A SERIOUS DISEASE OF *ELMERRILLIA* SEEDLINGS CAUSED BY *PHYTOPHTHORA* SPECIES

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The most economically important tree species in the Minahassa region, northern Sulawesi is *Elmerrillia tsiampacca*, a member of the *Magnoliaceae*, with the vernacular name "Wasian". *Elmerrillia tsiampacca* occurs also in Central Sulawesi, Sumatra, Borneo, Ambon, Buru, Irian Jaya and New Britain. The extensive use of Wasian in North Sulawesi has led to scarcity of the wood and may cause erosion of the gene pool. Many large- and small-scale nurseries have been established since 1990. Wasian thus has become a common crop for farmers in the Minahassa. A closely related species is *E. ovalis* which, in Sulawesi, is called "Cempaka". According to Lemmens *et al.* (1995) Cempaka has a more limited distribution and occurs only in Sulawesi and the Moluccas. Both species are fast growing and produce durable and expensive wood used, among others, for furniture, house construction and carving. Outside its natural geographic range *E. ovalis* has been planted in Java. Although producing equally valuable timber, *E. ovalis* is less popular because farmers say it often suffers from heart rot. The seeds of *E. tsiampacca* are collected from September to February. They are recalcitrant (i.e., during drying they lose viability) and therefore should be sown within one week after collection. The seedlings emerge two to three weeks after sowing. One month later they are transplanted into plastic containers and placed in the nursery. After six months they will have grown up to 50 cm height and may be planted in the field. In February 1997 a serious attack of seedlings occurred in nurseries in North Sulawesi. This paper described the occurrence and the causal agent of this hitherto unknown disease problem of *Elmerrillia*.

Observations of the disease development and the disease symptoms were carried out during two visits to Sulawesi, namely, in February and March 1997, covering 10 nurseries. Disease assessment was done by calculating the percentage of dead and diseased plants. During the visits diseased material was collected from two nurseries in the Kolongan and Kakaskawan village in the Minahassa region of North Sulawesi.

Diseased plants were examined microscopically at the Wanariset Samboja Research Station in East Kalimantan. From the material collected in February, 36 isolations were made on malt extract agar (MEA) (Oxoid) as follows: diseased plant material was thoroughly washed in demineralised water, followed by sterile water and finally blotted dry with sterile filter paper. Thereafter, thirty-six pieces of tissue (1 × 1 mm) from the edge of necrotic spots on leaves and stems were placed on MEA and incubated at 25 °C. Diseased leaf tissue was also inoculated into six carrot discs (previously surface heat sterilised by passing the discs through a flame) and then incubated at 25 °C in a Petri dish containing moist filter paper.

From the material collected in March, 60 isolations were made as described above on potato dextrose agar (PDA: 50 g of potatoes boiled and macerated in 500 ml of demineralised water, 1% glucose and 1.2% technical agar (Oxoid)). Six pieces of diseased leaves and stem tissues were inoculated into three apples, locally sold by the name "Red USA". After three

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days apple tissues from the edge of the necrosis were taken and placed on MEA plates. The species were identified by the Centraal Bureau voor Schimmelcultures, The Netherlands.

Four healthy seedlings of *E. tsiampacca* were inoculated by spraying the areal parts of the plants with a zoospore suspension obtained from a pure culture of cf. *P. botryosa*. Zoospores were obtained by cultivating the fungus on sterile apple pieces in Erlenmeijer flasks. Numerous sporangia developed on this medium and when the cultures were put in water at 5–10 °C the zoospores were released. After inoculation the plants were kept moist for 48 hours.

Re-isolations were made from the diseased leaf and stem tissue. The tissue was first disinfected in 3% H_2O_2 for 4 minutes, then rinsed in sterile water and blotted dry with sterile filter paper. Finally small pieces of necrotic tissue were placed on MEA plates.

The first visible symptoms of the disease were black spots, often starting at the cotyledons and spreading via the stem upwards and downwards. Black leaf spots were also observed on bigger leaves starting as small spots but finally covering the whole leaf, entering the stem through the petiole. In most cases this led to the death of the plant within three to five days.

The first occurrence of disease symptoms in nurseries was recorded in February 1997 at Kolongan village near Tomohon. This nursery had been used for the cultivation of *Elmerrillia* for four consecutive years, but this was the first year disease problems became evident. The nursery, located on a slope, was extremely wet and partly inundated due to an exceptional amount of rain during the two months preceding the appearance of the symptoms. Seedlings which were recently transferred into polythene bags were the most heavily attacked, first in one corner of the nursery and two weeks later, more than 80% of the 125 000 seedlings in this nursery were dead or showed disease symptoms. Four weeks after the first symptoms were detected, almost all the seedlings were dead. Seedlings up to 10 cm height were most susceptible to the attack. Plants older than three months were healthy or had only sporadic leaf spots. In this heavily infected nursery one-year-old plants about 1 m high showed sporadic spots on big leaves but the plants did not die. In three nurseries less than 1000 m away no symptoms were observed. In one other nursery 60% of the 100 000 seedlings were diseased or dead, most of them being in the downhill planting beds. Plantations of *Elmerrillia* were also visited but to date the disease had not been observed outside the nurseries.

On the abaxial surface of the symptomatic leaves, nearly invariably, sporangia of a *Phytophthora* species were present. Zoospores released from the sporangia were also observed. Fungus from the material collected in February could not be isolated on MEA. A number of fungi and bacteria were isolated, but not *Phytophthora* species. No growth was observed after one week in the carrot discs.

Isolations on PDA from the material collected in March 1997 all failed because of contamination with bacteria and unidentified fungi. However, the apple appeared to be an excellent isolation medium. Within three days a dark brown rot developed from all inoculation spots and when the apple tissue was taken from the border of the discoloured regions and placed on PDA, a white mycelium developed. Within four days these cultures produced mature sporangia of *Phytophthora* on the PDA. On the exposed surfaces of the decaying apples sporangia were produced in excess, and when they were placed in a drop of water and kept at 5 °C for 10 minutes zoospores were readily released. When decayed apple tissue was put in water in a Petri dish and left at ambient temperature for three days, many oospores were formed on the mycelium in the water. The isolations resulted in five pure cultures obtained from two different locations: Kakaskawan and Kolongan.

The isolates could not yet be identified with certainty; the fungus appeared to be closely related to *Phytophthora botryosa* Chee. *Phytophthora botryosa* was isolated from *Hevea brasiliensis* (Chee 1969).

Three days after inoculation, three of the four plants developed typical leaf spots starting at the tip of the leaf, and spreading through the petiole to the stem. Four days after inoculation the three plants were dead. In one plant the symptom started at the stem; this plant was dead within two days after the first symptoms appeared. Re-isolations from these plants resulted in pure cultures identical to the original isolates. Control plants showed no symptoms.

The disease symptoms in *Elmerrillia* observed in several nurseries in North Sulawesi in 1997 were caused by a *Phytophthora* species because the fungus was isolated from the diseased tissue. Furthermore, inoculation of healthy plants with pure cultures of the isolated fungus reproduced similar symptoms in the plants. From those plants the fungus was re-isolated, satisfying Koch's postulates. The observed disease symptoms were similar to those described for various other *Phytophthora* species (Erwin *et al.* 1989). We observed mass production of zoospores in pure cultures and on dead leaves in the nursery. These spores were likely the most important source of infection of plants in the nursery. This also explained the heavy infection in wet nurseries because zoospores need water for dispersion. Our literature research had not revealed any record of an attack of *Elmerrillia* by a *Phytophthora* species. A molecular genetic study including a number of related *Phytophthora* species for comparison may reveal that we are dealing with a hitherto undescribed species (de Cock, pers. comm.). Finally, the host range of this pathogen remained to be identified by cross inoculations.

Phytophthora species are fungi that are dispersed by water and persist in the soil for long periods. They spread by means of zoospores which are produced in the sporangia on dead plant material. To prevent attack by *Phytophthora* it is therefore recommended not to establish nurseries in areas with risk of inundation and to ensure good drainage in the nurseries. Plants showing symptoms must be removed immediately together with the soil around it and destroyed in order to prevent spread of the disease in the seedbeds. Heavily infected nurseries should not be used for the production of *Elmerrillia* seedlings for at least one year in order to decrease the inoculum potential.

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