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Phytophthora stem rot of purple passionfruit in Vietnam

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Abstract In August 2012 a severe stem rot (stem canker) disease was observed in a purple-fruit variety of passionfruit, *Passiflora edulis*, in Nghe An province, Vietnam. The stem rot progressed rapidly along the stem affecting branches and fruit, leading to chlorosis, wilting and death of the distal part of the stem. It did not progress below the graft junction into the stem or roots of the rootstock, *Passiflora edulis var. flavicarpa*. The disease spread within and between plantings causing 100 % loss of some plantings. *Phytophthora nicotianae* was consistently isolated from diseased tissue and the morphological identification was confirmed by sequencing, and two cultures were deposited in the Murdoch University Culture Collection as MUCC707 and MUCC708. The former culture proved pathogenic in stem inoculations and *P. nicotianae* was reisolated fulfilling Koch's postulates. This report represents a new record of Phytophthora stem rot of purple passionfruit in Vietnam.

Keywords *Passiflora edulis* · Stem rot · *Phytophthora nicotianae*

Passionfruit production has been promoted in Que Phong district in the mountainous region of western Nghe An province in north-central Vietnam as part of a poverty alleviation project. A purple-fruit variety (*Passiflora edulis*) from Taiwan is being used as the scion and is grafted onto a local yellow fruit variety (*P. edulis var. flavicarpa*). *Phytophthora nicotianae* and *P. cinnamomi* have been reported as root and stem rot (stem canker) pathogens of passionfruit (*P. edulis*) (Young 1970; Cole et al. 1992; Amata et al. 2009). However the rootstock, *P.edulis* var. *flavicarpa*, is resistant to Phytophthora root rot.

In Vietnam, *Phytophthora nicotianae* has been reported previously as a pathogen of tobacco (NIPP 1975) and pineapple (Dang 2007), to be non-pathogenic to weakly pathogenic on black pepper (Truong et al. 2008), and has been isolated from citrus (Drenth unpublished, noted in Dang et al. 2004). Several surveys and pathogenicity studies have shown *P. nicotianae* to be the dominant *Phytophthora* species associated with heart rot of pineapple in the North Central provinces of Vietnam, together with *P. cinnamomi* (Dang 2007). Truong et al. (2008) conducted an extensive survey of the incidence of foot rot of black pepper in the central and south-east regions. They isolated 57 isolates of *P. capsici* and 2 isolates of *P. nicotianae* from the root samples. These reports indicate that *P. nicotianae* is distributed across a significant region of Vietnam.

In August 2012 a stem rot disease of grafted passionfruit was observed in small-holder farms in Tri Le commune in Que Phong district following the onset of the rainy season. The disease was characterized by a stem rot (stem canker) (Fig. 1) that progressed along the stem in both directions from the initial infection site and into branches. It did not progress below the graft junction and the stem and root system of the rootstock remained symptomless. The stem rot sometimes caused stem splitting (Fig. 2) as well as leaf chlorosis, wilting, leaf and fruit drop distal to (above) the stem rot (Fig. 3). The stem rot also progressed through side branches leading to fruit rot (Fig. 4). The incidence of the disease gradually increased within plantings and spread to other farms. In 2013, the

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Fig. 1 Purple passionfruit stem rot in main stem and branch

disease was observed in all small-holder farms in Tri Le commune, again following the onset of the rainy season. It was estimated to have caused the loss of 5 ha out of the 100 ha grown, based on an ad hoc survey with 100 % loss in some plantings (Fig. 5).

In December 2013 diseased stem and fruit samples were collected in Tri Le commune and forwarded to the Nghe An Plant Protection Sub-Department for determination of the pathogen. The nature of the symptoms and the observations that the disease developed in the wet season, indicated that a *Phytophthora* species was the putative pathogen. The isolation methods were adapted from Dau et al. (2008). Sections of the diseased stems each including a junction with symptomless tissue were removed, washed in tap water, then washed in sterile water, surface sterilized in 70 % ethyl alcohol for 5 s, rinsed again in sterile water and damp-dried on sterile paper tissue. A

Fig. 2 Purple passionfruit stem rot and associated stem cracking (*splitting*)

Fig. 3 Early symptoms of purple passionfruit stem rot (*indicated by arrow*), and including leaf chlorosis and wilting

thin transverse segment was removed from each sterile section at the junction of diseased and symptomless tissue and plated on a Phytophthora Selective Medium (PSM), and incubated at room temperature. The PSM was prepared from agar 12 g, carrot 20 g as puree, potato 20 g diced and strained, hymexazol 0.05 g, pimaricin 0.4 ml, rifampicin 0.05 g in 1 L of water. The colonies that developed from the segments were sub-cultured to potato carrot agar (PCA) (Burgess et al. 2008) and grown in the dark at room temperature prior to purifying by hyphal tipping. The pure cultures were putatively identified as *Phytophthora nicotianae* based on morphological characteristics as described below. Cultures isolated from diseased fruit were also identified morphologically as *P. nicotianae*.

Two cultures from diseased stem tissue were forwarded to the Centre of Phytophthora Science and Management (CPSM) at Murdoch University, Perth, Australia, for confirmation of identification based on morphological and molecular markers. Cultures were deposited in the Murdoch University culture collection as MUCC707 and MUCC708. DNA was extracted from pure cultures and the ITS and β-tubulin gene regions amplified and sequenced as described previously (Aghighi et al. 2012). The isolates were confirmed as *Phytophthora nicotianeae* based on 100 % identity of sequences to those available on GenBank, both isolates were

Fig. 4 Purple passionfruit fruit rot showing mycelium of *Phytophthora nicotianae* on the fruit surface

Fig. 5 Trellised purple passionfruit vines, all dead from stem rot caused by *Phytophthora nicotianae*

identical (ITS sequence of MUCC707 = KP663606). The sporangia were typically papillate and predominantly ovoid, 50–75 × 35-45um, and non-caducous (Fig. 6a,b). The morphology of sporangia was within the range of that reported for *P. nicotianeae* (Erwin and Ribeiro 1996). Corolloid hyphae were also observed (Fig. 6c).

The pathogenicity of MUCC707 to *P. edulis* was tested at the Plant Protection Sub-Department in Vinh City, Nghe An province using a stem inoculation technique similar to that described by Dau et al. (2008) for testing the pathogenicity of *P. palmivora* to *Telosma cordata*. Well-rooted young vines of *P. edulis* were established in natural sandy soil in small pots from cuttings from symptomless mother stock. A colony of

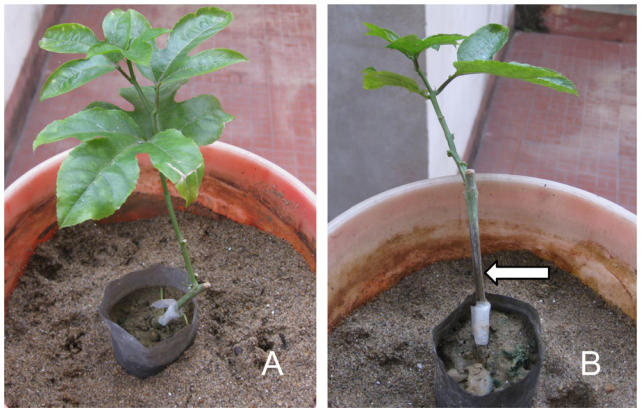

Fig. 7 Pathogenicity test of *Phytophthora nicotianae* (isolate MUCC707) on purple passionfruit using stem inoculation technique: **a** Control; **b** Inoculated stem showing progressive stem rot (*indicated by arrow*) after 15 d

MUCC707 was grown on PCA) for 7d. Three stems were inoculated by placing a small block (~5 mm□5 mm) of agar from the margin of the colony on a small cut (~2 mm long) in the stem. A strip of Parafilm^{R} was wrapped around the site of inoculation. Three other stems were used as controls by placing a small block of sterile PCA on the cut and wrapping the site with Parafilm^{R}. The plants were then placed in indirect sunlight at daily temperatures fluctuating within the range 20 to 28 °C. A progressive necrotic lesion (stem rot) typical of the field symptoms had developed from the site of inoculation with MUCC707 after 15d in all three inoculated plants (Fig. 7). The control plants were symptomless. Colonies of *P. nicotianae* were reisolated from the three diseased stems 5 cm from the site of inoculation, fulfilling Koch's Postulates.

The finding that *P. nicotianae* was the cause of the stem rot disease was not surprising as this pathogen is common in the region as outlined above. Furthermore the third author has isolated *P. nicotianae* from the Nghean Seed Centre in Vinh city where passionfruit vines were originally produced for use in Que Phong district. Passionfruit production at this Centre was devastated by the stem rot. This Centre was the likely source of the pathogen.

The small-holder farmers in Que Phong district were advised to ensure that the passionfruit fields were well-drained to

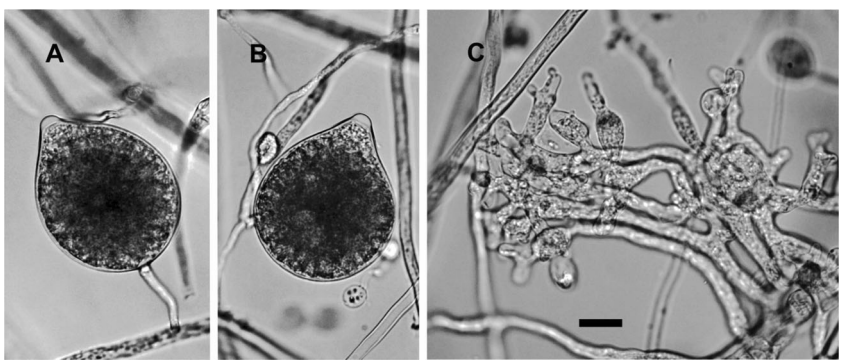

Fig. 6 *Phytophthora nicotianae* (**a**, **b**) typical ovoid papillate sporangia formed on V8 agar flooded with soil extract; (**c**) coralloid hyphae on V8 agar. Scale bar =25 μm on Fig. 6c also applies to Fig. 6a,b

minimize the risk of splash dispersal of the pathogen onto the stems above the graft junction. In addition they were advised to paint Alliette 80 WP (Fosetyl Aluminium) on diseased stems. Field observations in 2013 and 2014 indicated that this treatment was effective in reducing the incidence and severity of the stem rot (authors' unpublished data). However field trials are planned to clarify the efficacy of this fungicide. Farmers were also advised not to grow non-grafted seedlings.

Further studies are needed to refine the integrated disease management strategies, and clarify the distribution of this disease in purple passionfruit in other production areas in Vietnam such as Lam Dong province where a stem rot disease of unknown aetiology has caused a significant decline in production (authors' unpublished data). Furthermore surveys for the presence of Fusarium wilt in purple passionfruit plants that have not been grafted onto a resistant rootstock are justified, as Fusarium wilt and Phytophthora stem rot are known to co-occur in the same region in Kenya (Amata et al. 2009).

This report represents a new record of Phytophthora stem rot disease of purple passionfruit in Vietnam.

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