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Cadophora luteo-olivacea on apple and kiwifruit: characterization of selected strains and evaluation of fungicides for their control

S. Tomada · E. Staffler · G. Dionis · S. Baric · A. Di Francesco

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Abstract *Cadophora luteo-olivacea* has been reported as an emerging postharvest pathogen of pome fruit and kiwifruit with the potential to have a significant economic impact. To date, the biology and epidemiology of *C. luteo-olivacea* has been poorly investigated. Therefore, the present study aims to gain knowledge on *C. luteo-olivacea* biology, by analyzing the parameters that can influence the fungal growth and virulence and by getting information about the sensitivity to fungicides. A mycelial growth study at different temperatures was conducted with five *C. luteo-olivacea* isolates belonging to different host plants (apple and kiwifruit). The optimum fungal growth was observed in a temperature range between 20 °C and 25 °C, with *C. luteo-olivacea* isolates from kiwifruit that resulted in faster growth than apple isolates. The pathogenicity of *C. luteo-olivacea* isolates was evaluated on detached apple and kiwifruit twigs, on 'Golden Delicious' and 'Fuji' apples, and on 'Hayward' and 'Sungold' kiwifruit, stored both at 0 °C and 20 °C. The pathogenicity on fruit and on woody twigs was variable, depending on the host cultivar, with a minor effect related to the fungal isolate. Moreover, the efficacy of ten different plant protection products (PPPs) against the conidial germination of the isolates was determined. Few PPPs were found to be effective (e.g. fludioxonil, dithianon, and cyprodinil) against *C. luteo-olivacea*. These results represent a starting point for further research on the biology and epidemiology of *C. luteo-olivacea* and the development of effective management strategies.

Keywords Fruit · Storage · Fungicides · Epidemiology · Vegetative compatibility

S. Tomada (✉)
Servizio Fitosanitario E Chimico, Ricerca, Sperimentazione E Assistenza Tecnica. Agenzia regionale per lo sviluppo rurale del Friuli Venezia Giulia – ERSA, Gorizia, Italy
e-mail: selena.tomada@ersa.fvg.it

S. Tomada · E. Staffler · S. Baric
Free University of Bozen-Bolzano, Bolzano, Italy

G. Dionis · A. Di Francesco (✉)
Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy
e-mail: alessandra.difrancesco@uniud.it

Introduction

Climate change and the widespread global commercialization of plant materials and products are contributing factors to the outbreak of emerging or re-emerging fungal pathogens (Engering et al., 2013). In recent years, *Cadophora luteo-olivacea* (J.F.H. Beyma) T.C. Harr. and McNew, a fungus usually associated with trunk diseases of woody crops (Gramaje et al., 2011; Diaz et al., 2021), has been reported as an emerging postharvest pathogen of pome fruits (*Malus x domestica* Borkh) and kiwifruit (*Actinidia* spp.) (Amaral Carneiro et al., 2022; Di

Francesco et al., 2022; Grantina-Ievina, 2015; Wenneker et al., 2016).

There is increasing evidence that *C. luteo-olivacea* is a pathogen characterized by a long quiescent period on fruit infected in the field (Di Francesco et al., 2021, 2022, 2023), followed by a transition to necrotrophic colonization, usually after several months of cold storage (Köhl et al., 2018; Di Francesco et al., 2019). It has to be noted, that this kind of infections can arise from bloom to maturity at different phenological stages of fruit development (Harteveld et al., 2014; Nemsa et al., 2012; Di Francesco et al., 2023). Nevertheless, the increasing disease incidence observed on fruit represents a critical problem during the postharvest storage with economically significant losses reported during the shelf-life of the produce (Spadaro et al., 2011; Wenneker & Thomma, 2020). Cold storage represents a crucial phase for the control of postharvest pathogens because of the lack of effective fungicides and the few active ingredients allowed in this phase of the production chain (Romanazzi et al., 2016). *C. luteo-olivacea* is the causal agent of side rot and skin pitting of apple and kiwifruit, respectively. On pome fruits, *C. luteo-olivacea* induces dark brown circular to oval lesions that commonly appear after at least three months of cold storage (Spadaro et al., 2011; Sugar, 2014; Wenneker et al., 2016). Instead, symptoms on kiwifruit develop after four months of cold storage as circular-oval skin depressions under which little brown spots are visible (Di Francesco et al., 2023). In both cases, on apple and kiwifruit, the symptomatic tissues have a spongy and dry appearance. The infection biology of *C. luteo-olivacea* may contribute to a variable incidence of the pathogen over time, which is furthermore influenced by environmental conditions (Spadaro et al., 2010). The epidemiology of *C. luteo-olivacea* is still poorly investigated, which makes the management of the disease in the field even more challenging. In order to gain knowledge on the biology of *C. luteo-olivacea* and its sensitivity to fungicides, the present study was set up to: i) explore the pathogenicity of five fungal isolates during cold-storage and shelf-life on different apple and kiwifruit cultivars; ii) investigate the impact of different temperatures, ranging between 0 °C to 35 °C, on the mycelial growth; iii) investigate the vegetative compatibility and the possibility of coexistence between *C. luteo-olivacea* isolates belonging to different hosts; iv) investigate the effect of different fungicides on the conidial germination of the pathogen.

Materials and methods

Characterization of fungal isolates

The isolates of *C. luteo-olivacea* used in this study were isolated from cold stored symptomatic apple and kiwifruit collected in different Italian packing houses at different times (Table 1).

Symptomatic fruit portions (3 × 3 × 3 mm) were transferred onto Potato Dextrose Agar (PDA, 39 g/L of distilled water) (Sigma, St. Louis, MO, USA) at 20 °C for 10 days, subsequently, the isolates, were purified, and single spores were grown on new PDA plates. The isolates 19-DSS-BS-3–012, 18-DSS-CAFA-2–001, 19-DSS-KA-4–060 obtained from symptomatic apples belong to the mycological collection of University of Bolzano (Amaral Carneiro et al., 2022). The isolates CAD20 and CAD21 derived from symptomatic kiwifruits, belong to the mycological collection of Di4A-University of Udine (Di Francesco et al., 2023). Morphological differences among

Table 1 *Cadophora luteo-olivacea* isolates isolated from cold-stored apple and kiwi fruits

Nr	Isolate code	Plant material	Cultivar	Sampling year	Growing area	Farming system
1	CAD20	Kiwi fruit	Hayward	2022	Friuli Venezia Giulia	Integrated
2	CAD21	Kiwi fruit	Hayward	2022	Friuli Venezia Giulia	Integrated
3	19-DSS-BS-3–012	Apple fruit	Roho 3615/ Evelina®	2019	South Tyrol	Organic
4	18-DSS-CAFA-2–001	Apple fruit	Granny Smith	2018	South Tyrol	Integrated
5	19-DSS-KA-4–060	Apple fruit	Golden Delicious	2019	South Tyrol	Integrated

isolates were determined on PDA and Oatmeal Agar (OA) (60 g oatmeal, 12.5 g agar technical per 1 L of distilled water) (Sigma, St. Louis, MO, USA). Inoculated dishes were incubated at 20 ºC for two weeks. Five replicates of each isolate were grown on either culture medium. Each isolate was assigned to a specific morphological group according to the classification of Gramaje et al. (2011).

Plant materials and plant protection products

Fruits of apple cultivars 'Golden Delicious' and 'Fuji' were harvested at commercial maturity in the experimental orchard of the University of Udine (46°01′56.4"N13°13′23.6"E). Kiwifruit of both 'Hayward' and 'Sungold' cultivars were harvested in orchards located in Sedegliano (Udine, Italy, 46°02′19.08″N, 12°57′33.66″E) at commercial maturity. Fruits were selected to be homogeneous in size and injury-free, and immediately processed. One-year-old lignified twigs of a length of 50 cm of 'Golden Delicious' apple and 'Hayward' kiwifruit plants were collected, and stored at 5 °C for one month before use. Ten commercial plant protection products (PPPs), belonging to different fungicide classes, were selected (Table 2). The PPPs were used

Table 2 Plant protection products used in the study

N	Active substance	Commercial name	Recommended dose	Crop[a]	Application	Target pathogens[a]	Pre-harvest interval (days)
1	Captan	Merpan® 80WDG	1.60 g/L	pome fruit	open field	*Venturia* spp.; *Gleosporium*; *Stemphylium vesicarium*	21
2	Boscalid	Cantus®	0.25 g/L	apple kiwi	open field post-harvest	*Alternaria alternata* *Botrytis cinerea*	7 60[b]
3	Fludioxonil	Geoxe®	0.30 g/L	apple	open field	*Gloeosporium* spp., *Penicillium* spp., *Alternaria* spp., *Botrytis cinerea*	3
4	Orange Oil	Prev-AM® plus	3.00 mL/L	pome fruit	open field	*Venturia* spp.	3
5	Thymol, Geraniol, Eugenol	3logy®	4.00 mL/L	kiwi	open field	*Botrytis cinerea*	3
6	Dithianon	Kuki 70	0.50 g/L	pome fruit	open field	*Venturia* spp.; *Schizothyrium pomi*; *Gloeodes pomigena*; *Botrytis cinerea*	42
7	Fluazinam	Nando® Maxi	1.00 mL/L	apple	open field	*Venturia inaequalis*; *Alternaria alternata*	63
8	Dodine	Syllit® 65	1.20 g/L	pome fruit	open field	*Venturia* spp.; *Stemphylium vesicarium*	40
9	Copper sulfate	Poltiglia disperss®	5.00 g/L	pome fruit	open field	*Venturia* spp.; *Nectria* spp.; *Pseudomonas* spp.; *Xanthomonas* spp.; *Erwinia amylovora*; *Stemphylium vesicarium*	7[c]
10	Cyprodinil	Chorus	0.50 g/L	apple	open field	*Venturia* spp.	21

[a]target crops and pathogens as reported in the label

[b]days before commercialization

[c]winter treatments allowed without pre-harvest interval

at different concentrations against pathogen conidial germination to establish the EC_{50} values.

The effect of temperature on fungal growth

To investigate the impact of different temperatures on the mycelial growth of *C. luteo-olivacea* isolates, mycelial plugs (6 mm Ø) from the active edges of 10 days-old fungal colonies were inoculated on PDA and incubated at different temperatures ranging between 0 °C and 35 °C. Colony growth was measured 14 days post inoculation (dpi) at 10, 15, 20, 25, 30, and 35 °C and after 21 and 30 dpi at 5 °C and 0 °C, respectively. The diameter of radial growth was measured at two perpendicular axes and reported as mm/day of growth. Sample unit for each isolate and temperature was represented by five Petri dishes and the experiment was conducted twice.

Vegetative compatibility

The vegetative compatibility between *C. luteo-olivacea* isolates was checked on two different agar media: PDA and OA. Fungal mycelial plugs (4 mm Ø) were cut from the edge of active colonies and placed on each medium following a pattern whereby all plugs were spaced 1 cm apart and paired among themselves. After 14 days of incubation at 20 °C, the vegetative compatibility/incompatibility was macroscopically detected by barrage formation. The sample unit was represented by three Petri dishes. The experiment was conducted twice.

Pathogenicity test on detached fruits

Apple ('Golden Delicious' and 'Fuji') and kiwifruits ('Hayward' and 'Sungold'') were surface-sterilized by immersion for 1 min in 1% sodium hypochlorite solution and washed twice in sterile water. Fruits were wounded once by a sterile needle ($2 \times 2 \times 2$ mm) at the equatorial region and inoculated with 20 µL of *C. luteo-olivacea* spore suspensions (1×10^5 conidia/mL). The inoculum was prepared by using sterile water and by scraping sporulating fungal colonies of 10 days, adjusting to the final concentration by using a hemocytometer. Fruit wounds inoculated with sterile water were used as negative control. A batch of fruits was stored at 20 °C and 80% of relative humidity (R.H.) for four weeks, another batch was stored for four months at 0 °C, R.H. 90%. The sample unit was represented by 30 fruits per isolate at each storage condition.

The disease severity was assessed as lesion diameter (mm) with a caliper on apple and kiwifruit and recorded at different storage temperatures 0 °C and 20 °C after 4 months and 1 month, respectively. The experiment was performed twice.

Pathogenicity tests of detached twigs

Thirty twigs of both 'Golden Delicious' and 'Hayward' cultivars were cut in 10 cm segments, sterilized with 90% ethanol, rinsed with tap water, surface disinfected with 1% hypochlorite, rinsed with tap water and air dried. Three little portions of the bark were lifted by using a sterile scalpel and inoculated with 10 µL of spore suspension (1×10^5 conidia/mL) of each isolate. Wounds were covered with Parafilm® (Pechiney Plastic Packaging, USA) and the twigs were inserted in glass dishes containing sterile filter paper on the bottom, soaked daily with 1 mL of sterile water. Twigs inoculated with sterile water were used as negative controls. Twigs were incubated at room temperature (20 °C), with 12-h dark and 12-h light at 70% of RH for 1 month, and then the lesions were macroscopically evaluated. The pathogens were reisolated from the necrotic lesions on PDA and morphologically (conidia and mycelium) compared with the original strains. Symptoms images were taken by a digital camera (Canon E0S 3 Digital) and lesion color intensity was analyzed by using ImageJ software (1.8.0) to evaluate the severity intensity index (SI). The SI was rated on a scale from 0 to 100, where values close to zero denoted darker lesion and higher disease severity index and values close to 100, clearer tissue and lower disease severity index. The experiment was repeated twice.

Efficacy of plant protection products (PPPs) on conidial germination of *C. luteo-olivacea*

The sensitivity of *C. luteo-olivacea* to a selection of commercial PPPs, usually used in apple and kiwifruit orchards, was assessed. PDA medium was amended with each formulated commercial product at different concentrations by evaluating the inhibitory effect on each isolate conidial germination.

Starting from the recommended application dose of each PPP (Table 2), other 4 doses (2x, 0.5x, 0.25x, 0.12x) were used to amend liquid PDA medium. One hundred µL of spore suspensions (1×10^3 conidia/mL) of each isolate were spread on amended media and incubated at 20 °C for seven days. The medium without any addition of PPPs was used as control. The sample unit was represented by five dishes for each combination of isolate × PPP dose. The experiment was conducted twice.

Statistical analysis

Data were subjected to one-way ANOVA analysis. The separation of means was performed with a Tukey's test ($\alpha < 0.05$) by using the software MiniTab.16. For the *in vivo* assays, data were submitted to two-way ANOVA analysis. Least significant difference (LSD) at $P < 0.05$ was used to separate means of significant ANOVA factors (cultivar, isolate, temperature). The EC_{50} value of each PPP was calculated using the probit analysis applied to the percentage of inhibition of conidial germination (Lesaffre & Molenberghs, 1991).

Results

Isolates morphological characteristics

Considering the morphological characteristics of the fungal colonies, isolates 19-DSS-BS-3–012, 18-DSS-CAFA-2–00, 19-DSS-KA-4–060 could be assigned to group 4, while CAD20 and CAD21 to groups 2 and 3, respectively (Table 3). C*adophora luteo-olivacea* colonies on PDA varied in color from white to grey-olivaceous and the mycelium resulted flat, felty, and cottony in the center with an even edge. Instead, fungal colonies grown on OA varied in color, from olivaceous-buff to greenish olivaceous. *Cadophora luteo-olivacea* isolates, 18-DSS-CAFA-2–001, 19-DSS-BS-3–012, 19-DSS-KA-4–060 and CAD21, produced yellow pigmentation on OA as shown in Fig. 1. Isolate CAD21 produced yellow pigmentation also on PDA.

Table 3 Morphological classification of *Cadophora luteo-olivacea* isolates grown on Oatmeal Agar (OA) or Potato Dextrose Agar (PDA) according to the classification of Gramaje et al. (2011)

Isolate code	Morphological group	Yellow Pigmentation	
		OA	PDA
CAD20	Group 2	No	No
CAD21	Group 3	Yes	Yes
19-DSS-BS-3–012	Group 4	Yes	No
18-DSS-CAFA-2–001	Group 4	Yes	No
19-DSS-KA-4–060	Group 4	Yes	No

The effect of temperature on radial growth of *C. luteo-olivacea*

The effect of temperature on mycelial growth of *C. luteo-olivacea* isolates was assessed considering the temperature range between 0 °C and 35 °C. The optimum growth temperature for the five *C. luteo-olivacea* isolates was observed in a range of 20 °C to 25 °C with 2.52 ± 0.11 mm/day on average of growth (Fig. 2A). The colony growth of the isolates significantly decreased both below 15 °C and over 30 °C. Indeed, at 10 °C and 5 °C, the fungal growth was of 1.66 ± 0.08 and 0.62 ± 0.02 mm/day, respectively. Interestingly, at 0 °C mycelial growth was observed in a range of 0.25 to 0.46 mm/day. At 35 °C no fungal growth was observed. Moreover, the fungal development at cold storage temperature was further monitored. After two- and three-months *C. luteo-olivacea* colonies reached a diameter on average of 27.4 ± 1.4 and 40 ± 1.9 mm, respectively (data not shown). By grouping the fungal isolates according to their host source, significant differences between groups were observed at 10 °C, 20 °C, 25 °C, and 30 °C, with kiwifruit isolates showing a faster growth compared to the isolates derived from apple (Fig. 2B).

Vegetative compatibility

All *C. luteo-olivacea* isolates were paired with each other in a vegetative compatibility test conducted on PDA and OA media. Table 4 reports the results of the pairings among the isolates on OA, the only of the two media that resulted in the production of clear barrages between the tested isolates (Fig. 3). Four

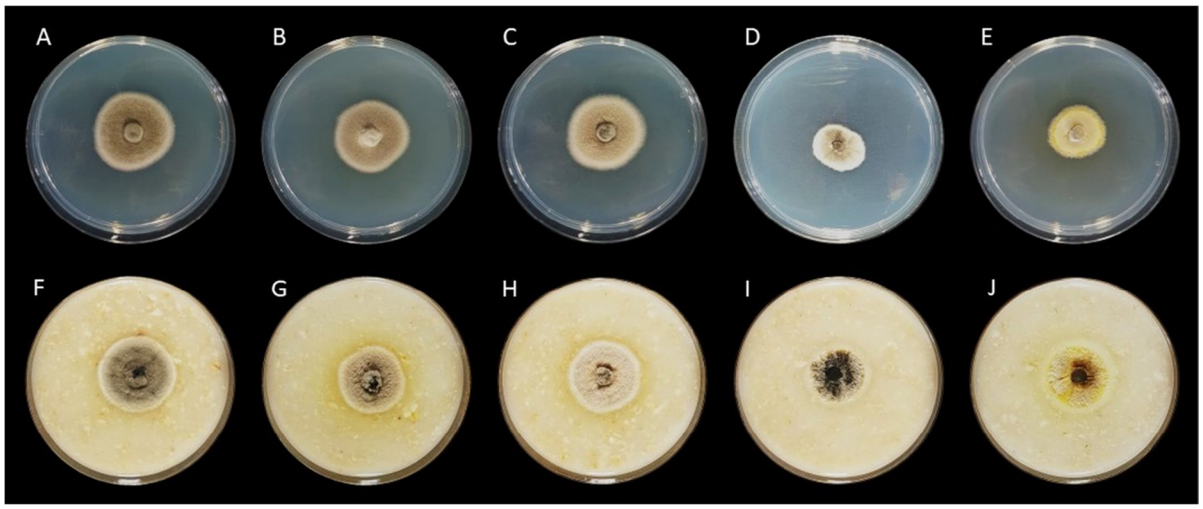

Fig. 1 Morphology of *Cadophora luteo-olivacea* isolates grown on Potato dextrose agar (first row) and Oatmeal agar (second row) for 14 days at 20 °C. The isolates are reported in the following order: 18-DSS-CAFA-2–001 (A, F), 19-DSS-BS-3–012 (B, G), 19-DSS-KA-4–060 (C, H), CAD20 (D, I), CAD21 (E, J)

pairings were compatible (C). Vegetative compatibility among all the three *C. luteo-olivacea* isolates from apple was observed. Isolate CAD21 from kiwifruit resulted incompatible (I) with all the other tested isolates. Conversely, the kiwifruit isolate CAD20 resulted compatible only with the apple isolate 19-DSS-KA-4–060.

Pathogenicity tests on detached fruits and twigs

Fungal isolates successfully infected both apple and kiwifruit. The lesions slowly developed on both fruit species and at the two different tested storage temperatures (0 °C and 20 °C).

Taken together, 'Golden Delicious' displayed a higher susceptibility compared to 'Fuji' apples, with lesion diameters of 5.9 ± 0.41 mm and 3.3 ± 0.31 mm, respectively (Table 5a). Isolate 18-DSS-CAFA-2–001 resulted in the greatest lesion diameter on both 'Golden Delicious' and 'Fuji' apples. The storage conditions did not significantly impact the activity of the isolates and the lesion diameter, even if it must be considered that the incubation time was different. Indeed, at cold storage temperature after four months of incubation reached the value of 4.6 mm, approximately the same value was obtained after one month at shelf-life temperature. Regarding kiwifruit, only the cultivar represented a significant discriminating parameter. 'Hayward' was susceptible, whereas 'Sungold' showed a high resistance to skin pitting disease, with lesion diameters of 3.5 ± 0.32 mm and 0.0 ± 0.0 mm, respectively. In kiwifruit, CAD20 and CAD21 produced lesion diameters slightly larger than the isolates from apple (Table 5b). On the fruits used as the negative control, no rotten tissue appeared.

The pathogenicity of *C. luteo-olivacea* isolates on twigs of the apple cultivar 'Golden Delicious' was observed. Specifically, all the inoculated isolates produced visible necrosis on twigs. The isolates 18-DSS-CAFA-2–001 and 19-DSS-BS-3–012 displayed a higher SI with respect to the untreated control (Table 6 and Fig. 4). Interestingly, no visible symptoms were detected on the twigs of the kiwifruit cultivar 'Hayward'.

Efficacy of plant protection products (PPPs) on the inhibition of conidial germination of *C. luteo-olivacea*

The derived EC_{50} values for conidial germination inhibition of the tested *C. luteo-olivacea* isolates ranged between 0.0001 and > 10 g/L of PPP. Results reported in Table 7 show that Geoxe (Fludioxonil), 3logy (Thymol, Geraniol, Eugenol), Kuki (Dithianon), Syllit (Dodine), and Chorus (Cyprodinil) were the most effective PPPs on conidial growth inhibition,

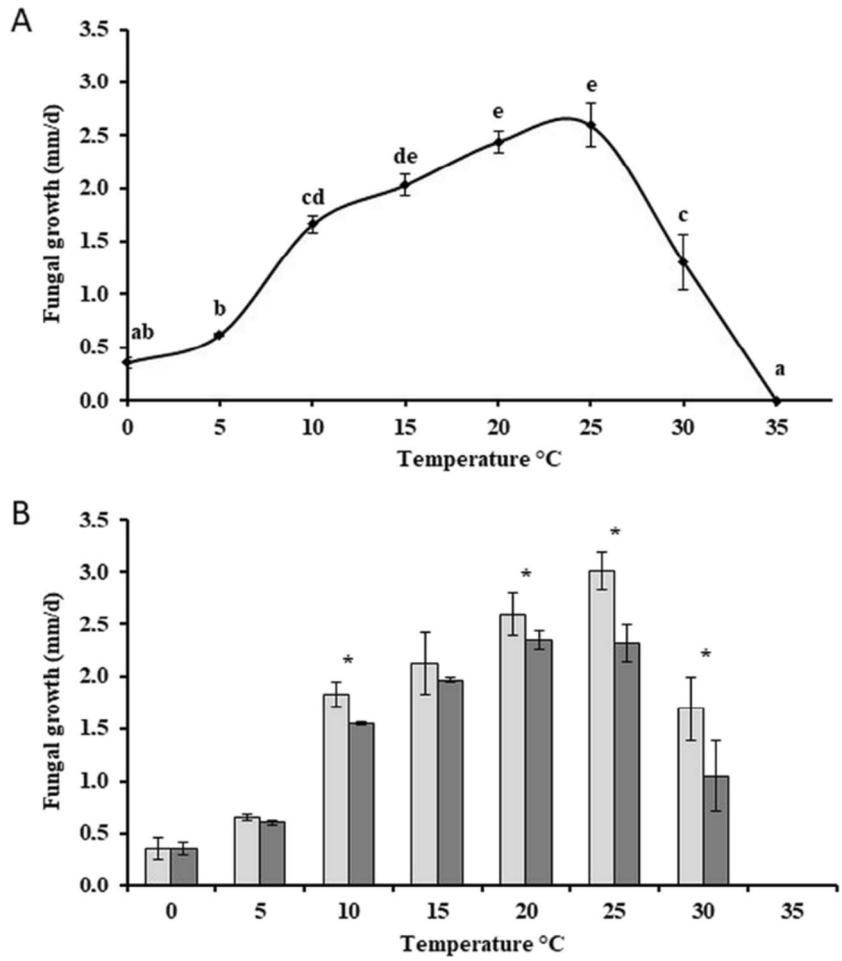

Fig. 2 Temperature influence on *Cadophora luteo-olivacea* isolates growth. The five *Cadophora luteo-olivacea* isolates were grown on Potato dextrose agar. Colony growth (mm/d) was measured 14 days post inoculation at 10, 15, 20, 25, 30, and 35 °C and after 21 and 30 days at 5 °C and 0 °C, respectively. (A) Mean value (± standard error) of fungal growth calculated among the five isolates per temperature. Different letters indicate significant differences according to *Tukey's test* ($\alpha = 0.05$). (B) Mean value (± standard error) of fungal growth calculated among the *C. luteo-olivacea* isolates from kiwifruit (light grey) and the apple fruit subgroup (dark grey). Asterisks indicate values that differ significantly in the pairwise comparison of kiwi-and apple fruit isolates subgroup

Table 4 Vegetative compatibility among *Cadophora luteo-olivacea* isolates tested on Oatmeal Agar. Symbols: (C) compatible and (I) incompatible

Isolate code	CAD20	CAD21	19-DSS-BS-3–012	18-DSS-CAFA-2–001	19-DSS-KA-4–060
CAD20	C	-	-	-	-
CAD21	I	C	-	-	-
19-DSS-BS-3–012	I	I	C	-	-
18-DSS-CAFA-2–001	I	I	C	C	-
19-DSS-KA-4–060	C	I	C	C	C

showing the total suppression of all *C. luteo-olivacea* isolates at low concentrations (< 0.0001 g/L).

Poltiglia dispress (Copper sulfate), Nando (Fluazinam), and Cantus (Boscalid) displayed the lowest antifungal effect against the tested isolates of *C. luteo-olivacea*. For these PPPs, EC_{50} ranged from 2.37 to > 10.0 g/L: values above the recommended application dosages provided on the official labels (Table 7). The isolates CAD20 and 18-DSS-CAFA-2–001 were found to have a more pronounced resistance against the tested PPPs, while CAD21 and 19-DSS-BS-3–012 were observed to be more sensitive (Table 7).

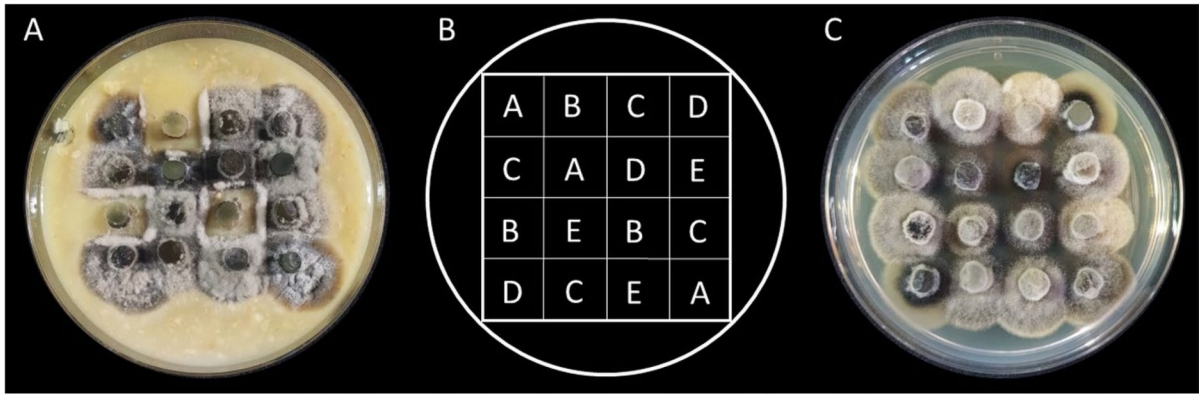

Fig. 3 Vegetative compatibility test of *Cadophora luteo-olivacea* isolates on A) Oatmeal agar and C) Potato dextrose agar. The inoculated plates were incubated for 14 days at 20 °C. Scheme of the isolates combination B): CAD20 (A); CAD21 (B); 19-DSS-BS-3–012 (C), 19-DSS-KA-4–060 (D) and 18-DSS-CAFA-2–001 (E)

Discussion

The present study aimed to characterize selected isolates of *C. luteo-olivacea* to induce rot on different cultivars of apple and kiwifruit, and to cause lesions on lignified branch tissue. The results indicate that the pathogenicity of different isolates on fruits was highly influenced by the host and intrinsic characteristics of each isolate.

In fact, *C. luteo-olivacea* isolates showed differences of virulence by considering fruit infection and wood colonization. Regarding the latter, only twigs obtained from apple trees that were artificially inoculated with conidial suspension of *C. luteo-olivacea* showed necrosis, highlighting the ability of the fungus to penetrate via wounds into lignified tissue. In contrast, no necroses were observed on kiwifruit twigs in the current experiment, even though *C. luteo-olivacea* and *Cadophora melinii* were isolated from the wood of kiwifruit trees affected by trunk hypertrophy and longitudinal bark crack (Prodi et al., 2008). The observed necroses on apple twigs could serve as an overwintering site for *C. luteo-olivacea*, as suggested in the case of other pre- and postharvest pathogens, like *Neonectria ditissima, Colletotrichum acutatum,* and *Colletotrichum gloeosporioides* (Shuttleworth, 2021; Wenneker & Thomma, 2020). Considering the fruit, the apple cultivar 'Golden Delicious' showed a higher susceptibility to side rot displaying lesion diameters 1.7-fold larger with respect to 'Fuji'. Spotts et al. (1999) reported that the main commercial apple cultivars displayed a different degree of susceptibility to fungal pathogens. It has been reported that in Europe several late-harvest apple cultivars, such as 'Golden Delicious' and 'Fuji', are particularly susceptible to fungal diseases like bull's eye rot and bitter rot (Neri et al., 2009; Velho et al., 2015). However, the limited information available about the relative resistance of apple cultivars toward the main postharvest diseases are often contradictory, due to the lack of a standardized pathogenicity test design and different plant material (e.g., origin of the fruits from different management practices and/or fruit ripeness degree) (Konstantinou et al., 2011; Spotts et al., 1999).

Looking at kiwifruit, 'Sungold' was found to be resistant to the tested isolates of *C. luteo-olivacea*. Conversely, skin pitting symptoms were detected in yellow-fleshed cultivar 'Hort16A' by Manning et al. (2003). Whereas a high susceptibility was confirmed for 'Hayward' as reported by Spadaro et al. (2010) and Di Francesco et al. (2022). The effect of host genotype on disease resistance was also reported considering *Botrytis cinerea* infection. 'Hort16A' showed a greater resistance compared to 'Hayward', both in terms of disease severity and incidence (Wurms, 2005).

The genetic differences among strains belonging to the same species can represent another important factor, that could influence their virulence and aggressiveness on a specific host. Diversity within species is the result of continuous processes of mutation and subsequent selection (Van Rossum et al., 2020). In our study, the tested isolates showed morphological variation, low differences in aggressiveness (except

Table 5 Disease severity evaluated as average lesion diameter (mm) ± standard error on 'Golden Delicious' and 'Fuji' apples (a) and on 'Hayward' and 'Sungold' kiwifruit (b) recorded at different storage temperatures 0 °C and 20 °C after 4 months and 1 month, respectively

a) Factor	Lesion diameter (mm)
Cultivar	
Golden Delicious	5.9 ± 0.41^{b}
Fuji	3.3 ± 0.31^{a}
p	*
Isolate	
CAD20	3.8 ± 0.67^{a}
CAD21	4.2 ± 0.72^{a}
19-DSS-BS-3–012	4.3 ± 0.50^{a}
18-DSS-CAFA-2–001	6.0 ± 0.15^{b}
19-DSS-KA-4–060	4.7 ± 0.64^{a}
p	*
Temperature	
0 °C	4.6 ± 0.46
20 °C	4.6 ± 0.40
p	*n.s*
b) Factor	Lesion diameter (mm)
Cultivar	
Hayward	3.5 ± 0.32^{b}
Sungold	0.0 ± 0.0^{a}
p	*
Isolate	
CAD20	2.5 ± 0.87
CAD21	2.5 ± 0.92
19-DSS-BS-3–012	1.6 ± 0.91
18-DSS-CAFA-2–001	1.6 ± 0.92
19-DSS-KA-4–060	1.3 ± 0.69
p	*n.s*
Temperature	
0 °C	2.5 ± 0.60
20 °C	1.4 ± 0.62
p	*n.s*

The asterisk indicate significance at $p < 0.05$ and *n.s.* means no significance. Different letters indicate statistical differences among group according to LSD test at $p < 0.05$

Table 6 Pathogenicity of *Cadophora luteo-olivacea* isolates on apple twigs. Average value (± SE) of lesion color intensity were evaluated by the software ImageJ 1.8.0. Significant differences could be distinguished by different letters in the same columns according to LSD test ($p < 0.05$). Severity intensity index (SI) was rated on a scale 0–10 (+ + + + +); 11–35 (+ + + +); 36–50 (+ + +); 51–75 (+ +); 76–100 (+). Values close to zero denote darker lesion and higher disease severity, values close to 100 denote clearer tissue and lower disease severity. Different letters represent significant differences among the lesions intensity caused by the tested isolates. No symptoms were detected on kiwifruit twigs

Isolate code	Intensity (unweighted)	Severity intensity index (SI)
Control	53 ± 2.0^{a}	+
CAD20	36 ± 3.0^{b}	+ + +
CAD21	36 ± 3.4^{b}	+ + +
19-DSS-BS-3–012	34 ± 1.5^{b}	+ + + +
18-DSS-CAFA-2–001	25 ± 1.5^{c}	+ + + +
19-DSS-KA-4–060	37 ± 3.1^{b}	+ + +

for the isolate 18-DSS-CAFA-2–001), marked vegetative compatibility and the ability to infect different hosts (cross-infection ability). Even though, the relevance of the morphological categories is questionable (Gramaje et al., 2011), and the genetic base of these differences and possible relation between morphotype and virulence has to further investigate (Kowalski & Cramer, 2020). The cross-infection ability represents a very important topic for understanding pathogens dissemination and evolution (Leslie, 1993). To better characterize *C. luteo-olivacea* isolates, a vegetative compatibility test was performed. A high level of hyphal anastomosis was detected between the isolates belonging from the same isolation host except for kiwifruit isolates that resulted incompatible within each other. Nevertheless, the co-existence of different isolates, also belonging to different hosts (e.g., CAD20 and 19-DSS-KA-0–060, isolated from kiwifruit and apple fruit, respectively) may have implications to be considered for establishing effective control strategies. In fact, different isolates may manifest different sensitivity to different fungicides.

Cadophora luteo-olivacea is often considered a pathogen of minor importance and therefore limited information about control strategies are currently available (Di Francesco et al., 2021). Some previous studies investigated the use of different biocontrol agents, basic substances, and physical treatments to control *C. luteo-olivacea* in the field (Di Francesco et al., 2021, 2022, 2023). No studies conducted *in vitro* and *in vivo*, so far, were focused on the effectiveness of common fungicides used in different agricultural systems (organic, conventional, and integrated) to potentially control *C. luteo-olivacea*. The most effective active substances inhibiting the conidial germination of the isolates

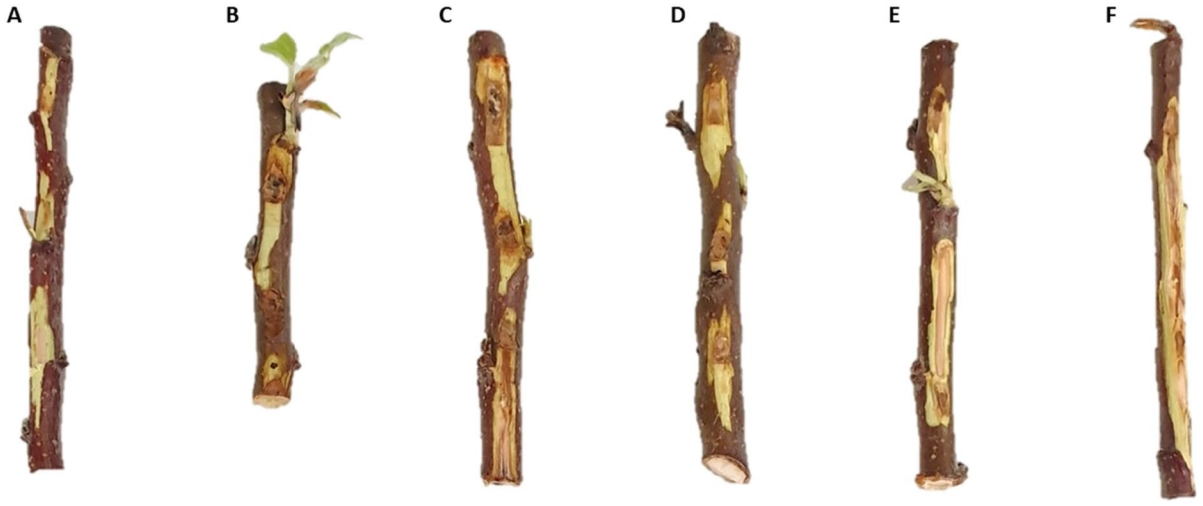

Fig. 4 Pathogenicity of *Cadophora luteo-olivacea* isolates on apple twigs. The twigs were artificially wounded and inoculated with the spore suspension (1×10^4 conidia/mL) of each *C. luteo-olivacea* isolate: 18-DSS-CAFA-2–001 (B), 19-DSS-BS-3–012 (C), 19-DSS-KA-4–060 (D), CAD21 (E), CAD20 (F). Twigs inoculated with sterile water were used as negative control (A). Necroses were assessed after 30 days at 20 °C

Table 7 EC50 values (g/L or mL/L) calculated for ten plant protection products on *Cadophora luteo-olivacea* inhibition of conidial germination. The evaluations were carried out on PDA amended with the plant protection product after 7 days of incubation at 20 °C

Active substance	EC50 values (g/L or mL/L) per isolate				
	CAD20	CAD21	19-DSS-BS-3–012	18-DSS-CAFA-2–001	19-DSS-KA-4–060
Captan	1.18	< 0.0001	< 0.0001	1.22	< 0.0001
Boscalid	7.79	> 10.0	> 10.0	2.37	> 10.0
Fludioxonil	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Orange Oil	0.04	< 0.0001	< 0.0001	2,68	0,15
Thymol, geraniol, eugenol	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Dithianon	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Fluazinam	> 10.0	> 10.0	> 10.0	> 10.0	> 10.0
Dodine	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Copper sulfate	> 10.0	> 10.0	n.i.*	> 10.0	n.i.*
Cyprodinil	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*n.i. no inhibition observed

were fludioxonil, dithianon, dodine, and cyprodinil, which represent the most important fungicides in apple crop protection (Ticha et al., 2008). These active substances are mainly applied in the field to control *Venturia* spp. but were reported to act *versus* a plethora of pathogens of pome fruit like *Alternaria* spp., *B. cinerea*, *Gloeosporium* spp., and *Penicillium* spp. (Table 2). Interestingly, fludioxonil was reported to be effective in controlling *Phlyctema vagabunda* on apple as postharvest treatment (Lolas et al., 2015; Russouw et al., 2021). In fact, the use of fludioxonil is also admitted for postharvest applications. However, the concern for its potential impact on human health has recently emerged (Brandhorst & Klein, 2019). The preharvest application of dodine, showed variable results in controlling storage fruit rot (Minář, 2006, Ticha et al., 2008), even if it was recently reported as a promising active substance for controlling the emergent white haze disease, caused by the epiphytic

fungus *Tilletiopsis* spp. on apple in Northern Italy (Angeli et al., 2022). Promising results were revealed for a product based on three plant terpenes (eugenol, thymol, geraniol), which is authorized to be used in organic agriculture to control gray mold on kiwifruit. The commercial product allowed in organic agriculture and based on geraniol, thymol and geraniol showed similar efficacy in inhibiting the conidial germination as some of the most effective synthetic fungicides.

The less effective active compounds against *C. luteo-olivacea* conidial germination were boscalid, fludioxonil, orange oil, fluazinam, and copper sulfate. Field experiments reported that boscalid did not provide a consistent significant reduction of many apple rots (Everett & Timudo-Torrevilla, 2007). The only reduction of rot incidence and severity were achieved probably for the persistence of this fungicide, able to prevent some later infections (Everett & Timudo-Torrevilla, 2007). Copper sulfate resulted non-effective against other apple pathogens, such as *C. acutatum* (Everett et al., 2015). This PPP has the potential to damage the fruit skin and to promote pathogen entry (Everett et al., 2015). Moreover, *Cadophora* spp. has been reported to be tolerant to several heavy metals (Karunasekera & Daniel, 2013; Likar & Regvar, 2013). For instance, the gene expression of *Cadophora finlandica* encoding for several extracellular proteins and transporters was observed in response to cadmium exposition (Gorfer et al., 2009).

Conclusions

In conclusion, the findings of the current study provided new information on *C. luteo-olivacea* isolate diversity, mostly in terms of virulence and efficacy of several fungicides. With regard to this, more research into fungal biology and epidemiology is required to better understand the relationships among fungal virulence, latency, and cross-infection ability in relation to climate change, in order to reduce the risk of *C. luteo-olivacea* incidence in postharvest, and to determine the best time to implement sustainable disease management strategies.

Data availability Data supporting this research is available upon reasonable request from the last author.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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