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First report of stub dieback of poinsettia (*Euphorbia pulcherrima*) caused by *Sclerotinia sclerotiorum* in Vietnam

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Abstract Stub dieback of poinsettia caused by *Sclerotinia sclerotiorum* is reported from Vietnam for the first time. It was discovered in potted plants in a polyhouse in Dalat, Lam Dong Province, in December, 2010. The disease was scattered throughout the greenhouse, but the incidence was low. Infection appeared to have occurred though the cut surface of the stub, where the stem had been pruned to encourage branching. Large irregular black sclerotia were present on some diseased stems. There was no evidence of apothecia in the affected pots, or elsewhere in the greenhouse. Consequently it is postulated that infection was caused by ascospores that drifted into the polyhouse after release from apothecia in adjacent fields. Sclerotinia head rot of polyhouse, and field-grown cabbages and lettuce, is a serious problem in the Dalat area.

Keywords *Sclerotinia sclerotiorum* · Poinsettia · *Euphorbia pulcherrima* · Vietnam

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Sclerotinia sclerotiorum is an important foliar pathogen affecting a wide range of vegetable, ornamental and field crops in many countries. It has previously been reported in Vietnam from cabbages, peanuts and other crops from a number of provinces (Burgess et al. 2008; Luong et al. 2010; Ly et al. 2002; Thanh 2008). Potted plants of a locally adapted cultivar of poinsettia (*Euphorbia pulcherrima*) with symptoms of stub dieback (Fig. 1) were discovered in a polyhouse in Dalat, Lam Dong province Vietnam, in December 2010. The fungus colonized progressively down the stem into other side branches and the crown, leading to death of the plants. Large irregular black sclerotia, typical of *S. sclerotiorum*, were present on severely diseased stems (Fig. 2). The disease was distributed throughout the polyhouse at a low incidence, but sufficient to cause concern economically given the value of potted plants. We found no evidence of apothecia in the affected pots or adjacent pots. We also did not observe discrete infections of intact stems or branches.

Pots of diseased plants were removed to the laboratory in Dalat. Diseased stems were then excised from the plants, washed in tap water, surface-sterilized in 70% ethyl alcohol for 30 s, immediately rinsed in sterile water and damp-dried on sterile paper tissue. Small sections from the margins of healthy and diseased stem tissue were plated on a one-quarter strength potato dextrose agar, emended with streptomycin sulphate (1.0 g/L) and neomycin sulphate (0.12 g/L). Fungal colonies that developed from the sections were sub-cultured to potato dextrose agar (PDA) (Burgess et al. 2008). All colonies recovered were typical of *S. sclerotiorum* and produced abundant irregular large black sclerotia on PDA. A representative colony was purified by transfer of a hyphal tip onto PDA (Burgess et al. 2008), and the pure culture accessioned as PPRI I305 in the culture collection of the Plant

Fig. 1 Advanced symptoms of stub dieback of poinsettia caused by *Sclerotinia sclerotiorum* in a polyhouse in Dalat, Vietnam

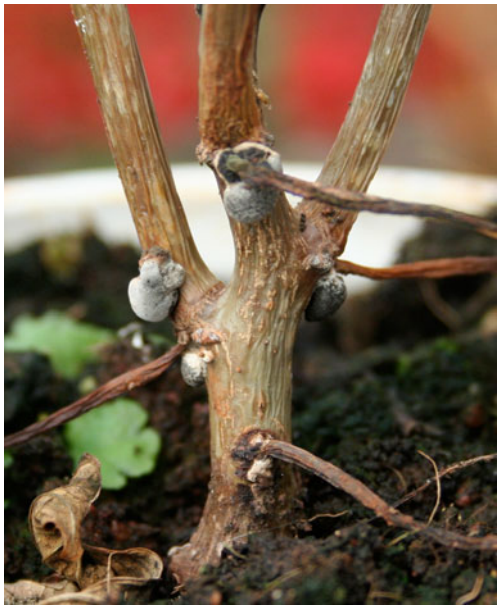

Fig. 2 Large irregular white to black sclerotia of *Sclerotinia sclerotiorum* in poinsettia severely affected by stub dieback, in a polyhouse in Dalat, Vietnam

Protection Research Institute (PPRI) in Hanoi. Two specimens of diseased plants were deposited in the Plant Disease Herbarium at PPRI under accession numbers PPRI 697 and PPRI 698.

Culture PPRI 1305 was forwarded to the CBS-KNAW Fungal Biodiversity Centre, Utrecht the Netherlands (CBS), for confirmation of identification and accessioning (PPRI305 = CPC 19039 = CBS 131900). Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog and Gerrits van den Ende 1998) and LR5 (Vilgalys and Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3′ end of the 18S nrRNA gene, the first internal transcribed spacer (ITS1), the 5.8S nrRNA gene, the second ITS region and the 5′ end of the 28S nrRNA gene. The primer ITS4 (White et al. 1990) was used as internal sequence primer to ensure good quality sequences over the entire length of the ITS region. A megablast search of the NCBI's GenBank nucleotide database using the ITS sequence generated in this study (GenBank JQ618848) revealed 100% identity over 539 nucleotides to publicly available sequences of *Sclerotinia sclerotiorum* (e.g. GenBank JN013184, HQ833450 and EF091809).

Culture PPRI 1305 was used to inoculate the stubs of two newly cut stems of each of three plants of the same cultivar of poinsettia as affected by the disease in the polyhouse, to prove pathogenicity. A small block (2 mm wide) was removed from the margin of a colony on water agar (WA) and placed on the freshly cut surface of the stub. Three control plants were similarly treated except they were inoculated with small blocks of sterile WA. Each potted inoculated plant was misted with water and then incubated in a pre-misted plastic bag for 4 weeks in diffuse daylight at room temperature that varied from approximately 10 to 16°C each day. Typical stub dieback symptoms as observed in the polyhouse were reproduced, and the pathogen formed typical sclerotia of *S. sclerotiorum* on the diseased tissue. Symptoms were not observed on the control plants. The fungus was re-isolated from the inoculated plants into pure culture, fulfilling the requirements for Koch's postulates.

Flower and vegetable crops are intensively cultivated in the Dalat area for domestic consumption in Vietnam, and for export. Flower crops are grown mainly in polyhouses while the vegetable crops are grown mainly in small-holder field plots, often adjacent to polyhouses. Cabbages are a major crop grown mainly in small plots, occasionally in polyhouses. We have regularly observed serious losses from

Sclerotinia head rot in cabbages in small plots, and in ground beds in polyhouses in Dalat. We have also observed the disease in this area in other crops such as field grown lettuce, and occasionally on *Lisianthus* in polyhouses (authors' unpublished data). There was no evidence of apothecia in the affected pots of poinsettia, or elsewhere in the polyhouse. Consequently it is postulated that infection was caused by ascospores that drifted into the polyhouse after release from apothecia in adjacent field plots where cabbages are grown. The preceding cool wet and humid weather would have provided ideal conditions for production of apothecia and release of ascospores (Abawi and Grogan 1979). We concluded that the disease was a consequence of opportunistic infection of cut branches, as there was no evidence of infection of intact plant parts. It was recommended that cabbages not be grown in close proximity to the polyhouses to minimise the risk of infection, given the high value of the poinsettias, and that a protectant fungicide be applied after pruning if stub dieback reoccurs.

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