Effects of drought stress and plant growth-promoting rhizobacteria on sudden-death disease (*Cytospora euginae*) development of clove

F S Budi¹ and Widodo²

¹Graduate student of Phytopathology Program, Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, West Java 16680, Indonesia
²Department of Plant Protection, Faculty of Agriculture, IPB University, JL. Kamper, Kampus IPB Darmaga, Bogor, West Java, 16680, Indonesia.

Corresponding author email: widodo@apps.ipb.ac.id

Abstract. Recently, a sudden-death disease caused by *Cytospora euginae* has been reported in several major clove-producing areas in Indonesia. Previous surveys indicated that drought stress factors are suspected of having an essential role in disease predisposition. This study aimed to know the effect of drought stress and PGPR treatments on disease development. The experiment was arranged in two randomized block designs with two treatments, drought stress, and PGPR. Drought stress treatment consisted of three levels, i.e., W1 = control or without drought stress on 80% field capacity (FC), W2=60% FC, and W3=50% FC, while the PGPR treatment consisted of two levels P1=with PGPR and P2=without PGPR. The results showed that a single treatment of drought stress significantly affected all the indicators of disease development. Meanwhile, its interaction with PGPR treatment only considerably delayed the development of bark canker symptoms. Drought on 60% and 50% field capacity significantly shortened the disease incubation period at 14.4 and 9.7 DAI, increased the disease incidence until 100%, increased area under the disease progress curve (AUDPC) namely 505.8 and 572.2, and reduced plant defense response as indicated by a small number of callus formation namely 0.8 and 0.1 callus per plants.

Keywords: disease predisposition, PGPR, stem canker

1. Introduction
Clove (*Syzygium aromaticum* L. Merr. Perry) is an endemic spice plant from Indonesia, especially the Maluku Islands. The economic value of clove is high and is one of the spices with various benefits such as kitchen spices, medicinal and perfume ingredients, eugenol, and raw materials for the cigarette industry. Indonesia is the largest clove-producing country globally, with 134 792 tons produced in 2019 [1]. The existence of plant diseases in cloves threatens the sustainability of production. Several important plant diseases of clove, namely Sumatra disease, *Phyllosticta* leaf disease, leaf spot, and leaf and twig blight have been reported [2]

Recently, one of the clove diseases that attacks a large area in Indonesia is the sudden-death disease which is characterized by symptoms of leaf shedding, die-back, and stem canker. The disease incidence has been reported in three districts of Central Java and East Java; Semarang, Tegal, and Trenggalek, at 78%, 98.5%, and 57%, respectively [3]. The study also found the influence of biotic factors and cultivation methods on the development of disease incidence and severity. Based on the pathogenicity
test and the molecular identification, the primary pathogen of sudden disease is *Cytospora euginae* which is a non-aggressive pathogen and only cause severe disease when plant under environmental stress [3] [4].

Drought stress reduces plant fitness and increases the disease development. Schoeneweiss reported that plant under environmental stress conditions leads to predispose of stem canker by *Cytospora* [5]. Based on that, enhancing plant fitness is alternative management, considering that physical and chemical control is relatively difficult to manage plant diseases caused by *Cytospora* sp. [6] [7]. Plant Growth Promoting Rhizobacteria (PGPR) is an alternative biological control by utilizing living bacteria in the rhizosphere, which can trigger plant growth and increase production [8]. This study aims to determine the effect of PGPR and drought stress on the development of clove disease caused by *C. euginae*.

2. Methodology

2.1 Determination of field capacity (FC) and drought stress levels

Field capacity (% FC) was determined by the Alhricks method. A 500 ml beaker was filled with quartz sand as high as 2 cm, and then a gauze was placed on top to prevent the soil from sinking. After that, the plastic pipe was placed perpendicular to the surface of the quartz sand, then air-dried soil was placed as high as 3.5 cm into the beaker. Water was sprayed with a sprayer onto the soil surface, the water that wets the soil must be controlled, so it does not wet the quartz sand. The beaker was covered with plastic and incubated for 24 hours, then 50 g of soil samples were taken at a depth of 2.5 cm from the surface. The soil water content measurement method used is a modified gravimetric method [9]. The soil was dried at a temperature of 105 °C in an oven for 24 hours until the soil weight was constant, and then the absolute dry soil was weighed. The water content was calculated by the following formula:

\[ \text{Field Capacity (FC)} = \frac{\text{Initial weight of soil (g)} - \text{Absolute dry soil weight (g)}}{\text{Initial weight of soil (g)}} \times 100\% \]

Drought stress treatment consisted of three levels, i.e., W1 = control or without drought stress on 80% field capacity (FC), W2=60% FC, and W3=50% FC. Five plants in the preliminary test were first watered to field capacity conditions based on the previously measured % FC. The test plants were placed in a greenhouse, and the water requirements were kept until they reached a predetermined level of drought stress. The water content in the pots was measured every 24 hours using the same formula as the determination of %FC

\[ \text{Water content to be added} = (\%\text{FC}) - (\%\text{FC drought stress}) \]

The water condition was returned to the original field capacity water content, and the drought stress treatment was repeated until the research observations were completed. The volume of water added was calculated based on the difference between %FC and %FC drought stress multiplied by the average weight of the growing media in one pot. Table 1 shows the moisture content of the drought stress treatment, dry days, and added water requirements.

| Drought stress levels | Water content (%) | Time from FC (days) | Water volume to be added (ml) |
|-----------------------|-------------------|---------------------|-------------------------------|
| FC                    | 34.4              | -                   | -                             |
| 80% FC                | 28.3              | 2                   | 162                           |
| 60% FC                | 20.7              | 8                   | 364                           |
| 50% FC                | 17.8              | 10                  | 440                           |

FC = Field Capacity

2.2. Plants preparation

This test was carried out in an experimental field size of 7 m x 4 m, modified with the greenhouse plastic and paranet as a shade and buffer against rainwater splashes. One-year-old Zanzibar variety clove seeds
with a minimum height of 60 cm were planted in 20 cm x 15 cm polybags filled with soil and manure in 1:1 ratio, then acclimatized for four weeks and fertilized with NPK 16:16:16 given up to 3 g/plant.

2.3. Preparation and propagation of pathogen isolates
The pathogen isolate was obtained from the collection of the Laboratorium Klinik Tanaman of IPB University which was isolated from a plantation in Trenggalek Regency and was identified as *C. euginae* based on pathogenicity test and molecular identification. *C. euginae* was rejuvenated and propagated in PDA media at 25 °C under continuous light to stimulate sporulation and incubated until pycnidia containing conidia ready to be harvested [10].

2.4. PGPR treatment
The PGPR used is a flour formulation commercial product with active ingredients *Rhizobium* sp., *Bacillus polymixa* and *Pseudomonas flourescens*. PGPR treatment was carried out by pouring the suspension formulation (5 g/liter of water) once a day before inoculation and drought stress treatment. The mixture was poured at the base of the stem with the volume of PGPR pouring per plant according to the water needs that must be added to achieve the water capacity of the field conditions.

2.5. Pathogen inoculation
Pathogen inoculation was carried out before the plants received drought stress treatment on stems at the height of 8 cm, 16 cm, and 24 cm from the media surface. The stem of plants was penetrated with a sterile needle 15 times, then the bark and phloem tissue from the cuts were taken off. The cuts were disinfected with 70% ethanol and set aside for five minutes to evaporate. Afterwards, the cuts were inoculated with 7.5 mm diameter PDA pieces covered with *C. euginae* colonies. The inoculated cuts were covered with wet cotton to prevent dryness and contamination. The moisture of the cotton swab was maintained with sterile water for seven days after inoculation.

2.6. Experimental design and data analysis
The experiment was arranged in two randomized block designs with two treatments, drought stress, and PGPR. Randomized Block Design was used because of the different sunlight intensity in each replicate block. Drought stress treatment consisted of three levels, i.e., W1 = control or without drought stress on 80% field capacity (FC), W2=60% FC, and W3=50% FC, while the PGPR treatment consisted of two levels P1=with PGPR and P2=without PGPR. The experiment was carried out in three replications, with five plants per treatment unit. The indicators observed were the incubation period of the disease, incidence and severity of the disease. The calculation of disease severity is based on the percentage of the number of fallen leaves and or hanging wilts to the total number of leaves. The disease severity value is used to calculate the area under the disease progress curve (AUDPC) using the Simko and Piepho formula [11]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i)$$

*y*: severity of the disease in each observation,
*t*: time for each observation,
*n*: is the observation number.

Other indicators of disease development observed were the number of stem callus, the length of the bark canker, and the depth of stem canker necrosis. The effect of PGPR and drought stress on the development of sudden-death disease was analyzed for variance, and the treatment with a significant effect was further tested with the Tukey test at $\alpha =5\%$ using the XLSTAT 2018 software.
3. Result and discussion

3.1 Morphological characteristics of *C. euginae* and symptoms of sudden-death of cloves

The fungus *C. euginae* on PDA medium had white to yellowish-white colonies on the fourth day of incubation and changed colour to greenish-yellow on the sixth day (Figure 1). The mycelium is sparse, thin on PDA medium and microscopically septa hyaline hyphae. Pycnidia *C. euginae* were black, round to oval in shape, scattered separately or in groups of concentric circles on PDA media which began to grow on the sixth day and emerged on the surface of the media at 30 days of incubation. Small pycnidiospores are spherical and have a single cell, whereas perithecia were not formed in culture media.

![Figure 1](image1.png)

**Figure 1.** Morphology of *C. euginae* in the form of colonies on PDA medium (A) and conidia (B).

Symptoms of the sudden death of cloves caused by *C. euginae* begin with a change in colour of the leaves from dark green to copper, then the base of the leaves shrinks, and the leaves fall off quickly causing the plant to become bald. Some of the test plants experienced sudden wilting with leaves drying and hanging. Symptoms of necrosis in yellow spots develop at the point of inoculation and extend circularly on the plant stem (Figure 2). The bark colonized by pathogenic fungi will exfoliate and form a bark canker on the 49th day after inoculation, necrosis from the surface of the bark spread to the xylem tissue.

![Figure 2](image2.png)

**Figure 2.** Symptoms of *C. euginae* infection on the stems of clove plants at 53 days after inoculation (DAI) in the form of necrosis and peeling of the bark (A), the callus on the stem (B), and withering of the plant crown (C).

Symptoms of the sudden death of cloves caused by the pathogenic fungus *C. euginae* reported by Nutman and Sheffield [12] include massive green leaf loss, followed by sudden wilting of the remaining
leaves, drying of the remaining leaves on the tree, and the colour changes to copper. Another symptom found in the field on young plants is slow decline, which is a slowdown in growth that is characterized by progressively reduced the number and size of foliage as well as the lighter colour that lasts steadily and continuously. These symptoms are often found in plants on the soil that have previously been infected with sudden death. However, on the soil that was free from disease investment, and there are no symptoms of slow decline. Symptoms that appear are also similar to those reported by Schoeneweiss [13] that the wood tissue near the cambium tissue is extensively colonized by pathogens and will cause changes in the colour of the bark.

3.2 Effect of PGPR and drought stress on disease development
The summary of the results of a two-way analysis of the influence of PGPR and drought stress factors and the interaction of the two factors on indicators of disease development is shown in Table 2. The treatment of PGPR factors on disease development indicators was not significantly different except for the vertical length of the bark canker, while the drought stress factor had a significant effect except for the depth of necrosis. The interaction of PGPR factors and drought stress was significantly different only in the vertical and horizontal lengths of stem bark canker. Factors that showed a significant effect were further tested with the Tukey test (HSD) at $\alpha=5\%$.

| Response variable          | Treatment | W         | PGPR X W |
|----------------------------|-----------|-----------|-----------|
| Incubation period          | 0.629     | 0.0002$^b$| 0.673     |
| Disease incidence          | 0.621     | 0.0001$^b$| 0.640     |
| AUDPC value                | 0.833     | 0.0004$^b$| 0.782     |
| Total Callus               | 0.121     | $<0.000^b$| 0.670     |
| Bark canker horizontal length | 0.116   | 0.002$^b$ | 0.015$^a$|
| Bark canker vertical length | 0.033$^a$| 0.006$^b$ | 0.010$^a$|
| Necrotic depth             | 0.855     | 0.121     | 0.640     |

Number indicates based on Tukey Test
$^a$ significantly different at $P < 0.05$
$^b$ significantly different at $P < 0.01$

3.3 Incubation period
Drought stress treatment had no significant effect on the incubation period of the disease, while drought stress single had a significant effect on these components (Table 2). The two levels of drought stress (50% FC and 60% FC) can accelerate the incubation period of the disease and are significantly different from those without stress (80% FC). At 50% FC, the fastest incubation period was 9.7 DAI, followed by 60% FC and without stress, 14.4 DAI and 28.5 DAI, respectively.

| Treatment | Incubation period (days) |
|-----------|---------------------------|
| 80% FC    | 28.5 a                    |
| 60% FC    | 14.4 b                    |
| 50% FC    | 9.7 b                     |

*numbers followed by the same letter are not significantly different (Tukey test, $\alpha=0.05$)

The incubation period of the inoculated plants with drought stress was shorter, the higher the water stress level, the shorter the incubation period (Table 3). A short incubation period in the treatment with high levels of drought stress triggers the emergence of predisposing factors when the plant experiences
a weakened defence system, creating a state that is more susceptible to pathogens [14], whereas the incubation period is longer in conditions of sufficient water. This indicates that plants can carry out defense mechanisms against pathogenic infections.

3.4 Disease incidence and severity

The appearance of symptoms is indicated by the change of leaves colour to copper and experience loss. Disease development based on the observed incidence varies, and groundwater conditions have a strong influence (Figure 3).

![Figure 3. Progression of the incidence of sudden-death disease by C. euginae 6 to 49 days after inoculation. P1W1 = PGPR and moisture content of 80% FC (without stress), P1W2 = PGPR and water content of 60% FC, P1W3 = PGPR and water content of 50% FC, P2W1 = without PGPR and water content of 80% FC (without stress), P2W2 = without PGPR and water content of 60% FC, and P2W3 = without PGPR and water content of 50% FC.](image)

The incidence of disease reached 100% in the four treatment combinations PGPR+60% FC (P1W2), PGPR+50% FC (P1W3), without PGPR+60% FC (P2W2), and without PGPR+50% FC (P2W3). The incidence of the disease reached 100% in the fastest time, namely 21 DAI occurred in the 50% FC stress level treatment, both with and without PGPR treatment (P1W3 and P2W3), while the treatment with 60% FC stress level, both with and without PGPR (P1W2 and P2W3), P2W2) took a longer time to reach 100% incidence, occurring at 28 DAI and 35 DAI, respectively. Treatment without water stress (80% FC) with and without PGPR showed a lower incidence of disease compared to other treatments.

Drought stress was significantly able to increase the development of disease severity, while plants with healthy vigor had a lower severity. This shows that a plant with a good state of vigor can slow down the disease development after pathogens infections. The development of sudden death of the fungus C. euginae is influenced by environmental conditions such as temperature and humidity. According to Chen et al. [15], Cytospora sp. is able to grow in plant xylem tissue at a temperature range of 5 °C to 35 °C with an optimum temperature of 20 °C however, the differences in air humidity only had a small effect on pathogenic conidia infection in plant xylem tissue. The findings of this study also support the report of Schoeneweiss that drought stress is a major factor in the occurrence of canker-predisposing processes caused by the pathogenic fungus Cytospora. The requirement for the drought stress predisposition to increase the disease development by weak pathogens in perennial plants is they must exceed a certain drought threshold.
Drought stress treatment had a significant effect on the AUDPC value, while PGPR and its interaction with drought stress did not significantly affect the AUDPC value (Table 2). The stress treatment of 50% FC water content significantly showed the highest AUDPC value, while the lowest AUDPC value was in the treatment without water content stress of 80% FC.

**Table 4.** Area under disease progress curve (AUDPC) value on various PGPR treatments, drought stress, and interactions.

| Treatment               | AUDPC  |
|-------------------------|--------|
| PGPR                    | 399.3 a|
| Without PGPR            | 409.6 a|
| Water content           |        |
| 80% FC                  | 235.3 c|
| 60% FC                  | 405.8 b|
| 50% FC                  | 572.2 a|
| Interaction             |        |
| PGPR + 80% FC           | 206.1 b|
| PGPR + 60% FC           | 410.3 ab|
| PGPR + 50% FC           | 581.3 a|
| Without PGPR + 80% FC   | 264.5 b|
| Without PGPR + 60% FC   | 401.2 ab|
| Without PGPR + 50% FC   | 563.1 a|

*Numbers followed by the same letter in the same treatment column show no significant difference (Tukey test, α =0.05)*

The effect of PGPR on the AUDPC value tends to suppress drought stress on 80% FC moisture content compared to other treatments (Table 4). The mechanism of PGPR in suppressing the effect of drought stress was increasing antioxidant activity such as catalase enzyme production, modifying phytohormonal activity, and morphologically influencing plant absorptive root formation [16]. The researchers showed that *Pseudomonas* increased the activity of the catalase enzyme when plants were under water stress. It was the main enzyme to detoxify hydrogen peroxide, which is toxic to plants.

### 3.5 Callus number, bark canker length, and necrosis depth

The drought stress factor significantly affected the number of callus formed, while the PGPR factor and its interaction with drought stress did not significantly affect the number of callus (Table 2). Treatment without drought stress (80% FC) showed the highest callus occurrences, namely 2.43 callus per plant, while the lowest callus number was 0.10 in 50% moisture stress treatment (Table 5).

**Table 5.** The number of callus of clove stems in various drought stress treatments.

| Treatment | Callus number/plant |
|-----------|---------------------|
| 80% FC    | 2.4 a               |
| 60% FC    | 0.8 b               |
| 50% FC    | 0.1 c               |

*Numbers followed by the same letter are not significantly different (Tukey test, α =0.05)*

The highest number of callus was found in plants without dry stress or 80% FC. Drought stress causes the moisture in the bark to decrease until it reaches a critical point and allows a higher predisposition to pathogen attack [17]. Plants in four seasons countries will actively form callus in the spring when water resources and growth factors are maximally available, while in tropical climates, callus formation can occur throughout the year. The development of the largest canker size in the treatment with the highest
stress was 50% FC and 60% FC. Canker formation in bark cracks was triggered by extensive colonization of the *Cytospora* fungus in the bark tissue. Drought stress in plants causes an increase in sugar but a decrease of starch content in the bark tissue [18]. This is one of the causes for the different sizes of bark cankers that form.

The interaction of PGPR factors and drought stress showed a significant effect on the horizontal and vertical length of stem bark canker (Table 2). The 50% FC stress treatment without PGPR treatment showed the lowest stem bark canker length and was significantly different from the other treatments, namely 0.59 mm horizontally and 0.72 mm vertically (Table 6).

**Table 6.** Stem bark canker length on various treatments of PGPR interaction and drought stress.

| Treatment               | Horizontal length (mm) | Vertical length (mm) |
|-------------------------|------------------------|----------------------|
| PGPR + 80% FC          | 4.6 a                  | 5.4 a                |
| PGPR + 60% FC          | 4.0 a                  | 5.3 a                |
| PGPR + 50% FC          | 3.8 a                  | 5.2 a                |
| Without PGPR + 80% FC  | 5.5 a                  | 6.2 a                |
| Without PGPR + 60% FC  | 3.9 a                  | 4.7 a                |
| Without PGPR + 50% FC  | 0.6 b                  | 0.7 b                |

*Numbers followed by the same letter in the same treatment column show no significant difference (Tukey test, α = 0.05)*

Based on analysis of variance (Table 2), the effect of PGPR application, drought stress, and the interaction of these two factors on the depth of necrosis showed no significant effect. The development of necrosis in stem tissue tends if the higher the stress level of the drought level, the deeper the necrosis is formed (Table 7). Most of the necrosis in the 80% water content stress treatment stopped at the stem cortex tissue and did not reach the xylem vascular tissue, while the depth of necrosis at 60% FC and 50% FC moisture content could reach the vascular tissue causing the water supply and plant nutrients to be disrupted. So that the plant wilts, the crown withers, and dies.

**Table 7.** Depth of necrosis in various drought stress treatments.

| Treatment | Necrosis depth (mm) |
|-----------|---------------------|
| 80% FC    | 2.2 a               |
| 60% FC    | 3.3 a               |
| 50% FC    | 3.8 a               |

*Numbers followed by the same letter are not significantly different (Tukey test, α = 0.05)*

Callus formation on treatment without water stress 80% FC tend to have a lower depth of necrosis. From these results, it is suspected that there are a relation between the size of the bark canker with the number of callus formations and the depth of necrosis in the stem tissue. The larger the size of the bark canker in the treatment without moisture content stress (80% FC) tend to have a shallower depth of necrosis (Table 7), because in the treatment without drought stress, the plants were able to form a physical barrier network in the form of more callus (Table 5). Thus preventing necrosis into the vascular tissue and tends to only develop on the surface of the bark (Figure 4).
Figure 4. Cross-section of clove stem: necrosis is stopped by cork tissue, and at the edges of the necrosis side, there is the regeneration of healthy tissue (A), necrosis reaches xylem vessel tissue (B).

The condition of plant vigor is an important factor in the defence mechanism against biotic and abiotic stresses. The high-water content supports the formation of additional effective defence strategies against infection by canker pathogens (*Cytospora* sp.) with increasing water supply to the stem bark and maintaining cell turgor. The plant defence to suppress the rate of necrosis by *Cytospora* sp. is in the form of cork tissue used as a barrier between the healthy and diseased tissue, as well as to regenerate healthy tissue of the host plant at the edges of necrosis. When plants are subjected to drought stress, fewer resources are invested by plants in the process of preventing canker development, including the production of suberin and lignin as mechanical barriers, nonspecific wound healing, and the synthesis of secondary metabolites as defence compounds [19]. Infection of *Cytospora* sp. as in forest plants are also influenced by climatic conditions. Warm and dry temperatures will stress the host plant, thereby increasing its susceptibility to infection and canker growth caused by *Cytospora* sp [20]. Meanwhile, more humid conditions and sufficient water can reduce drought stress on the host, thereby slowing or stopping canker growth. The development and typical necrosis is clearly illustrated by Nutman.

4. Conclusion
Drought stress had a significant effect on the development of sudden-death disease caused by *Cytospora euginae*, while PGPR and its interaction with drought stress had no significant effect. Drought stress on 60% and 50% field capacity significantly shortened the disease incubation period at 14.4 and 9.7 DAI, increased the disease incidence until 100%, increased area under the disease progress curve (AUDPC) namely 505.8 and 572.2, and reduced plant defence response as indicated by small number of callus formation namely 0.8 and 0.1 callus per plants.

References
[1] Food and Agriculture Organization 2019 *Production quantities of Cloves by country* http://www.fao.org/faostat/en/#data/QCL/visualize
[2] Semangun H 2008 *Plantation plant diseases in Indonesia* (Yogyakarta: Gadjah Mada University Press) p 458
[3] Widodo, Supriyanto, Prasetyo G D, Triwidodo H 2020 *IOP Conf. Ser.: Earth Environ. Sci.* 418 012082
[4] Nutman F J, Roberts FM 1954 *Ann. Appl. Biol.* 41 23–44
[5] Schoeneweiss D F 1983 *Plant. Dis.* 65 308–14
[6] Peng H X, Wei X Y, Xiao Y X 2016 *Plant. Dis.* 100 884–9
[7] Zhang J X, Gu Y B, Chi F M, Ji Z R, Wu J Y, Dong Q L, Zhou Z S 2015 *Bio. Cont.* 8 1-7
[8] Ahemad M and Kibret M 2014 *J. King. Saud. Univ. of Sci.* 26 1–20
[9] Tan K H 2005 Soil Sampling, Preparation, and Analysis 2nd Ed Boca Raton: CRC Press Taylor and Francis Group p 96
[10] Zang R, Yin Z, Ke X, Wang X, Li Z, Kang Z, Huang L 2012 *Plant. Dis.* 96 1645-52
[11] Simko I, Piepho H P 2012 *Phytopathol.* 102 381-9.
[12] Nutman F J and Sheffield F M L 1949 *Ann. Appl. Biol.* 36 419-34
[13] Schoeneweiss D F 1981 *Plant. Dis.* 65 308-14
[14] Pandey P, Irulappan V, Bagavathiannan M V, Kumar M S 2017 *Plant. Sci.* 8 1-15
[15] Chen C, Li B H, Dong X L, Wang C X, Lian S, Liang W X 2016 *Plant. Dis.* 100 2 394-401
[16] Heidari M, Golpayegani A 2012 *J. Soc. Agr. Sci.* 11 57-61
[17] Bier J E 1964 *Phytopathol.,* 54 250-253
[18] Hodges J D Lorio P L 1969 *J. Bot.* 47 1651-7
[19] Richey J K R, Mulder C P H, Winton L M, Stanosz G 2010 *New Phytol.* 189 295-307
[20] Kliejunas J T 2011 *A Risk Assessment of Climate Change and the Impact of Forest Diseases on Forest Ecosystems in the Western United States and Canada.* (Albany: United States Departement of Agriculture)