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Heat treatment effect on *Cadophora luteo-olivacea* of kiwifruit

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Abstract

Heat treatment represents an alternative method to fungicides to control latent fruit pathogens, such as *Cadophora luteo-olivacea* of kiwifruit. This study reports the efficacy of different hot water (HW) and hot air (HA) treatments (45, 50, 55, 65, and 70°C with durations of 10, 15 or 20, 5 or 10, 5, and 3 min, respectively) on the conidial and mycelial growth of fungal isolates by in vitro assays. Both treatments at 70°C were the most effective in inhibiting conidial and mycelial growth of *C. luteo-olivacea* isolates. Treatment efficacy for HW and HA was on average 88% and 71%, respectively, on mycelial growth, and 100% and 91.3%, respectively, on conidial growth. A significant reduction of xylanase and pectinase enzyme activities of the isolates was detected after HW and HA treatment (70°C × 3 min). In most cases, both treatments showed a total reduction, sometimes varying with the target isolate. HA treatment was the most efficient treatment for reducing the skin pitting incidence in in vivo experiments, showing an average efficacy of 46.6%. Results show that heat treatments are effective against *C. luteo-olivacea*, but the main challenge will be to develop the optimal time × temperature combinations.

KEYWORDS
FT-IR, heat treatment, kiwifruit, postharvest, skin pitting

1 | INTRODUCTION

Kiwifruit cv. Hayward has been planted worldwide in temperate-zone countries (Michaelidis & Elmer, 2000), becoming a significant commercial planting (Di Francesco, Mari, Ugolini, et al., 2018). Skin pitting of kiwifruit is a postharvest disease caused by the pathogen *Cadophora* (Spadaro et al., 2010). This pathogen belongs to a group of pathogens that infects developing fruits during the growing season, remaining quiescent, and then appearing during the postharvest phase (Köhl et al., 2018) in cold storage.

The incidence of the pathology is very variable over time, strictly connected to field conditions; however, it can cause considerable economic losses. Commonly, *Cadophora luteo-olivacea* is associated with trunk disease of grapes (Gramaje et al., 2011; Manning & Mundy, 2009) and more recently has been reported by Wenneker et al. (2016) as causing side rot of pears in the Netherlands.

Commonly, the risk of postharvest diseases is approached with fungicide treatments before harvest (Palm & Kruse, 2012), but recently, issues related to chemical residues on fruit make these treatments challenging to justify.

Among alternative methods to control fruit postharvest diseases, heat applied as forced hot air or by dipping fruit in hot water appears to be one of the most promising methods (Lurie, 1998; Mari et al., 2007). Hot water (HW) treatment has been reported to be effective in managing postharvest decay in a variety of fruits (Chen et al., 2015) in managing several postharvest diseases and physiological disorders (Aghdam & Bodbodak, 2014; Fallik, 2004). The efficacy of HW treatment for the management of postharvest fungal decay has been reported for

various fruits, including apple (Maxin et al., 2012a), pear (Bai et al., 2006), peach (Liu et al., 2012), mandarin (Hong et al., 2007), and mango (Dessalegn et al., 2013). Heat treatment can also prevent storage decay by stimulating the host defence responses in fruit tissue (Nunes et al., 2007; Palou et al., 2008) and acting directly on fungi by inhibiting spore germination or disease symptoms (Di Francesco, Mari, Roberti, et al., 2018; Liu et al., 2012), or by accumulating reactive oxygen species (ROS) causing oxidative damage of proteins and lipids (Zhao et al., 2014). Kiwifruit, often subjected to postharvest fungal decay, is almost always treated with heat treatments to control grey mould, caused by *Botrytis cinerea*, the most significant postharvest disease of kiwifruit (Bautista-Banos et al., 1997; Williamson et al., 2007). Heat treatments (temperature and time) are variable depending on kiwifruit cultivar and target pathogen (Chen et al., 2015). As far as we know, there are no studies on the role of fruit tissue treated with heat treatments on pathogenesis enzyme production of postharvest pathogens and their influence both on fungal mycelial and fruit nutraceutical composition.

The present study aimed to determine the direct effect of different HW and hot air (HA) treatments on *C. luteo-olivacea* isolates by in vitro assays and indirectly on kiwifruit by in vivo assays. More specifically, this study tested the effects of both HW and HA treatments on (a) conidial germination and mycelial growth of *C. luteo-olivacea* isolates by in vitro assays, and (b) kiwifruit artificially inoculated by in vivo assays, (c) xylanase and pectinase activity of fungal isolates by enzyme assays, and (d) *C. luteo-olivacea* mycelial structure and kiwifruit chemical composition through FT-IR analysis.

2 | MATERIALS AND METHODS

2.1 | Fungal isolates

C. luteo-olivacea isolates (CadA, CadB, and CadC) were obtained from the Criof fungal collection (Di Francesco et al., 2021). Each isolate was grown on potato dextrose agar (PDA) and incubated at 20°C. Conidial suspensions were prepared from 20-day-old colonies by scraping and suspending conidia in sterile distilled water with 0.05% (vol/vol) Tween 80 and adjusting to a final concentration of 10^5 conidia/ml with a haemocytometer.

2.2 | Kiwifruit

Hayward kiwifruit (*Actinidia deliciosa*) obtained from an orchard located in Faenza (Italy) were used. Fruits were harvested at commercial maturity (7.2 °Brix) and immediately artificially inoculated, heat-treated, and stored.

2.3 | HW and HA treatments

Sterile tubes, each containing 10 mycelial plugs (6 mm diameter) and 1 ml of spore suspension for each isolate, were treated by dipping in hot water in a 10-L stainless steel tank or exposed to heated air in an oven. The water and air temperatures were 45, 50, 55, 65, and 70°C and the treatment durations were 10, 15 or 20, 5 or 10, 5, and 3 min, respectively.

The water and air were heated by a digital thermostat (ScanVac SHC 2000, Linge DK) with temperature stability +0.01°C.

2.4 | In vitro assay

After treatments, mycelial plugs (one per plate) and conidial suspensions (100 µl per plate) were inoculated on PDA plates and incubated at 20°C. After 2 weeks of incubation, the mycelial growth (diameter, mm) and the colony-forming units (cfu) of each isolate were measured and counted. Ten plates represented a sample unit for mycelial plugs and conidial suspensions. The experiment was performed three times. Untreated mycelial plugs and conidial suspensions represented the controls.

2.5 | In vivo assay

Kiwifruits were treated with HW and HA heated at 70°C × 3 min. After 1 h, kiwifruits were wounded by a sterile nail (3 × 3 × 3 mm) at the equator (one wound per fruit) and then inoculated with 20 µl conidial suspension of each isolate. After the artificial inoculation, all fruits were stored at 0°C for 4 months. Disease severity was assessed after one week of incubation at 20°C by counting the number of lesions on fruits and the percentage of incidence with respect to the untreated fruits (control). Three replicates of 20 fruits each represented a sample unit, and the experiment was performed twice.

2.6 | Effect of HW and HA treatment on xylanase and pectinase enzyme activity

Xylanase and pectinase enzyme activity of the three *C. luteo-olivacea* isolates (CadA, CadB, and CadC) was evaluated after exposure of mycelial plugs and conidial suspensions to the most efficient HW and HA treatment (70°C × 3 min) in vitro. The assay was performed in Petri dishes containing a specific agar medium described by Di Francesco, Mari, Roberti, et al. (2018). Mycelial plugs and 40 µl aliquots of conidial suspension, both heat-treated, were placed on agar and in holes (each spaced 3 cm apart) punched in the medium with a 5-mm sterile cork borer. Control plates were inoculated with nontreated mycelial and conidial suspensions. After incubation at 20°C for 7 days and 48 h for mycelial plugs and conidial suspensions, respectively, the xylanase and pectinase enzyme activity was determined with a specific colourimetric method, visualizing a clear zone of substrate degradation around the holes (Di Francesco, Mari, Roberti, et al., 2018). The diameter (mm) of the degradation halos was measured with a ruler, and the halo area was calculated.

2.7 | Fourier-transform infrared spectroscopic analysis

Mycelial plugs of each isolate were inoculated on a sterile cellophane disc (Courtauld Films; 90 mm diameter), laid down on a PDA plate and incubated at 20°C for 14 days. The mycelium grown on the cellophane was then removed and positioned in a sterile tube. Mycelia were heat-treated (either HW or HA, 70°C × 3 min), collected in sterile tubes (2 ml), stored at −80°C and suddenly lyophilized by freeze-drying (FD-10 Freezing Dryer, Lab kits) under vacuum (<20 Pa) at a temperature of −36°C, and freeze-dried for 7 days to avoid water spectroscopic interferences. The sample unit was represented by five tubes (replicates) for each treated isolate and the control. The assay was repeated twice.

Fifty grams of heat-treated (HW or HA, 70°C × 3 min) and non-treated kiwifruit were collected in sterile tubes (50 ml) stored at −80°C, and immediately lyophilized as described above. The sample unit was represented by three tubes (replicates) for each heat treatment and the control.

Samples were analysed by Fourier-transform infrared (FT-IR) spectroscopy to obtain a rapid and nondestructive characterization of their main molecular components. Infrared spectra were recorded with a Bruker ALPHA series FT-IR spectrophotometer (Bruker) equipped with an apparatus for attenuated total reflectance (Diamond crystal). IR spectra were averaged over 100 scans at a resolution of 4 cm^{-1}; four spectra were measured for each sample for each sampling time.

2.8 | Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA, $p < 0.05$). The separation of means was performed using the Tukey test at $\alpha < 0.05$. All analyses were performed using Statgraphic Plus v. 2.1 (Statistical Graphics Corp.).

3 | RESULTS

3.1 | In vitro treatments

Almost all tested HW treatments showed a reduction in mycelial growth of the three *C. luteo-olivacea* isolates. In particular, the 65 and 70°C temperatures displayed the highest reduction values compared to the control, with growth reduced by 58.6% and 64.4%, 56.7% and 100%, and 100% and 100% for isolates CadA, CadB, and CadC, respectively (Figure 1a). The 45 and 50°C HW treatments were less effective against all isolates. However, all three isolates showed a significant reduction in mycelial growth with the 55°C HW treatment for 10 min compared to the same temperature treatment conducted for 5 min, of 11.1%, 14.5%, and 12.5% (CadA, CadB, and CadC, respectively).

Similar results were detected for HA treatments, with 70°C × 3 min the most efficient treatment, reducing mycelial growth of isolates CadA, CadB, and CadC by 58.1%, 100%, and 54.7%, respectively, compared to the control (Figure 1b). However, the remaining HA treatments were less efficient in reducing *C. lutea-olivacea* with respect to HW treatments. In fact, HW treatments were effective in totally inhibiting colony growth (cfus) of the three tested isolates (data not shown).

In the case of the HA treatments (Figure 2), 50°C × 20 min and 70°C × 3 min resulted in the most efficacious conditions for controlling CadA, CadB, and CadC cfu growth with respect to the control: 61.1% and 74.4%, 88% and 100%, and 100% and 100%, respectively.

3.2 | In vivo treatments

After 4 months of storage, heat treatment efficacy was verified on kiwifruit. HA treatment was more efficient than HW treatment, reducing the skin pitting incidence of isolates CadA, CadB, and CadC by 81.6%, 36.7%, and 21.6%, respectively, compared to the control; conversely, HW seemed to stimulate incidence in isolate CadA (Figure 3). The lesion diameter was measured by removing the skin of 15 infected fruits for each isolate, and results are reported in Figure 4. Results confirmed HA as the most active treatment against *C. luteo-olivacea*, which was able to reduce aggressiveness of isolates CadA, CadB, and CadC by 18.6%, 41.1%, and 68.2%, respectively, compared the control.

3.3 | Heat treatment effect on *C. luteo-olivacea* pathogenic enzyme production

The effect of heat treatments on mycelial and conidial enzyme activity was evaluated as virulence key factors of *C. luteo-olivacea* isolates. Two lytic enzymes, xylanase and pectinase, were considered. Of these, xylanase activity produced by *C. luteo-olivacea* isolates was the most prevalent, with control samples showing an average of 243.6 and 763.6 mm^2 mycelial and conidial halo area, respectively, whereas pectinase showed an average of 75 and 99 mm^2 mycelial and conidial halo area, respectively; Table 1). For both enzymes, activity was greater in the conidia than the mycelia. The treatments completely inhibited pectinase in the isolates, hence only the control values are reported (Table 1). However, both treatments showed a substantial reduction of xylanase, with variation between isolates. In fact, HW treatment on *C. luteo-olivacea* mycelial xylanase activity exerted a reduction of 100% (both CadA and CadC isolates) and 95.3% (isolate CadB), and on conidial activity by 96.7% (CadA), 100% (CadB), and 100% (CadC), respectively.

3.4 | FT-IR analysis of heat treatment effect on *C. luteo-olivacea* and kiwifruit

Figure 5 shows the effects of the thermal treatments on the chemical composition of kiwifruit skin (a,b) and pulp (c,d). FT-IR spectroscopic investigation was focused on the following biochemical

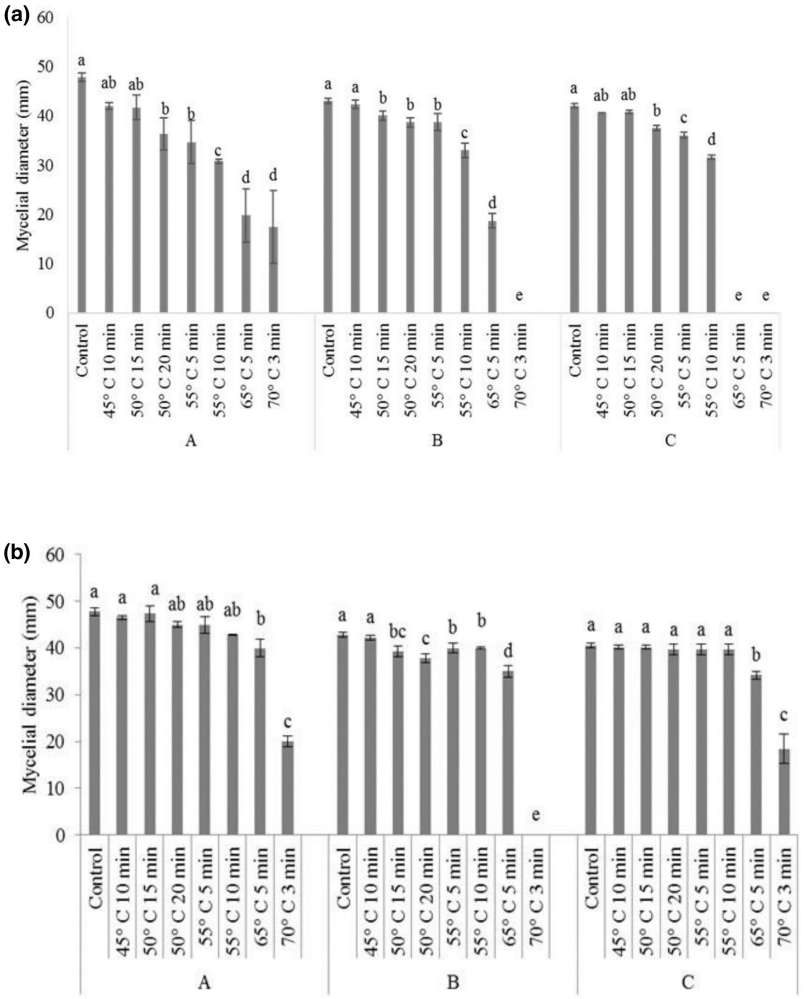
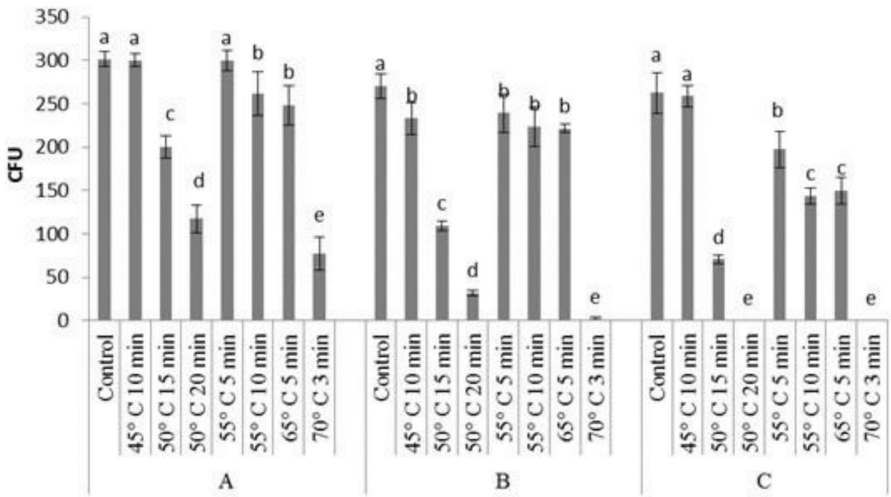


FIGURE 1 Effect of hot water (a) and hot air (b) treatments (45, 50, 55, 65, 70°C for 10, 15 or 20, 5 or 10, 5, and 3 min, respectively) on mycelial growth (diameter, mm) of *Cadophora luteo-olivacea* isolates (A, CadA; B, CadB; C, CadC). Each value is the mean of three replicates ± standard error. Within the same isolate, different letters represent significant differences according to Tukey's HSD test ($\alpha = 0.05$)

FIGURE 2 Effect of hot air treatments (45, 50, 55, 65, 70°C for 10, 15 or 20, 5 or 10, 5, and 3 min, respectively) on colony-forming units (cfu) of *Cadophora luteo-olivacea* isolates (A, CadA; B, CadB; C, CadC). Each value is the mean of three replicates ± standard error. Within the same isolate, different letters represent significant differences according to Tukey's HSD test ($\alpha = 0.05$)



compounds: proteins (amide bands in the 1700–1500 cm^{-1} region), carbohydrates (mainly cellulose, hemicellulose, pectin, and monosaccharides such as glucose or fructose), aromatic compounds (phenolics in the 1620–1580 and 1210–1180 cm^{-1} regions, and lignin at 1510 cm^{-1}), and cuticular waxes (mainly on fruit skin, at 2920–2850 and 1730 cm^{-1}; Di Francesco et al., 2020). In order to visualize the chemical differences induced by thermal treatments more easily, difference spectra are reported: positive and negative peaks are

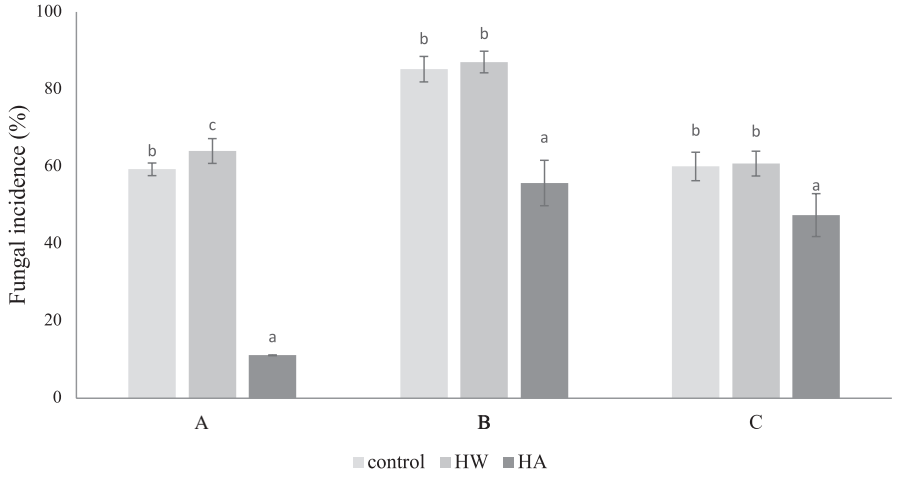


FIGURE 3 Hot water (HW) and hot air (HA) treatments (70°C × 3 min) were conducted on kiwifruits, artificially inoculated with 20 μl of a conidial suspension of *Cadophora luteo-olivacea* isolates (A, CadA: B, CadB; C, CadC; 10^5 conidia/ml). Fruits were stored for 4 months at 0°C and 90% RH. Untreated fruits were used as control. Each value is the mean of three replicates (60 fruits) ± standard error. Different letters represent significant differences within the treatments among the fungal isolates according to Tukey's HSD test (α = 0.05)

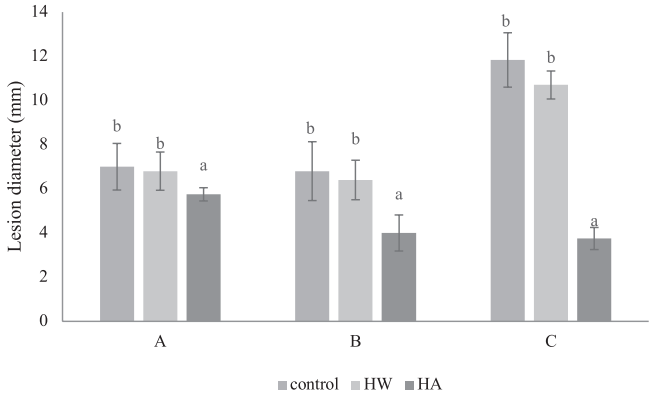


FIGURE 4 Lesion diameter (mm) caused by *Cadophora luteo-olivacea* isolates (A, CadA; B, CadB; C, CadC) on kiwifruit treated with hot water (HW) and hot air (HA) (70°C × 3 min), after 4 months of storage at 0°C and 90% RH. Untreated fruits were used as control. Each value is the mean of 15 fruits with symptoms ± standard error. Different letters represent significant differences within the treatments among the fungal isolates according to Tukey's HSD test (α = 0.05)

associated with a higher or lower content of the associated compounds, respectively.

Figure 5a presents the spectrum of kiwifruit skin after HA treatment (continuous line) and the difference spectrum with the control (dashed line). The HA treatment induced a decrease in cuticular waxes (2918, 2850, 1732, and 1165 cm^{-1}) and cellulose (1437, 1315, 1103, 1057, 1028, 657, 582, and 567 cm^{-1}) bands, while the bands associated with aromatic compounds, such as phenols and flavonoids, increased (1575, 1558, and 1145 cm^{-1}), except for lignin (slight decrease at 1502 cm^{-1}). Similarly, glucose bands at 918 and 773 cm^{-1} slightly increased because of the possible degradation of polysaccharides induced by HA treatment. The behaviour of pectin bands was more complicated: a more detailed investigation of these bands revealed that pectin bands showing a negative peak in the difference spectrum were associated with the degree of polymer esterification. In particular, the bands at 1745, 1234, and 943 cm^{-1} come from the ester group vibrations. Another chemical modification of pectins is reflected in the increase of the 1396 cm^{-1} band, attributed to the carboxyl group vibration, and the 688 cm^{-1} band, attributed to pectin rings caused by pectin degradation.

HW treatment caused similar effects on kiwifruit skin (Figure 5b): in particular, waxes and cellulose bands decreased, while phenolics, flavonoids, and glucose bands increased. Again, pectin bands showed both positive and negative bands in the difference spectrum (dashed line); interestingly, the 986 cm^{-1} band attributed to pectin methyl esters showed a marked decrease. Another interesting feature of HW treatment was the increase of the 853 cm^{-1} band of hemicellulose, which could reflect a chemical modification of cellulose moieties after HW treatment. In Figure 5a, the difference spectrum between HW and HA treatments (dashed and dotted line) help to compare the effects of both treatments. Phenolics/flavonoids and wax content are higher after HW treatment, while cellulose showed the opposite trend. As previously mentioned, hemicellulose and glucose content was higher after HW treatment, which could have caused a higher degradation of carbohydrates. The degree of pectin esterification was higher in HA treatment: negative peaks appeared in the difference spectra at 1717, 1225, and 972 cm^{-1}, with the latter attributed to methyl ester vibrations.

Figure 5c,d show chemical differences induced in fruit pulp. In general, fewer differences were observed compared to fruit skin. Pectin appeared to be the compound most affected by HA treatment (Figure 5c): the esterification degree increased (positive peak at 1717 cm^{-1}), but the negative band at 999 cm^{-1} (decreased methyl esters) indicated a critical chemical variation occurring after treatment. Celluloses were also affected by HA treatment, with a decrease in both cellulose and hemicellulose bands.

TABLE 1 Halo area of conidial and mycelial xylanase and pectinase activity of *Cadophora luteo-olivacea* isolates after exposure to hot water and hot air treatment (70°C × 30 min)

		Isolate					
		CadA		CadB		CadC	
Enzyme	Treatment	Conidia	Mycelia	Conidia	Mycelia	Conidia	Mycelia
Xylanase (mm^2)	Control	697.4 ± 70.2 a	232.6 ± 22.4 a	826.1 ± 92.4 a	272.1 ± 26.5 a	768.0 ± 64.7 a	227.5 ± 26.6 a
	Hot water	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 b	12.7 ± 4.4 c	0.0 ± 0.0 b	0.0 ± 0.0 c
	Hot air	22.6 ± 5.5 b	85.1 ± 9.0 b	0.0 ± 0.0 b	49.1 ± 1.2 b	0.0 ± 0.0 b	61.0 ± 5.9 b
Pectinase (mm^2)	Control	114.0 ± 22.0 b	95.3 ± 12.2 a	92.0 ± 12.7 b	69.8 ± 20.0 a	92.3 ± 17.8 b	61.0 ± 6.0 a

Note: Each value is the mean of three replicates ± standard error. Within the same isolate and form of propagation (conidia or mycelia), different letters represent significant differences according to Tukey's HSD test (α = 0.05).

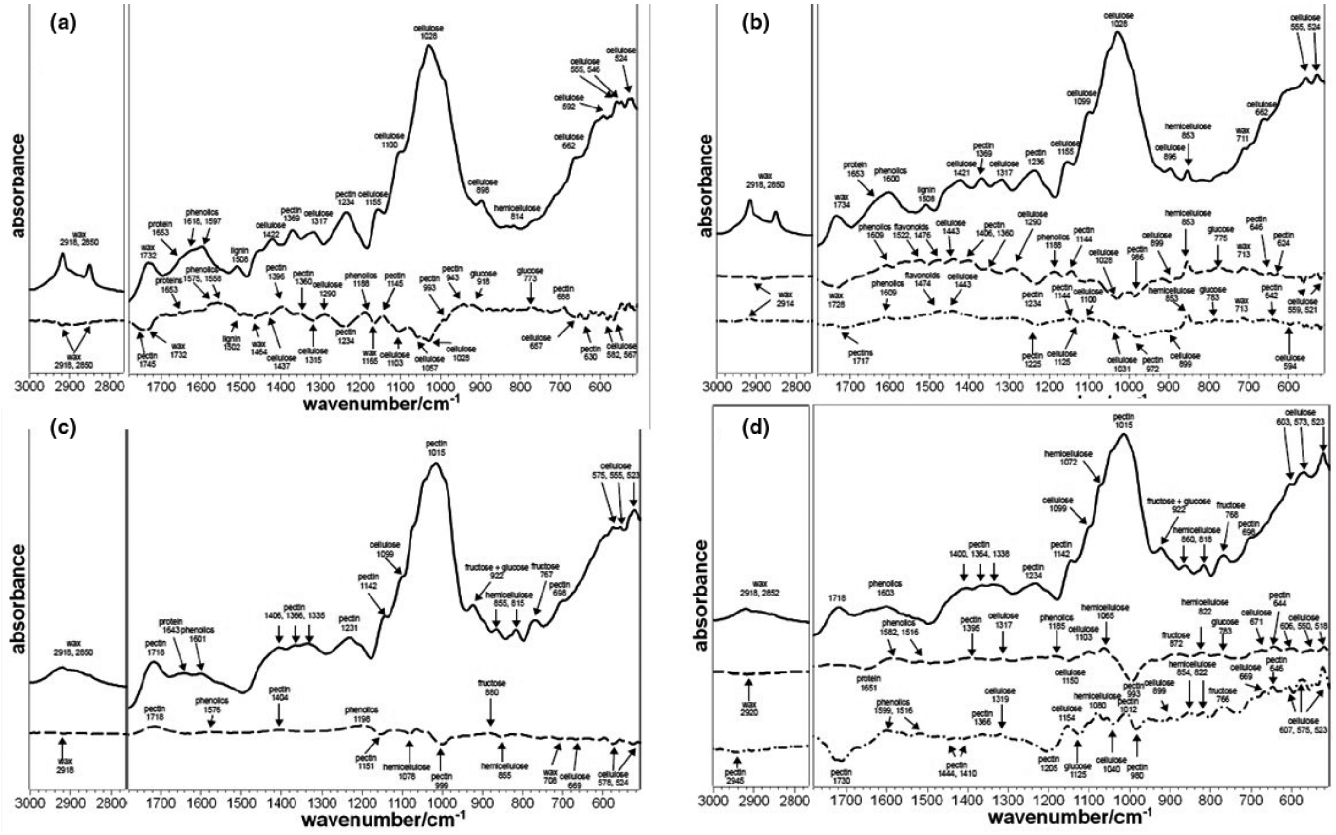


FIGURE 5 Fourier-transform infrared (FT-IR) spectroscopy spectra of the effects of the thermal treatments on the chemical composition of kiwifruit skin (a, b) and pulp (c, d) after hot air (HA) or hot water (HW) treatments (70°C × 3 min). (a) Continuous line: kiwifruit skin after HA treatment; dashed line: difference spectrum between HA-treated and control kiwifruit skin. (b) Continuous line: kiwifruit skin after HW treatment; dashed line: difference spectrum between HW-treated and control kiwifruit skin; dashed and dotted line: difference spectrum between HW- and HA-treated kiwifruit skin. (c) Continuous line: kiwifruit pulp after HA treatment; dashed line: difference spectrum between HA-treated and control kiwifruit pulp. (d) Continuous line: kiwifruit pulp after HW treatment; dashed line: difference spectrum between HW-treated and control kiwifruit pulp; dashed and dotted line: difference spectrum between HW- and HA-treated kiwifruit pulp

HW treatment on pulp generally had similar effects (Figure 5d). Comparing the two treatments gave interesting indications (dashed and dotted lines): phenolics gave more intense bands after HW treatment, while the degree of esterification of pectins was reduced after HW treatment. Other carbohydrates, such as cellulose and hemicellulose, gave more positive peaks after HW treatment (i.e., 1319, 1154, 899, 669, 607, 575, and 523 cm^{-1} for cellulose; 1080, 854, and 822 cm^{-1} for hemicellulose; 766 cm^{-1} for fructose).

IR spectra were also recorded on *Cadophora* mycelia before and after the heat treatments (Figure 6). IR spectra are dominated by the biochemical compounds present in the cell wall. In the ascomycete *Paracoccidioides brasiliensis*, Ferreira et al. (2016) reported that the mycelium contains up to 40% proteins, 40% glucans (mainly

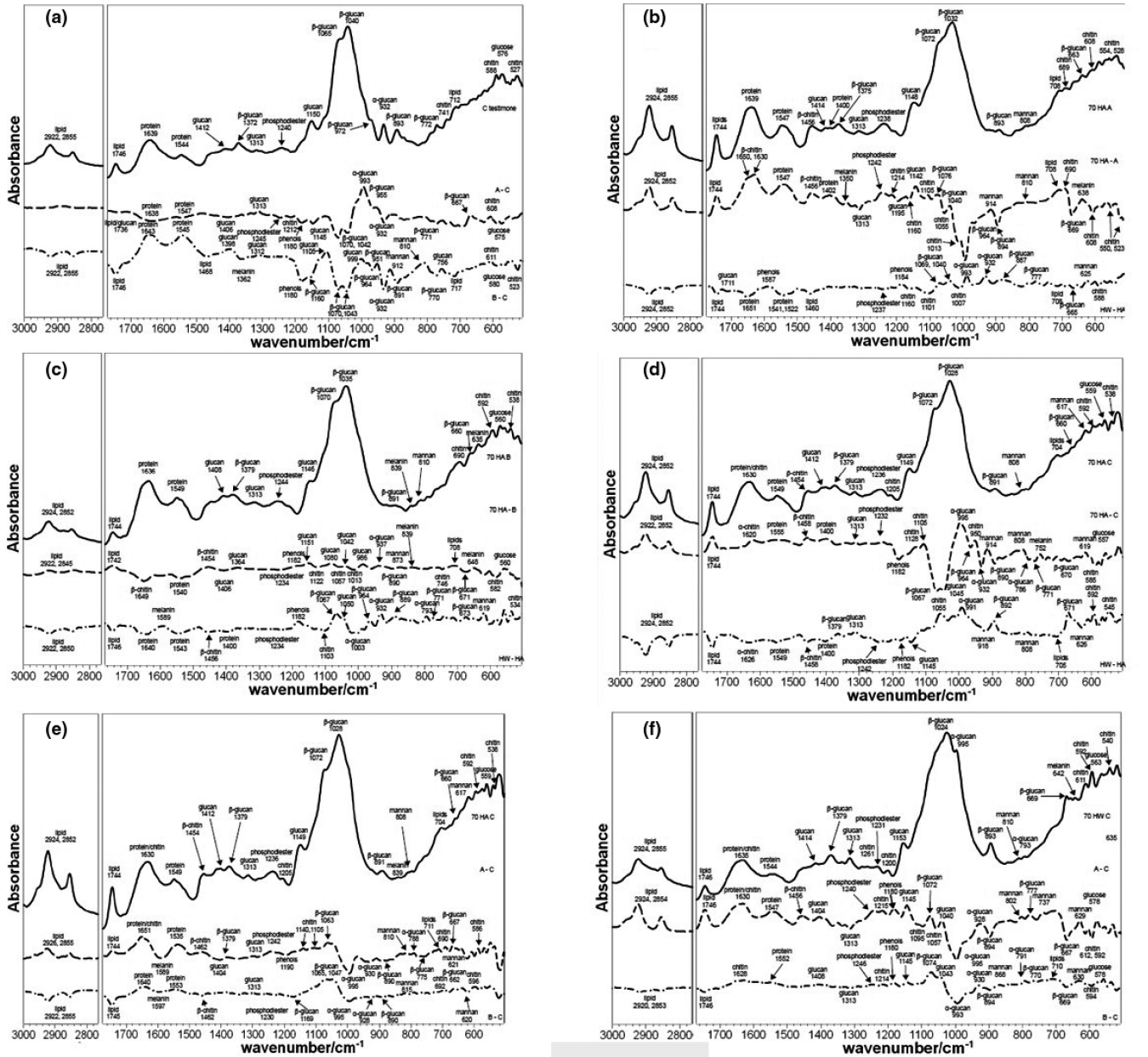


FIGURE 6 Fourier-transform infrared (FT-IR) spectra of the effects of the thermal treatments on the chemical composition of mycelium of *Cadophora* isolates (A, CadA; B, CadB; and C, CadC) before and after hot air (HA) and hot water (HW) treatments (70°C × 3 min). (a) The main biochemical differences between the three isolates before heat treatments. (b, c, d) Differences in mycelial biochemical composition after the heat treatments at 70°C × 3 min for both HA and HW. (e, f) Comparison between the effects of HA (e) and HW (f) on the three isolates. (a) Continuous line: isolate C before treatment; dashed line: difference spectrum between isolates A and C before treatment; dashed and dotted line: difference spectrum between isolates B and C before treatment. (b) Continuous line: isolate A after HA treatment; dashed line: difference spectrum between isolate A HA treatment and control; dashed and dotted line: difference spectrum between isolate A HW treatment and HA treatment. (c) Continuous line: isolate B after HA treatment; dashed line: difference spectrum between isolate B HA treatment and control; dashed and dotted line: difference spectrum between isolate B HW treatment and HA treatment. (d) Continuous line: isolate C after HA treatment; dashed line: difference spectrum between isolate C HA treatment and control; dashed and dotted line: difference spectrum between isolate C HW treatment and HA treatment. (e) Continuous line: isolate C after HA treatment; dashed line: difference spectrum between isolate C HA treatment and isolate A HA treatment; dashed and dotted line: difference spectrum between isolate C HA treatment and isolate B HA treatment. (f) Continuous line: strain C after HW treatment; dashed line: difference spectrum between isolate C HW treatment and isolate A HW treatment; dashed and dotted line: difference spectrum between isolate C HW treatment and strain B HW treatment

β-glucans), 18% chitin, and traces of lipids, mannans and melanins as an organic pigment. IR spectra allowed discrimination of the main biochemical differences between the three isolates before heat treatments (Figure 6a): based on the 1650–1530 cm^{-1} spectral region, the protein content follows the order: CadC > CadB > CadA, while CadC showed the highest lipid content, aromatic compounds

(i.e., phenols) and glucans. Interestingly, CadB showed the most significant variations compared to CadC, with a higher content of proteins and mannans and a high reduction in lipids and glucan contents.

Figure 6b–d shows the differences in mycelial biochemical composition after the heat treatments at the highest temperature (70°C for both HA and HW). In isolate CadA, the HA treatment affected mainly glucans. As a matter of fact, the main bands at 993 cm^{-1} (α-glucan) and 890 cm^{-1} (β-glucans) showed negative peaks. On the other hand, HW treatment induced a decrease in many biochemical components: lipids, proteins, and chitin, while glucans were better preserved compared to the HA treatment (positive peaks). HA and HW treatments on isolate CadC (Figure 6d) showed a similar trend, while HA treatments showed lower effects on isolate CadB, mainly on protein and chitin content, while confirming the effects mentioned above after HW treatment.

In Figure 6e,f, the comparison between the effects of HA (Figure 6e) and HW (Figure 6f) on the three isolates is presented. Taking isolate CadC as a reference, the main positive peaks refer to proteins (in the 1650–1530 cm^{-1} spectral region) and β-glucans (1065 cm^{-1}), while the main negative peaks refer to α-glucans (995 cm^{-1}) and mannans (620 cm^{-1}). Lipid bands behaved differently between isolate CadA (relative content increased, positive peaks at 1744 cm^{-1}) and isolate CadB (negative peak at 1744 cm^{-1}).

Generally, HW treatment showed more considerable differences between the isolates than HA treatment (Figure 6f) but similar trends compared to HA treatment were observed. Interestingly, HW treatment also affected β-glucans content, as shown by the negative peaks at 894 and 668 cm^{-1}.

4 | DISCUSSION

Heat treatments are alternative methods to synthetic fungicides for sustainably containing fungal pathogen infections. In this study, significant differences and responses were found in the resistance of *C. luteo-olivacea* conidia and mycelia to different temperatures and exposures to heat treatments. *C. luteo-olivacea* is quite tolerant of different temperature × time combinations (Gramaje et al., 2010). Significant differences were found between different temperatures and times on the viability of conidia and mycelia for all tested fungal isolates. Results confirmed that higher temperatures such as 65 and 70°C are needed to reduce or inhibit mycelial growth significantly. IR spectra focused on the biochemical effects on mycelia after heat treatment. All isolates showed that proteins and glucans, the main components of the mycelial cell wall (accounting for up to 80% of the total composition; Ferreira et al., 2016), were strongly affected by both heat treatments at the highest temperature. Gramaje et al. (2010) reported that the colony growth rate decreased with increased temperature and time combinations. Mycelia were generally less susceptible to heat treatments than conidia.

Conversely, a lower temperature can inactivate almost all conidia of the tested isolates of this species (50°C). The present study was conducted to investigate the development of an effective control strategy to reduce the incidence and aggressiveness of *C. luteo-olivacea* infection in kiwifruit. In fact, studies conducted to reduce the incidence of *C. luteo-olivacea* in kiwifruit are still scarce. Most studies conducted in recent years with heat treatments have involved stone fruit and pome fruit, showing excellent efficacy, especially on the latter fruit species (Maxin et al., 2012b). Studies have shown that short-term heat treatments at relatively high temperatures effectively reduce microbial contamination and insect infestations, maintaining the storage quality of fruits (Kabelitz & Hassenberg, 2018; Lurie, 1998; Rux et al., 2019). Therefore, the efficacy of a short exposure at the highest temperature treatment (70°C × 3 min) was tested on the enzyme activity of three isolates, both on the infective potential of the mycelium and the conidia. As for *B. cinerea* and *Neofabraea vagabunda* (Di Francesco, Mari, Roberti, et al., 2018), the pathogenesis enzymes most produced by *C. luteo-olivacea* were confirmed to be xylanase and pectinase, mainly produced by the fungal mycelium. Pectinases are usually the enzymes most produced by fungal pathogens (Miedes & Lorences, 2004) and, together with xylanases, give pathogens high virulence and aggressiveness (Kikot et al., 2009). Heat treatments at high temperature (70°C × 3 min) for a short time on both sources of fungal inoculum (mycelia and conidia), showed a direct effect on enzyme production. This finding was further confirmed by the reduction of protein bands observed after heat treatment by FT-IR spectroscopy.

We focused our analysis on pectinases and xylanases because they represent the major plant cell wall-degrading enzymes (CWDEs) in fungal pathogens. The heat treatments increased the inhibition of enzyme activity of *C. luteo-olivacea* mycelia and conidia. It has also been demonstrated that the effect of heat treatments can be directly related to inhibition of pathogen spore germination (Di Francesco, Mari, Roberti, et al., 2018). The results obtained in vitro support the hypothesis that heat treatments can act on the fungus, a hypothesis that should be correlated with in vivo efficacy. However, conversely to what was seen in in vitro experiments, HW treatment (70°C × 3 min) on kiwifruits displayed increased incidence of fungal infection. In particular, HW treatment seems to have influenced some biochemical aspects of the fruit, as confirmed by FT-IR analyses. The spectra show that the heat treatments can determine an increase in the degree of esterification of pectins in the pulp of treated fruits (Le Cam et al., 1994; Marty et al., 1997; McMillan et al., 1993; Wydra & Beri, 2006), and a reduction in the content of superficial waxes in the peel, data closely related to a probable influence on the susceptibility of the host to a fungal attack. Also, there was an evident increase in aromatic compounds and flavonoid production. Ruan et al. (1995) showed that some flavonoids produced by plants could stimulate the conidial germination of fungal pathogens. In agreement with these results, Spadoni et al. (2015) showed that peaches treated with HW and subsequently inoculated with *Monilinia fructicola* stimulated pathogen conidial germination. It can be deduced from the obtained results that the most active heat treatments in in vitro experiments, unlike in in vivo conditions, can increase pathogen virulence.

Interestingly, HA treatment slowed down the fungal incidence and reduced isolate virulence, leading to a reduction in lesions, on average, of 43.5% with respect to the control. These results show how heat

treatments could provide a good efficacy in the containment of fungal development and affect the biochemical composition of the fruit, an aspect closely correlated to the host–pathogen interaction. A similar outcome was detected by Maxin et al. (2012a), where complete control of *Penicillium expansum* was achieved in vitro after a HW treatment of 3 min at 70°C, but caused fruit physiological disorder such as heat scald. Also, the ability of fungi to grow on heat-damaged fruits was directly correlated with the heat tolerance of spores in vitro.

Ambuko et al. (2012) reported that high-temperature preconditioning with HA has the potential to impart tolerance to storage injuries in some commodities, probably strictly related to the fruit heat-shock response (Maxin et al., 2012b) and antioxidant capacities of fruits during storage. For instance, HA treatment has also been reported to enhance the total phenolics and flavonoids in cherry tomato fruit and induce disease resistance by activating phenylpropanoid metabolism (Wei et al., 2017). Our study demonstrated that HA treatment significantly reduced the rate of softening during kiwifruit storage (data not shown) with respect to control fruit and HW treated fruits, in accordance with Chang et al. (2019).

These findings show that heat treatments are effective against *C. luteo-olivacea* growth, but in kiwifruits the development of time × temperature combinations remains the main challenge to ensure quality and resistance during and after cold storage.

These results could help in understanding the principles of heat treatments on kiwifruit to reduce the occurrence of skin pitting symptoms, and indirectly to reduce food waste by increasing consumer satisfaction with chemical-free and environmentally friendly technology. The possible use of other heat treatments or alternative fungal containment systems will be necessary for larger scale applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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