Pathogenicity of Phytopythium chamaehyphon: a new player in Kiwifruit Vine Decline Syndrome of Actinidia chinensis var. deliciosa (A. Chev.) A. Chev. 'Hayward' in Italy

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Pathogenicity of *Phytopythium chamaehyphon*: a new player in Kiwifruit Vine Decline Syndrome of *Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev. 'Hayward' in Italy

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Abstract

Kiwifruit Vine Decline Syndrome (KVDS) is a serious soil-borne disease that degrades the fine roots of both *Actinidia chinensis* var. *deliciosa* and var. *chinensis*. The disease seems to be the result of an interaction between several soil-borne pathogens, mostly oomycetes, and waterlogging. This work investigates the pathogenicity of the oomycete *Phytopythium chamaehyphon* recently isolated from roots of diseased plants. Pathogenicity was tested in 6-month-old and 1-year-old plants that after inoculation were flooded up to three times to induce symptom appearance. Leaf wilting and root rot typical of KVDS was observed in all the plants inoculated with *P. chamaehyphon* strain KD-15 (PCHA) and in all the positive controls potted in a mix of peat and soils collected in KVDS-affected orchards, while negative controls remained symptomless. Disease development on 6-months-old plants was characterized by unusual degradation of the not-lignified collar, occurring even in absence of flooding. Conversely, on 1-year-old plants, symptoms faithfully reproduced KVDS dynamics observed in orchard. This work confirmed the pathogenicity of *P. chamaehyphon* and raised new questions about the actual role of waterlogging in KVDS etiology.

Keywords: Kiwifruit Vine Decline Syndrome, oomycetes, waterlogging, pathogenicity test, root rot, collar rot

Italy is one of the major kiwifruit producer worldwide, satisfying by itself over 15% of the global market demand (FAOSTAT, 2020). A new disease known as Kiwifruit Vine Decline Syndrome (KVDS) has been reported in Italy since 2012 and currently affects 25% (6,160 ha) of Italian kiwifruit production area (Tacconi et al. 2020). KVDS mainly attacks the softer root tissues (fine roots) causing sudden and fast wilting of the canopy, usually visible after the first heat waves occur in late-spring and early-summer (June-August) (Tacconi et al. 2015; Savian et al. 2020a, b; Bardi 2020). KVDS etiology has not been fully clarified, but it seems the result of an interaction between waterlogging and soil-borne pathogens (Savian et al. 2020a). In particular, oomycetes (*Phytopythium* and

Phytophthora genera) seem to play a major role in KVDS (Savian et al. 2020a; Donati et al. 2020; Prencipe et al. 2020), however, also fungi (genus *Desarmillaria*) and bacteria (genus *Clostridium*) have been associated with the disease (Donati et al. 2020; Spigaglia et al. 2020).

The role of the interaction between soil-borne pathogens and waterlogging in the development of KVDS was demonstrated using soils from infected orchards of Friuli Venetia Giulia (FVG, NE Italy; Savian et al. 2020a). Two species, belonging to *Phytopythium* genus, were isolated and emerged as potential pathogens: *Phytopythium vexans* and *Phytopythium chamaehyphon*. Pathogenicity of *P. vexans* on kiwifruit vine and its association to KVDS was previously demonstrated by Prencipe et al. (2020), while the fulfillment of Koch's postulates for *P. chamaehyphon* was not determined and is the focus of the present work.

P. chamaehyphon was also isolated in Piedmont (NW Italy) from kiwifruit vines showing typical KVDS symptoms, in the framework of an extensive field monitoring of 18 orchards during 2016-2019 seasons. Root sampling and isolation were performed according to Prencipe et al. (2020). One representative isolate, grown on V8 agar, was observed morphologically according to de Cock et al. (2015). Colonies showed typical mycelia of a *Pythium* species, and older cultures showed sub-globose sporangia, (20.19 ± 0.36 µm), containing circular shaped zoospores (9–10 µm). DNA was extracted from isolates using Omega E.Z.N.A. Fungal DNA Mini Kit (VWR, USA), according to manufacturer's instructions. Species identification was confirmed by sequencing ITS region using primers ITS1/ITS4 (White et al. 1990), cytochrome oxidase I region (COI) using FM85mod/OomCOILevup primers (Robideau et al. 2011), and the large subunit (LSU) rDNA using NL1/NL4 primers (Baten et al. 2014). Sequences obtained from field isolate shared 99% identity for ITS region and 100% identity for LSU and COI with *P. chamaehyphon* isolates from Savian et al. 2020a.

P. chamaehyphon strain KD-15 from FVG (ITS region sequence under the accession number MN535819; Savian et al. 2020a), further identified based on sequencing the LSU and COI regions (accession numbers MW431329 and MW430118), was selected for the pathogenicity test.

Pathogenicity was tested twice on 1-year-old (trial 1) and 6-month-old (trial 2) *Actinidia chinensis* var. *deliciosa* (A. Chev) A. Chev. 'Hayward' plants potted in sterilized perlite-peat mixture (1:2 volumetric ratio). The oomycete was prepared according to Prencipe et al. (2020), growing *P. chamaehyphon* (PCHA) on wheat and hemp seeds for 7 days, and mixing the inoculum to the soil at a rate of 6 g/liter. Negative controls (NC) were inoculated with 6 g/l of autoclaved seed mixture, while positive controls (PC) pots were filled with infected soil and sterile peat to a volumetric proportion of 1:1. Infected soils were collected in June 2019, close to symptomatic roots from three orchards of Pordenone province (FVG, NE Italy) clearly affected by KVDS. Each thesis included 6 plants in trial 1 and 12 plants in trial 2, potted on 6- and 3-liter vases, respectively. Both trials were kept in a greenhouse at 28±5°C. After transplanting, water content was maintained proximal to field capacity for 3 weeks for trial 1 and 4 weeks for trial 2, before applying three rounds of flooding and drainage to induce symptoms (Savian et al. 2020a). Wilting appearance was recorded after 25, 35, and 50 days post inoculation (dpi) in trial 1 and every 2-3 days in trial 2. To fulfil Koch's postulates, re-isolations were performed from PCHA symptomatic plants and the isolates were molecularly identified by amplification and sequencing of the ITS region.

Typical wilting symptoms occurred in plants of PC and PCHA of both trials, while all NC plants remained symptomless until the end of the experiment (58 dpi).

On plants of trial 1, the disease progression was similar to that observed in the field, with wilting appearing only after waterlogging when the fine roots were heavily compromised. Wilting appeared after the third flooding cycle (50 dpi) where PCHA plants showed 68% symptomatic leaves, while PC plants showed 77% of symptomatic leaves (Figure 1). Re-isolation from PCHA plants fulfilled Koch's postulate confirming the pathogenicity of *P. chamaehyphon*.

In trial 2 with younger plants, the symptoms evolution was much faster than in trial 1, probably because the infection took place in the non-lignified plant collar, causing a faster dieback. For this reason, not all the flooding cycles were applied to induce the disease (Figure 2). Wilting on PCHA plants appeared right after the end of the first flooding (30 dpi), and rapidly evolved to complete plant decline for 92% of the plants (Figure 2). The disease progression in PC thesis was even faster: the plants started to wilt at 17 dpi, and one week before the first flooding cycle (24 dpi) 75% of the plants were dead, while the remaining ones died shortly after the first flooding application (Figure 2). Re-isolation from PCHA plants confirmed the Koch's postulate and the pathogenicity of *P. chamaehyphon* as observed for 1 year-old inoculated plants.

To the best of our knowledge, this is the first report of pathogenicity of *P. chamaehyphon* on kiwifruit. *Phytopythium* is a newly established genus including species formerly classified as *Pythium* species. This genus can inhabit both water and soil, and requires free water for the zoospore movement and for the initiation of the infection, as many other oomycetes (de Cock et al. 2015). Previously other oomycetes have been associated to KVDS: *Phytopythium vexans* (Prencipe et al. 2020), *Phytopythium helicoides* (Donati et al. 2020), *Phytophthora infestans* (Donati et al. 2020), *Phytophthora citrophthora* and *Phytophthora cryptogea* (Tacconi et al. 2015). It may be speculated that KVDS is enhanced if several pathogenic species are simultaneously infecting the roots, since the speed of symptom appearance and the disease incidence were higher in PC treatments compare to PCHA. Co-occurrence of *P. chamaehyphon* strain KD-15 and *Phytopythium vexans* was indeed observed in Savian et al. (2020) with a frequency of 55%. However, although the concept of a "pathobiome" associated with KVDS is well accepted (Savian et al. 2020a; Donati et al. 2020; Prencipe et al. 2020; Spigaglia et al. 2020), we do not fully know yet the composition of the KVDS microbial community and the interactions occurring between these pathogens.

Our findings confirm some preliminary indications suggesting that pathogens aggressiveness is enhanced when waterlogging is applied (Savian et al. 2020a). Indeed, the application of the flooding cycle accelerated the disease development in both PC and PCHA plants, while those in NC remain symptomless (Figure 1). However, the high mortality rate of 6-month-old plants, observed before waterlogging application (24 dpi) in PC plants, permits to hypothesize that KVDS might develop not only during/after flooding, but also at soil water content proximal to field capacity. Furthermore, the sudden appearance of symptoms on 6-month-old PCHA plants, right after the first day of flooding, indicates that the infection had started before the flooding application. Previously, Donati et al. (2020) already showed that the disease could be observed also in plants maintained at 70% of the estimated field capacity, without flooding. Moreover, in the study of Savian et al. (2020a), the leaf/root ratio of plants in unsterile soil without flooding was found more unbalanced than that of plants in sterile soil with flooding. KVDS being active even at soil water content proximal to field capacity could explain the reason why agronomical practices so far implemented to control the disease (tensiometer-driven irrigation and raised beds) were only able to delay the appearance of the disease after replanting (Tacconi et al. 2019). These results raise new questions on the effective role of root oxygen depletion and consequently of strictly anaerobic microorganisms on the onset of the disease.

The symptoms on 1-year-old plants, normally used as propagation material, faithfully reflected the disease development in orchard (Tacconi et al. 2015), while the uncommon rotting of the collar observed on 6-month-old plants was not observed previously, but it is in accordance with KVDS development, that usually degrades non-lignified tissues such as fine roots (Savian et al. 2020a). Considering these results, plants with lignified collar should be preferred for pathogenicity tests related to KVDS. In case only younger plants are available, modifications on the timing and duration of flooding should be taken into consideration in order to control the symptom appearance.

In conclusion, this work clearly demonstrated the pathogenicity of *P. chamaehyphon* and provided indication regarding the planting material to conduct KVDS-related tests. Furthermore, knowing that KVDS can develop also at soil water content proximal or inferior to field capacity may have a significant impact on both disease etiology and management strategies.

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Figure 1. Kiwifruit Vine Decline Syndrome (KVDS) symptoms observed during the pathogenicity test. Negative control (NC) plants inoculated with sterile seed mixture are in a) and d); plants infected with P. chamaehyphon strain KD-15 (PCHA) are in b) and e); Positive control (PC) plants inoculated with soil collected from KVDS affected orchards are in c) and f). Wilting symptoms are shown for: 1-year-old plants at the end of the experiment in a-c); 6-month-old plants at 24, 28-31 and 45 dpi in d-f). On the right, the roots of 6-month-old plants at the end of the experiment are shown: g) healthy roots of NC plants; h-k) symptoms on the roots of PCHA and PC plants. h-i) typical symptoms of KVDS on feeding roots, red circles in i) indicate the areas selected for re-isolation. j-k) atypical rots observed on the collar.

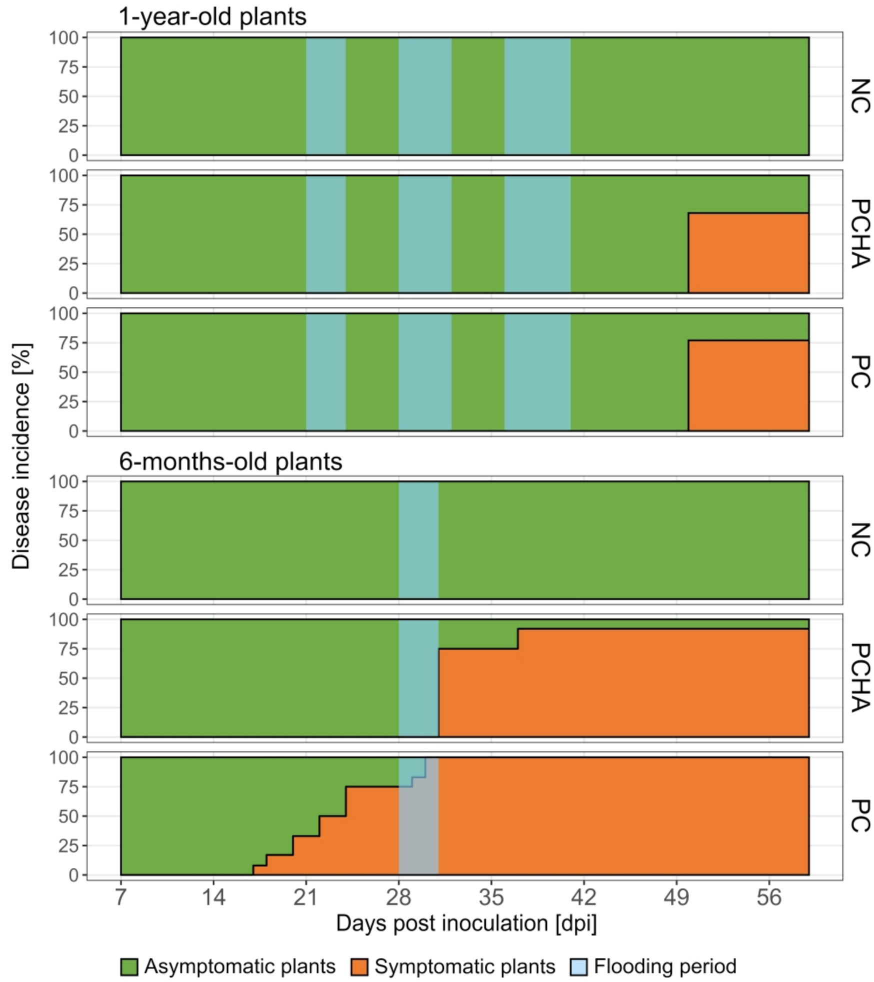

Figure 2. Percentage of plants with Kiwifruit Vine Decline Syndrome (KVDS) symptoms (disease incidence) during the pathogenicity test in 1-year-old and 6-month-old plants. Plants with KVDS symptoms are colored in orange while asymptomatic plants are in green. Shaded area indicates the application and duration of the flooding periods. NC, plants inoculated with sterile seed mixture; PCHA, plants inoculated with P. chamaehyphon strain KD-15; PC, plants potted on soil collected from KVDS affected orchards.