the elemental sulfur and gypsum effect on soil pH, EC, $SO_4$-S and available Mn content. *J Food Agric Environ* 10:572–575

Uchida R, NV Hue (2000). Soil acidity and liming. *In: Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical*, pp:101–112. Silva JA, RS Uchida (Eds). Collage of Tropical Agriculture and Human Resources, University of Hawaii, USA

Vásquéz-Jiménez, DP Bartholomew (2018). 8 Plant nutritions. *In: The Pineapple: Botany, Production and Uses*, 2nd ed, pp:175–202. Sanewski GM, DP Bartholomew, RE Paul (Eds). CABI Publishing, London

Velásquez AC, CDM Castroverde, SY He (2018). Plant–Pathogen Warfare under Changing Climate Conditions. *Curr Biol* 28:619–634

Vettraino AM, GS Lucero, PH Pizzuolo, A Vannini, S Franceschini (2009). First report of root rot and twigs wilting of olive trees in Argentina caused by *Phytophthora nicotianae*. *Plant Dis* 93:765–765

Wang B, L Rong, R Yunze, O Yannan (2015). Pineapple-banana rotation reduced the amount of *Fusarium oxysporum* more than maize-banana rotation mainly through modulating fungal communities. *Soil Biol Biochem* 86:77–86

Waterhouse GM, JM Waterson (1964a*). CMI Description of Pathogenic Fungi and Bacteria no 34 Phytophthora Nicotiana var*. Nicotiana. CABI, Wallingford, UK

Waterhouse GM, JM Waterson (1964b). *CMI Description of Pathogenic Fungi and Bacteria no 35 Phytophthora nicotiana var*. parasitica. CABI, Wallingford, UK

Weinert M, M Simpson (2016). *Sub Tropical Banana Nutrition– Matching Nutrition Requirements to Growth Demands*. New South Wales Department of Primary Industries. Wollongbar, Australia

Yergeau SE, CC Obropta (2013). Preliminary field evaluation of soil compaction in rain gardens. *J Environ Eng* 139:1–16

Yeo J, JE Weiland, DM Sullivan, DR Bryla (2017). Nonchemical, cultural management strategies to suppress *Phytophthora* root rot in northern highbush blueberry. *HortScience* 52:727–731

Zofio MN, S Larregla, C Garbisu (2010). Application of organic amendments followed by soil plastic mulching reduced the incidence of *Phytophthora capsici* in pepper crops under temperate climate. *J Crop Prot* 30:1563–1572

Zook MN, JS Rush, JA Kuć (1987). A role for $Ca^{2+}$ in the elicitation of rishitin and lubimin accumulation in potato tuber tissue. *Plant Physiol* 84:520–525