Elicitor Activity of Curdlan and Its Potential Application in Protection of Hass Avocado Plants against Phytophthora cinnamomi Rands

Nathalie Guarnizo 1,2,* 1, Andree Álvarez 1 2, Diego Oliveros 1, Oveimar Barbosa 1, Jordi Eras Joli 3, María Bianney Bermúdez-Cardona 4 and Walter Murillo-Arango 1, 2, *

1 Departamento de Química, Facultad de Ciencias, Universidad del Tolima, Ibagué 730006, Colombia; asalvarezg@ut.edu.co (A.Á.); dfoliverosg@ut.edu.co (D.O.); lobarbosaj@ut.edu.co (O.B.)
2 Departamento de Química, ETSEA, Universidad de Lleida, 25198 Lleida, Spain
3 Departamento de Química, Servicios Científico Técnicos-TCEM, Universidad de Lleida, 25198 Lleida, Spain; jordi.eras@udl.cat
4 Departamento de Producción y Sanidad Vegetal, Facultad Ingeniería Agronómica, Universidad del Tolima, Ibagué 730006, Colombia; mbermudez@ut.edu.co
* Correspondence: anguarnizop@ut.edu.co (N.G.); wmurillo@ut.edu.co (W.M.-A.); Tel.: +57-3003634776 (N.G.); +57-3147185845 (W.M.-A.)

Abstract: Phytophthora cinnamomi causes one of the most important diseases in avocado crop and its chemical management represents 25% of the production cost per year. Induction of plant defense responses by elicitors is a promising strategy that is compatible with sustainable agriculture. This study aimed to evaluate the effect of curdlan application on the induction of defense responses in avocado plants against P. cinnamomi. The trials were conducted under greenhouse conditions, and curdlan leaf spraying was performed one day before the inoculation of the pathogen. The results showed that the application of elicitor significantly increased the protection of avocado plants against P. cinnamomi, decreasing the injury and wilting. The Curd + Phy treatment improved the defenses of plants by increasing the enzymes peroxidase (POD) and polyphenol oxidase (PPO) in the first 3 h after inoculation and increasing the enzymes superoxide dismutase (SOD) and phenylalanine ammonium lyase (PAL) 144 h after inoculation (p < 0.05). Also, chlorophyll and carotenoid content increased or remained stable in Curd + Phy treatment. Therefore, these results suggest that curdlan increases the protection against P. cinnamomi and its protection could be due to an increase in the activity of the enzymes related to the phenylpropanoid pathway as well as the effect on chlorophyll and carotenoids.

Keywords: avocado; Phytophthora cinnamomi; induced resistance; curdlan; defense-related enzymes

1. Introduction

The avocado (Persea americana) is an important fruit appreciated for its taste and for its high nutritional value and bioactive compounds with health benefits, such as antioxidants, phenolic compounds, fiber, vitamins, and low sugar content [1,2]. Recently, it has been a highly profitable crop on the national and international market, which increased the areas for its cultivation worldwide [3]. The “Hass” variety is the most common worldwide [4]. It has been suggested that organoleptic characteristics and better post-harvest conservation of Hass avocado fruit has influenced its increased production worldwide [5]. However, there are phytosanitary problems caused by the oomycete Phytophthora cinnamomi Rands, the main avocado Hass crop pathogen, causing losses of up to 90% [6–9]. P. cinnamomi is also the species with the widest host range within its genus and is considered an invasive species and one of the most destructive phytopathogens, known by some as the “biological bulldozer” [8,10].
The cost for chemical management of root rot disease caused by *Phytophthora* is important; the fungicide market represents over 25% of production costs per year. However, there are phylogenetical differences between *P. cinnamomi* and true fungi, implying an inefficient action of fungicides in general against this pathogen [11]; for that reason, many broad-spectrum fungicides are not effective against these organisms and few alternatives are available to manage this pathogen (e.g., metalaxyl, mefenoxam and fosetyl-Al). Nevertheless, the rapid regeneration times and exceptional adaptability of *Phytophthora* spp. have resulted in the development of fungicide resistance within specific pathogen populations [12].

On the other hand, new strategies are needed urgently to minimize the quantities of pesticides in the soil and their residues in food products. The stimulation of natural plants’ defenses by the use of elicitor molecules is considered one of the most promising alternatives that can be included in integrated pest management (IPM) [13].

These important molecules offer an interesting possibility that activates the defense genes, leading to a systemic acquired resistance [12,13]. Microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) can induce plant innate immunity to protect plants from microbial pathogens [14–16]. The recognition of a pathogen, after eliciting the plant, generates an improved defense response to prevent the development of the pathogen. One of the common responses is the accelerated generation of reactive oxygen species (ROS), which are second messengers in a variety of cellular processes in response to stress caused by pathogens. Their production regulates processes such as the upregulation of enzymes of the phenylpropanoid pathway that generate phenolic compounds with antioxidant activity that regulate ROS, production of antimicrobial compounds to attack the pathogen directly and cell wall strengthening to avoid the entry of the pathogen [17,18].

These elicitors include glycoproteins, carbohydrates (like β-1,3-glucan), chitin, fatty acids, proteins, glycosphinoglipids and peptides [19–21]. Curdlan (Curd), a linear water-insoluble β-1,3-glucan, was first detected in *Alcaligenes faecalis* var. *myxogenes* and belongs to microbial exopolysaccharides and also is produced by fermentation of *Agrobacterium* sp.; it is also approved as a safe food additive [22,23]. Curd activates the defense responses in tobacco (*Nicotiana tabacum* L.) cells [23], and it also elicits production of another defense response, such as capsaicin in *Capsicum frutescens* [24]. More recently, it has exhibited an activation effect on the early- and late-defense responses in potato leaves [22].

This study aimed to investigate the role of curd as a defense inducer in Hass avocado plants against *P. cinnamomi* under greenhouse conditions. To determine the effect of the inducer, differences in defense-related enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), phenylalanine ammonylase (PAL), peroxidase (POD), polyphenoloxidase (PPO), phenolic content and antioxidant activity were evaluated. The authors hope that this work can be of great value and can afford a theoretical basis for further studies on the possibility to include β-glucans in many integrated management programs in the avocado crop.

### 2. Materials and Methods

#### 2.1. Plant Material

Hass avocado plants were obtained from a plant nursery that has a registered and phytosanitary status, located in Libano, Tolima, Colombia. Young (six-month-old) plants were used for experimental analysis. The plants were kept in a greenhouse at ambient humidity and temperature conditions in the facilities of the University of Tolima (4.428242, −75.213478) at 1285 masl. They were maintained with a photoperiod of 12 h light and 12 h of darkness.

#### 2.2. Pathogen

The Hass avocado soil pathogen *P. cinnamomi* was supplied by Corporación de Investigaciones Biológicas (CIB), located in Medellín (Colombia). The culture was main-
tained on Potato Dextrose Agar (PDA) at 25 °C. Fifteen-day-old culture was used as an active mycelium.

2.3. Elicitor Preparation and Application

Curd was purchased from Sigma (Israel) and was dissolved in a solution of 1% DMSO and 0.05 mM NaOH at a concentration of 2658 mg/L. The solution was stored at 4 °C and no precipitation of the compound was observed. The application of the curd was made according to the methodology proposed by Li et al. [22], with some modifications. Hass avocado plants were treated in four different ways, including: (1) Plants sprayed with 5 mL of the solution, representing 13.29 mg of Curd 1 day before P. cinnamomi treatment (Curd + Phy); (2) Plants sprayed with 5 mL of the solution, representing 13.29 mg of Curd 1 day before without pathogen to establish an elicitor control (Curd); (3) Plants with the pathogen (Phy) and (4) Control plants (Control). A total of 120 Hass avocado plants were randomly used in each repetition of the experiment, 30 in each group. The experimental unit was composed of 2 plants, and 3 replicates were assayed.

Hass avocado plants were inoculated with P. cinnamomi according to the methodology of Dinis, et al. [25]. A “T” form incision was made in the plants, and a PDA plug with oomycete was incorporated. Inoculation was done directly in the stem. After inoculation, the incision was covered with a piece of cotton wool to ensure an adequate moisture level for infection and disease development. The whole assay was repeated three times, carried out in November 2018, March, and April 2019, and the leaves were collected between 10:00 and 13:00 at 0, 3, 24, 144 and 312 h after inoculation (hai).

2.4. Observation of Lesions Caused by Phytophthora Cinnamomi on Hass Avocado Plants under Greenhouse Conditions

To observe the development of lesions, the inoculated Hass avocado plant were inspected every three days for three times. The lesion was measured with a vernier caliper to see its progress. This process was done until day nine after inoculation.

2.5. Sample Preparation

The harvest was made by taking the whole leaves of two plants to make one sample. A total of three samples (from six plants) were established in each interaction between time and treatment to account for biological variations. Samples were quickly frozen with liquid nitrogen (shock freezing), then powdered under the same condition and subsequently stored at −80 °C [26].

2.6. Measurements of Active Defense Response-Related Enzymes, Antioxidant Enzymes Activities and Total Phenolic Content in Avocado Fruit

The enzyme extraction process was carried out as proposed by Sellamuthu, et al. [27]. SOD activity was assessed according to the methodology proposed by Beauchamp and Fridovich [28], with the modifications made by Demiral and Türkan [29]. APX activity was assayed according to Nakano and Asada [30]. POD activity was determined according to the Sellamuthu, et al. [27] method.

GR activity was determined as described by Smith, et al. [31], with some modifications. The standard reaction mixture contained 0.2 M potassium phosphate (pH 7.5), containing 1 mM EDTA, 3 mM DTNB, 2 mM NADPH and 20 mM GSSG. The specific activity of the enzyme was expressed as U mg⁻¹ protein.

PAL activity was assayed as follows: 1.0 mL homogenate was incubated with 1.0 mL of 50 mM borate buffer (pH 8.8) and 1.0 mL of 20 mM L-phenylalanine for 60 min at 37 °C. The reaction was terminated by the addition of 0.1 mL of 6.0 M HCl. PAL activity was determined based on the production of trans-cinnamate, measured by the absorbance change at 290 nm. The blank was the crude enzyme preparation mixed with L-phenylalanine with zero-time incubation. One unit of enzyme activity represents the amount of enzyme that produces 1.0 µmol of cinnamic acid per hour [32].
PPO activity was assayed spectrophotometrically according to the method of Xie, et al. [32] with a slight modification. The reaction mixture consisted of 20 µL homogenate and 200 µL substrate solution, containing 0.1 M catechol as the substrate and 0.1 M sodium phosphate buffer (pH 7.4). The reference cuvette contained only the substrate. The rate of oxidation of catechol was monitored at 410 nm at 25 °C for 1 min.

2.7. Protein Assay

The protein content in the extract was estimated using the protein-dye binding method of Bradford [33] with Bovine Serum Albumin (Sigma, St. Louis, MO, USA) as a standard.

2.8. Total Phenolic Content (TPC)

Total phenolic compounds were extracted according to the method of Makkar [34], with some modifications. A total of 50 mg of plant material was taken in a tube with 750 µL of 70% aqueous acetone and put on a shaker for two hours at room temperature. Then, the tubes were subjected to centrifugation at 3000 rpm for 10 min at 4 °C. The supernatant was collected and kept cool, and an aliquot of 80 µL was suspended in 920 µL of methanol. The Folin–Ciocalteu method was used for the determination of total phenols by some modification of the method of Cheplick, et al. [14]. A total of 150 µL of water, 20 µL of sample and 10 µL of Folin–Ciocalteu reagent were added to the well and allowed to stand for 6 min. Afterward, 20 µL of sodium carbonate (7.5%) solution was added to each well and incubated at 22 °C for 1 h in the dark, and the absorbance was measured at 760 nm (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Waltham, MA, USA). Standard curves were made using increasing concentrations of gallic acid in 95% ethanol. Absorbance values were converted to total soluble phenolic content and expressed as µg equivalents of gallic acid per gram fresh weight (FW) of shoot sample.

2.9. DPPH Free Radical Scavenging Activity

The activity of elimination of the free radicals of the extracts was determined based on the DPPH [35], with some modification. A 50 µL aliquot of the extract (obtained in Section 2.8) was added into 100 µL of a diluted DPPH solution in ethanol (0.0236 mg/mL), stirred, and incubated for 30 min in the dark, and the absorbance was measured at 517 nm. The standard curve was obtained with Trolox (5–80 µmol/L in ethanol). The results were expressed in mM Trolox/mg extract.

2.10. Determination of Chlorophyll and Carotenoid Content

Chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids were determined spectrophotometrically [36] and the results were calculated using the equations described in [37].

2.11. Statistical Analysis

Data from at least three replicates consisting of a pool of six plants were analyzed. All statistical analyses were performed with R software. All data underwent a one-way ANOVA, and differences between treatments and control data were assessed using the Tukey post-hoc test at a significance level of 0.05.

3. Results

3.1. Observation of Lesions Caused by Phytophthora Cinnamomi on Hass Avocado Plants under Greenhouse Conditions

Minimum length of the developed lesion was observed when the plants were sprayed with curdlan one day before the inoculation of *P. cinnamomi*, unlike in the plants in which only the pathogen was inoculated (Table 1). The plants treated with Curd were found to have dark brown lesions significantly smaller than those in untreated plants, and no sign of wilt was observed until the end of the trial (Curd + Phy). Otherwise, it was also possible
to observe a wilting in the leaves and branches of the plants inoculated with P. cinnamomi but without curdlan spray (Phy).

Table 1. Lesion length caused by P. cinnamomi on avocado plants sprayed with curdlan and on untreated plants inoculated with the pathogen.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Length of the Lesion (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 dai</td>
</tr>
<tr>
<td>Curd + Phy</td>
<td>2.32 ± 0.32 a</td>
</tr>
<tr>
<td>Phy</td>
<td>3.56 ± 0.24 b</td>
</tr>
<tr>
<td></td>
<td>6 dai</td>
</tr>
<tr>
<td>Curd + Phy</td>
<td>3.39 ± 0.34 a</td>
</tr>
<tr>
<td>Phy</td>
<td>4.53 ± 0.28 b</td>
</tr>
<tr>
<td></td>
<td>9 dai</td>
</tr>
<tr>
<td>Curd + Phy</td>
<td>3.90 ± 0.24 a</td>
</tr>
<tr>
<td>Phy</td>
<td>5.69 ± 0.31 b</td>
</tr>
</tbody>
</table>

Curd + Phy: Plants treated with the elicitor and inoculated with the pathogen. Phy: plants inoculated with the pathogen. Values are the mean ± standard deviation (n = 9). Means with same letters within the same column are not significantly different (ANOVA followed by Tukey’s test at p < 0.05).

3.2. Effect of Curdlan on the Activities of Disease-Resistant Enzymes

In plants, SOD catalyzes the dismutation of the superoxide radical into oxygen and hydrogen peroxide, which is closely related to disease resistance because of its antioxidant activity. The activity of SOD showed an increase in the plants inoculated with P. cinnamomi. The enzyme in Phy was significantly higher during the early stages of inoculation. After 24 h after inoculation (hai), the enzyme activity of Curd + Phy increased (Figure 1). The first peak in SOD activity occurred at 3 hai for Phy and 312 hai for Curd + Phy, which is statistically significant concerning Curd and Control groups (p < 0.05). Therefore, the curdlan treatment (Curd + Phy) appears to increase SOD activity at certain stages following inoculation with P. cinnamomi.

![Figure 1](image)

Figure 1. Effect of curdlan treatment on SOD activity from avocado plant leaves inoculated with P. cinnamomi. Curd + Phy, Phy, Curd and Control represent the application of Curd one day before inoculation of P. cinnamomi, inoculation of P. cinnamomi, application of Curd without inoculation of P. cinnamomi and the control (distilled water), respectively. Data are presented as the mean ± standard deviation for nine replicates. Means with the same letter are not significantly different (ANOVA followed by Tukey’s test at p < 0.05).

APX and GR enzymes were not detected. All relevant modifications were made to detect the activity but could not be measured.

PAL enzyme is important in the first step of phenylpropanoid pathway for the production of some metabolites including lignin (as part of cell wall strengthening). PAL activity was significantly higher (p < 0.05) in the pathogen at the early stage, until 24 hai. After 3 hai, Curd treatment (Curd + Phy) was slowly increased, and the enzyme activity peaked at 144 hai (p < 0.05) (Figure 2a).
POD is an enzyme known to be involved in increasing defenses against pathogens, because it catalyzes the final lignin synthesis. The most significant increase in POD activity was achieved by Curd + Phy at 3 hai with respect to control plants (Figure 2b), whereas the Curd control had a significant increase at 24 and 144 hai ($p < 0.05$). Meanwhile, at other times, the trend was similar among all treatments. However, at the last time, 312 hai, the activity of POD was the same between Curd + Phy and between Curd and Control. After the inoculation with *P. cinnamomi*, plants treated with curdlan increased POD activity at the early stage.

On the other hand, the enzyme PPO has been implicated in plant defense. The oxidation of polyphenols into quinones could have an antimicrobial effect and help avoid the spread of pathogens. In the initial stage, the PPO activity was low in Curd + Phy. After 3 hai, there was a significant increase in PPO activity in the same treatment to enhance toxicity ($p < 0.05$) (Figure 2c), with a maximum value at 3 hai. After that, the enzyme activity decreased. Moreover, the activity for the pathogen treatment was similar to the control group without any peak in the hours evaluated. Thus, curdlan applied before the inoculation with *P. Cinnamomi* increased the activity of PPO, providing an increase in activity at the early stage.

3.3. Effects of Curdan Treatment on Total Phenol Content and Antioxidant Activity

Plants inoculated with *P. Cinnamomi* significantly increased the total phenolic content ($p < 0.05$). The content in Phy accumulated rapidly during the early stages (3 hai). Other treatments did not cause many changes in the hours after inoculation (Figure 3a).
Figure 3. Effect of curdlan treatment on (a) TPC and (b) DPPH activity from avocado plant leaves inoculated with P. Cinnamomi. Curd + Phy, Phy, Curd and Control represent application of Curd one day before inoculation of P. Cinnamomi, inoculation of P. Cinnamomi, and the control (distilled water), respectively. Data are presented as the mean ± standard deviation for nine replicates. Means with the same letter are not significantly different (ANOVA followed by Tukey’s test at p < 0.05).

The antioxidant scavenging activity was found to be higher in plants inoculated with the pathogen (Phy) (p < 0.05). In general, the activity showed a similar trend to total phenolic compounds, so the treatment is similar except for Curd + Phy at 24 hai; at this time the activity is lower than that of other treatments (Figure 3b).

3.4. Effect of Curdlan on Chlorophyll and Carotenoid Content

A significant decrease in Chl a, Chl b and in carotenoid content was observed in the P. cinnamomi-infected plants at 3 hai. Treatment with Curd prior to oomycete inoculation elevated chlorophyll and carotenoid contents at 3 hai. Before and after 3 hai, there were no statistically significant differences between the plants treated with the elicitor and those that were not (Figure 4a–c).

Figure 4. Effect of Curd treatment on (a) chlorophyll a, (b) chlorophyll b and (c) carotenoids from avocado plant leaves inoculated with P. cinnamomi. Curd + Phy, Phy, Curd, and Control represent the application of Curd one day before inoculation of P. cinnamomi, inoculation of P. cinnamomi, application of Curd without inoculation of P. cinnamomi and the control (distilled water), respectively. Data are presented as the mean ± standard deviation for nine replicates. Means with the same letter are not significantly different (ANOVA followed by Tukey’s test at p < 0.05).
4. Discussion

Modern agriculture seeks to be environmentally friendly; therefore, resistance induction is an effective strategy that could be included in integrated disease management plans. As curdlan is an oligosaccharide used as a food additive and is not a potential environmental pollutant, we suggest it could be used in organic agriculture.

The induction of SOD in plant cells in response to different stressful environments reflects its important role in the defense mechanism of plants [38]. In the present study, the SOD enzyme was increased in plants inoculated with the pathogen (Phy) at early stages but decreased in the later stages, whereas plants inoculated with a previous application of Curd (Curd + Phy) had a sustained increase, maybe associated with increased resistance to the pathogen. In the same way, many authors have associated increased production of the SOD enzyme with resistance. For melon against Colletotrichum lagenarium, the resistant cultivar has a higher SOD activity than the susceptible cultivar [39]. Likewise, a study conducted with four cotton genotypes showed, after 90 days of inoculation of the pathogen, that resistant genotypes have a high superoxide dismutase activity compared to susceptible ones [40]. Furthermore, an investigation with salicylic acid as a resistance inducer in beans showed that the SOD enzyme increases in response to that induction, which was associated with a reduction in the susceptibility of this plant [41].

The enzymes APX and GR detoxify the hydrogen peroxide generated by the SOD enzyme. Measuring their activity would have been useful to know how much peroxide was transformed and how much was not. However, it was not possible to measure its activity. In this case, we could suppose that the avocado defense response under the treatment conditions is not associated with an increase of this enzymatic pathways, at least under the detection threshold of the applied techniques. All possible modifications suggested by Bisswanger [42] were performed, but the activity could not be measured. However, in previous works carried out in the research group, these two enzymatic activities have been measured in avocado, rice and tomato leaves (unpublished data), without problems.

Phenylpropanoid metabolism pathway in plants generates phenolic compounds, which have antimicrobial, anti-oxidative, and free radical elimination activity. They also act as phytoalexins, modulators of pathogenicity-signaling molecules [43] and are markers for resistance to pathogens [44] as well as the enzymes involved (PAL, POD and PPO). Consequently, the induction of resistance has been also measured with other defense-related enzymes that are activated when a pathogen is inoculated. PAL was expressed in the treatment with the pathogen (Phy) until 24 hai; after that, it is possible to see an effect of the application of curdlan (Curd + Phy) at 144 and 312 hai. This is an interesting result because many authors report the relation among a high production of PAL enzyme both in resistant genotypes [39] and plants with prior application of an inductor [13,22,45–47].

In this report, the treatment with Curd before the inoculation of P. cinnamomi increased PPO and POD enzymes with very similar behavior, and the highest activity of the enzyme was observed at 3 hai. These enzymes, PPO and POD, are involved in the production of quinones, H₂O₂ removal, oxidation and lignification [48]. Some studies show that the POD enzyme is associated with resistance to a specific pathogen [49,50]. The increase in POD activity indicates that it has a key role in local and systemic resistance; hence, an increase might be related to lignin biosynthesis, which prevents the entry of pathogenic [51] cells such as P. Cinnamomi. It does so through mechanical pressure. The use of elicitors has also been shown to increase POD activity, as the study conducted with exogenous caffeic acid and epicatechin to enhance resistance against Botrytis cinerea in apples [53] showed. Interestingly, another study using fungi as resistance inducers in
tomato plants found an increase in the resistance-related enzyme PPO and showed that these fungi, which are known to have the ability to produce β-glucan, can be used as inducers [54]. Furthermore, a specific study using β-glucan, Salecan, triggered the defense response of Arabidopsis thaliana Colo against Botrytis cinerea infection, related to an increase in different enzyme activities including PPO [55].

Phenolic compounds are a plant defense against pathogens either because there is an oxidation of them when there is a production of reactive oxygen species or because they have an antimicrobial function. In addition, they are precursors of structural polymers or serve as signaling molecules. Thus, when there is a decrease in phenols in plant–pathogen interaction, it is attributed to their oxidation [43]. The present study indicated that Curd treatment did not affect phenol production or antioxidant activity, contrary to the treatment that had only the inoculation of the pathogen. Similar results have been found in tomato against the pathogen Oidium neolycopersici, in which no increase in phenolic content was evident in the medium-resistant tomato variety. However, the resistant variety did increase phenolic content, but it was much higher in the susceptible variety. The authors concluded that this phenomenon is difficult to explain and could be related to the enzymatic transformation of phenols by peroxidase [56] or polyphenol oxidase (PPO) enzymes, which means that they are related to other defense mechanisms. It is also important to note that the total phenolic content only reflects some phenols that are quantified, those that the extraction methodology allows; many may not be observed because they are bound, as found in a study of fibers [37], or because they are generated in low quantities in the plant tissue and therefore are not detectable.

In plants, chlorophyll content is an essential factor in determining the growth capacity. Under normal physiological conditions, the major part of light absorbed by the photosynthetic pigments was used for photosynthetic quantum conversion. Thus, photosynthetic pigment content directly influenced the light absorption, transmission, distribution between the PSI and PSII, and energy conversion [58]. The curdlan foliar sprays noticeably enhanced the chlorophyll (a and b) accumulation under biotic stress at 3 hai in comparison to the plants that were only inoculated with the pathogen (Phy). However, in the other measurement times, there were no statistically significant differences, although at 312 hai, it is observed that the plants to which only curdlan was applied have a chlorophyll content similar to the control plants.

It was noticed that Curd showed significant superior carotenoids accumulation at 3 hai in the treatment Curd + Phy over the Phy. The possible reason is that carotenoids possess dual functions, such as the harvesting of light pigment and scavenging of free oxygen radicals at abnormal irradiance levels [59]. It is important to consider that the pathogen, when entering, covers the vascular beams and induces not only a high production of reactive oxygen species but also damage in the transport of water and nutrients, which in turn affects the process of photosynthesis.

The results found in this research correspond with those reported in the literature: when a foliar elicitor of oligosaccharide type has been applied to different stresses, the contents of chlorophyll and carotenoids have increased or remained stable [58,60–63]. The reactive oxygen species exert various effects on plant defense responses. In this case, the SOD activity remained high after several hours after inoculation of the pathogen in Curd treatments, suggesting that this compound can make the defense response persist over time. The production of ROS, in this case of H$_2$O$_2$ produced by SOD, may have several functions. It has been determined that biotrophic pathogens can be sensitive to H$_2$O$_2$ at micromolar concentrations or lower [64], whereas necrotrophs can be favored from that production [12]. In the case of P. cinnamomi, H$_2$O$_2$ seems not to be involved in avocado resistance to this pathogen [65]; however, this was proved in vitro and not directly in the plant. Nevertheless, this result is somewhat contradictory because in other investigations the effect of this ROS against Phytophthora infestans [66] has been proved as well as its participation in the resistance of Lomandra longifolia to P. cinnamomi [67]. In addition, that H$_2$O$_2$ generated can also be used by the POD enzyme as an electron acceptor to catalyze
the final step in the synthesis of lignin from the oxidation of cinnamyl alcohols, which as mentioned is an important defense response, acting as a physical barrier that blocks the entry of the pathogen [68].

Since this research seeks to understand the effect of Curd as a resistance inducer, it is important to mention that what has been observed suggests that it may confer systemic acquired resistance (SAR). This is because, on the one hand, the defense response was found in a different place than the point of inoculation; on the other hand, the increase of PAL in the final sampling time could indicate the participation of salicylic acid as a signaling agent [68]. It is possible to think that plant defenses were primed by curdlan because there is a rapid and intense response to the pathogen in the first 3 hai of POD and PPO enzymes [69]. Other enzymes such as SOD and PAL increased over time, which is similar to what has been observed in other investigations with inducers, such as that conducted by Eshraghi, et al. [70]. Furthermore, these results add to the shorter length of the lesion and the healthy appearance of the plants at the end of the trial. However, this requires other experiments using inhibitors of enzymes and analyzing the behavior of the plants.

Finally, although Curd had a positive effect on treated avocado plants, it may not be the β-glucan that best triggers the defense response in avocado plants. Despite the fact that a commercial one with a high cost was used, knowing that it works may be the basis for including it in integrated disease management to reduce the use of pesticides. Even other similar molecules can be sought in the framework of sustainable agriculture. Obtaining β-glucans from plant residues would provide an alternative for integral use in crops that can even be degraded by macromycetes fungi. In this process, both the transformed biomass and the fungus itself can be used, since they are recognized for producing β-glucans and it has been seen that they can trigger defense responses, such as those mentioned above.

Even in a specialized search on this topic, some molecule that further potentiates the response against this pathogen could be found. It has been observed that different modifications in the molecule can have a better response; they are like a key in a lock: many can be recognized, but the one with a precise shape generates a more effective defense response. This is something that turns out to be very specific in each pathosystem and that will be dependent on conditions such as adequate nutrition, stage of development, host genotype and pathogen, as well as abiotic stress [71].

5. Conclusions

The application of the elicitor curdlan on Hass avocado plants reduced the symptoms of the disease caused by *P. cinnamomi*. The response between plants inoculated with the pathogen with (Curd + Phy) and without the inducer (Phy) were mostly contrasting. Curdlan modulated the expression of different enzymes such as SOD, PAL, POD, PPO, total phenolic content and chlorophyll, having contrasting effects in these two treatments for almost all sampling times. Moreover, the plants with the pathogen died or wilted considerably, whereas those with the previous application of the inducer had no or minimal signs of wilting. All these results lead to the conclusion that there was a protective action of curdlan and that possibly what is required is that the expression of the metabolites occur at an adequate time and concentration, so that it effectively leads to a defense against the pathogen. However, further research is needed to establish when the application of curdlan should be repeated to maintain its effect, if it behaves similarly in the field or if it is affected by climatic conditions, and what its impact on crop productivity is. Likewise, it would be appropriate to know which other inducers work or which ones generate a better response than the one found in this research.

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