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Programas de fungicidas biológicos y químicos apoyados en trampas de esporas y detección basada en PCR para el manejo del oídio en huertos de avellano europeo

Tesis para optar al grado de Magíster en Ciencias Agronómicas

KARINA POLET MARION ROJAS DURÁN
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Profesor Guía: Ernesto Moya Elizondo
Dpto. de Producción Vegetal
Facultad de Agronomía
Universidad de Concepción

Esta tesis ha sido realizada en el Departamento de Producción Vegetal de la Facultad de Agronomía, Universidad de Concepción.

Profesor Guía

Dr. Ernesto Moya Elizondo
Facultad de Agronomía
Universidad de Concepción

Comisión Evaluadora:

Dra. Marisol Vargas Concha
Facultad de Agronomía
Universidad de Concepción

Dra. Inés Figueroa Heredia
Facultad de Agronomía
Universidad de Concepción

Dra. Daina Grinbergs Salas
INIA - Quilamapu

Director de Programa:

Dra. Macarena Gerding González
Facultad de Agronomía
Universidad de Concepción

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RESUMEN

La rápida expansión del cultivo de avellano europeo (*Corylus avellana* L.) en Chile ha favorecido la aparición y diseminación de enfermedades, destacando el oídio causado por *Phyllactinia guttata*. Este patógeno, reportado por primera vez en el país en 2006, ha mostrado en los últimos años infecciones más agresivas, provocando defoliación prematura y estrés fisiológico en las plantas, lo que amenaza la productividad y sostenibilidad del cultivo. En este estudio se evaluó la recurrencia de *P. guttata* en huertos del centro-sur de Chile y se diseñó un sistema de detección temprana basado en trampas de esporas y diagnóstico molecular por PCR. A partir de esta herramienta, se implementaron y compararon distintos programas fitosanitarios que integraron fungicidas químicos y biológicos, con el objetivo de establecer estrategias de manejo más específicas, eficientes y sostenibles. Los resultados confirmaron la presencia generalizada de *P. guttata* y demostraron que el monitoreo mediante trampas de esporas combinado con PCR permite detectar el patógeno antes de la manifestación de síntomas visibles. Los programas fitosanitarios basados en fungicidas químicos fueron los que presentaron la mayor eficacia en el control de la enfermedad, mientras que aquellos que incorporaron ingredientes activos biológicos mostraron una eficacia moderada, pero con un menor impacto ambiental potencial. Estos hallazgos evidencian que la integración de herramientas de detección temprana en programas fitosanitarios permite optimizar el momento de aplicación de fungicidas, mejorar la eficacia del control del oídio en avellano y reducir el uso innecesario de productos, contribuyendo así a un manejo integrado de enfermedades más sostenible en este cultivo en expansión en Chile.

ABSTRACT

The rapid expansion of European hazelnut (*Corylus avellana* L.) cultivation in Chile has favored the emergence and spread of diseases, particularly powdery

mildew caused by *Phyllactinia guttata*. This pathogen, first reported in the country in 2006, has shown increasingly aggressive infections in recent years, causing premature defoliation and physiological stress in plants, which threatens the productivity and sustainability of the crop. In this study, the recurrence of *P. guttata* was evaluated in hazelnut orchards in south-central Chile, and a detection system based on spore traps and PCR molecular diagnostics was designed. Using this tool, different plant protection programs integrating chemical and biological fungicides were implemented and compared, with the aim of establishing more specific, efficient, and sustainable management strategies. The results confirmed the widespread presence of *P. guttata* and demonstrated that monitoring using spore traps combined with PCR makes it possible to detect the pathogen before the appearance of visible symptoms. Plant protection programs based on chemical fungicides showed the highest efficacy in controlling the disease, while those incorporating biological active ingredients exhibited moderate efficacy but with lower potential environmental impact. These findings show that integrating detection tools into plant protection programs can optimize the timing of fungicide applications, improve the effectiveness of powdery mildew control in hazelnut, and reduce the unnecessary use of products, thereby contributing to a more sustainable integrated disease management in this expanding crop in Chile.

CAPÍTULO I

Introducción General

El avellano europeo (*Corylus avellana* L.) ha experimentado una expansión comercial exponencial en Chile durante las últimas cuatro décadas, desde la Región de O'Higgins hasta la Región de Los Lagos (CIREN, 2023), posicionando al país como el principal productor de este fruto seco en el Hemisferio Sur (Duran *et al.*, 2022). La mayor superficie plantada y el crecimiento más significativo se concentran en las regiones centro-sur y sur,

donde las condiciones climáticas favorecen el potencial productivo de este cultivo (INIA, 2018).

Actualmente, en Chile se cultivan aproximadamente 36.000 hectáreas de avellano, y se proyecta que esta cifra alcance las 60.000 hectáreas para el año 2030 (CIREN, 2023). Esta expansión está impulsada por la creciente demanda mundial, atribuida a las propiedades nutricionales beneficiosas de las avellanas (Ellena *et al.*, 2013), así como a los, relativamente, bajos costos de establecimiento y producción asociados a este cultivo (Duran *et al.*, 2022).

La rápida expansión de los cultivos hacia nuevos agroecosistemas favorece la aparición y desarrollo de plagas y enfermedades nuevas o reemergentes, especialmente bajo condiciones ambientales favorables (Gulcu, 2022). La producción de avellanas en Chile no ha sido la excepción. De hecho, se ha reportado una amplia gama de patógenos bacterianos y fúngicos que afectan este cultivo. Entre las enfermedades bacterianas se incluyen *Agrobacterium tumefaciens* Smith & Townsend, *Pseudomonas syringae* pv. *syringae* van Hall y *Xanthomonas arboricola* pv. *corylina* Halbert & Collmer (Guerrero *et al.*, 2014). Las enfermedades fúngicas están asociadas a especies como *Armillaria mellea* Vahl (Guerrero *et al.*, 2014), *Botrytis cinerea* Pers. (Guerrero *et al.*, 2014), *Chondrostereum purpureum* Pers. (Grinbergs *et al.*, 2024), *Cylindrocarpon* sp. Tul. and C. Tul. (Guerrero *et al.*, 2014), *Diaporthe foeniculina* Fuckel (Guerrero *et al.*, 2020), *Diplodia coryli* Fuckel (Guerrero y Pérez, 2012), *Diplodia mutila* Fr. (Moya-Elizondo *et al.*, 2022), *Fusarium culmorum* W.G. Smith, *F. avenaceum* Fr. (Moya-Elizondo *et al.*, 2025), *Phomopsis* sp. Fuckel (Duran *et al.*, 2020), *Phyllactinia guttata* Wallr., entre otros (Martínez *et al.*, 2006; Acuña, 2010). Entre estos patógenos, *P. guttata*—uno de los agentes causales del oídio en avellano (Hartney *et al.*, 2005)—fue reportado por primera vez en Chile en 2006, en material vegetal importado desde Argentina (Martínez *et al.*, 2006).

El oídio se caracteriza por el desarrollo de un micelio blanquecino en el envés de las hojas y, en estados avanzados, por la formación de cleistotecios

(chasmotecios) de color amarillo a negro. Cuando *P. guttata* infecta una porción significativa del follaje, la planta se debilita y presenta defoliación prematura (Houshyarfard, 2022). Aunque tradicionalmente no se ha considerado de importancia económica debido a su limitado impacto en el rendimiento de frutos, se han observado infecciones cada vez más agresivas que causan caída prematura de hojas y estrés fisiológico que puede retrasar el crecimiento de la planta (Sezer *et al.*, 2017; Erper *et al.*, 2012). *P. guttata* es un parásito obligado del avellano, caracterizado por poseer células peniciladas mucilaginosas en el ápice de sus ascocarpos—sitios clave para la colonización y posterior esporulación (Dugan y Glawe, 2006). Estas esporas desempeñan un rol crucial en el ciclo de infección del hongo, ya que se producen en etapas tardías de la infección y permanecen latentes hasta que se presentan condiciones favorables y tejidos hospederos susceptibles (Baykal, 2020).

Recientemente, *Erysiphe corylacearum* Braun & Takamatsu ha sido identificada como un nuevo patógeno del avellano en Turquía (Sezer *et al.*, 2017), Suiza (Beenken *et al.*, 2020), Italia (Mezzalama *et al.*, 2021), Hungría (Kalmár *et al.*, 2023) y República Checa (Šafránková *et al.*, 2024). A la fecha, este hongo oidiáceo, que afecta negativamente el rendimiento y calidad de las avellanas y puede causar la muerte prematura de las plantas (Erper *et al.*, 2012; Matić *et al.*, 2024), no ha sido reportado en Chile. Sin embargo, recientes observaciones de infecciones severas de *P. guttata* en huertos del centro-sur de Chile resaltan la necesidad de investigar la diversidad y prevalencia de especies de oídios en el país, así como desarrollar programas de manejo adaptados a las condiciones específicas de los huertos y a las características de los patógenos.

El oídio se maneja comúnmente mediante aplicaciones repetidas de fungicidas, lo que con el tiempo puede generar impactos ambientales adversos y el desarrollo de resistencia de los patógenos a los ingredientes activos (Vielba *et al.*, 2020; Kunova *et al.*, 2021; Gulcu, 2022). En Chile, sin embargo, no existen fungicidas registrados oficialmente ni programas de manejo basados en

fungicidas desarrollados específicamente para el control del oídio en huertos de avellano. Como resultado, los productores suelen aplicar fungicidas en exceso o recurrir a productos ineficaces contra la enfermedad. Esto puede tener consecuencias graves, como la aparición de cepas resistentes, la alteración del equilibrio ecológico y la disminución de la biodiversidad microbiana (Yildirim, 2022), lo que pone de manifiesto la necesidad de desarrollar estrategias de manejo sostenible para el control de la enfermedad. Entre ellas, los métodos de control biológico son especialmente prometedores debido a su carácter ambientalmente amigable. En contraste, Turquía—el mayor productor mundial de avellanas—ha aprobado solo tres ingredientes activos para el control del oídio: fluopiram, tebuconazol y triadimenol, siendo el azufre el agente de control principal utilizado. Por su parte, Italia ha autorizado un rango más amplio de fungicidas, incluidos boscalid, miclobutanil, piraclostrobina y metil tiofanato (SAGEA, 2016). Además, el oídio puede manejarse con fungicidas orgánicos como el azufre, ampliamente utilizado para el control de *Erysiphe necator* Schwein en vides (Möth *et al.*, 2023).

La detección temprana de patógenos vegetales es fundamental para determinar el momento óptimo de las aplicaciones de fungicidas dentro de programas de manejo integrado de enfermedades (Forrer *et al.*, 2021). Identificar un patógeno en un huerto o región específica antes de que se propague ampliamente es esencial para implementar estrategias efectivas de erradicación o contención (Parnell *et al.*, 2017). En el caso del oídio, las aplicaciones de fungicidas suelen iniciarse tras la detección visual de micelio fúngico en los tejidos vegetales (Forrer *et al.*, 2021). Aunque existen modelos predictivos que pueden estimar la propagación potencial de un patógeno, el único método confiable para confirmar la presencia de patógenos vegetales aerotransportados antes de la aparición de síntomas visibles es el uso de trampas de esporas (Jackson y Bayliss, 2011).

Los dispositivos de captura de esporas proporcionan datos valiosos sobre la epidemiología y la dinámica diaria y estacional de las esporas de fitopatógenos

aerotransportados. Por ejemplo, se ha documentado su uso para el monitoreo de *Fusarium graminearum* Schwein en trigo (Forrer *et al.*, 2021); *Pseudoperonospora cubensis* Berk. & Curtis (mildiu velloso) y *Podosphaera xanthii* Braun & Takamatsu (oídio) en pepino (Gao *et al.*, 2019; Bello *et al.*, 2021); y *Venturia inaequalis* Cooke en manzano (Prodorutti *et al.*, 2025). Además, el monitoreo de esporas permite cuantificar el inóculo presente en el aire y correlacionarlo con la severidad de la enfermedad, apoyando así el establecimiento de umbrales de acción y el momento óptimo de intervención. Sin embargo, hasta la fecha no existen estudios que reporten el uso de trampas de esporas para la detección temprana o predicción de *P. guttata*. Las técnicas actuales de captura de esporas incluyen portaobjetos recubiertos con vaselina (Quesada *et al.*, 2018), placas Petri con medios de cultivo selectivos (Schweigkofler *et al.*, 2004) y muestreadores volumétricos con cilindros rotatorios recubiertos con adhesivos (Karolewski *et al.*, 2012; Quesada *et al.*, 2018). Otras trampas emplean mecanismos de succión de aire para capturar partículas aerotransportadas en una matriz colectora (Quesada *et al.*, 2018). Si bien estas técnicas son eficaces para detectar esporas fitopatógenas en los huertos, requieren personal capacitado para el examen microscópico e identificación precisa de los patógenos (Karolewski *et al.*, 2012; Jiménez-Zapata *et al.*, 2022).

En este contexto, técnicas moleculares como PCR, qPCR, LAMP (amplificación isotérmica mediada por bucles de ADN) y CRISPR-Cas permiten detectar organismos objetivo incluso a bajas concentraciones de esporas en el aire, gracias a su capacidad de identificar de manera rápida y precisa secuencias específicas de ADN (Jalali *et al.*, 2017; Aguayo *et al.*, 2021; Shajith *et al.*, 2021; Arumugam *et al.*, 2024; Palanisamy *et al.*, 2025). Recientemente, Bilge *et al.* (2023) diseñaron partidores específicos para PCR capaces de diferenciar *P. guttata* de *E. corylacearum* utilizando tejido foliar de *Corylus avellana*. En Turquía (Baykal, 2020) y Estados Unidos (Shi *et al.*, 2009) también se han

reportado enfoques similares de detección molecular. Estos partidores podrían aplicarse para detectar conidias aerotransportadas de *P. guttata* y *E. corylacearum* recolectadas mediante trampas de esporas, permitiendo así determinar de manera precisa el momento óptimo para aplicar fungicidas. Este enfoque podría mejorar la eficacia del control de la enfermedad, al mismo tiempo que minimiza el uso de fungicidas.

HIPÓTESIS

La integración de herramientas de detección, como las trampas de esporas y el diagnóstico molecular por PCR, en conjunto con programas fitosanitarios permite optimizar el momento de aplicación de fungicidas, mejorando la eficacia del control de *Phyllactinia guttata* en huertos de avellano europeo en Chile.

OBJETIVO GENERAL

Desarrollar un sistema de detección para *P. guttata* mediante el uso de caza esporas y detección por PCR, y evaluar su influencia en la eficacia de distintos programas de manejo para el control de este patógeno

OBJETIVOS ESPECÍFICOS

- Determinar la recurrencia de *P. guttata* en huertos de avellano en el centro-sur de Chile
- Desarrollar un sistema de detección de este patógeno mediante el uso de trampas de esporas y diagnósticos moleculares basados en PCR
- Evaluar la eficacia de fungicidas químicos y biológicos seleccionados, con el fin de determinar su integración en programas de manejo de enfermedades para un control más eficiente y sostenible del oídio en avellano

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CAPITULO II

Biological and chemical fungicide programs supported by spore trapping and PCR-based detection for controlling Powdery mildew in hazelnut orchards

Rojas-Durán, K[1]., B. Ruiz[1], J. San Martín[1], I. Figueroa[1], M. Vargas[1], D. Grinbergs[2], M.J. Lisperguer[3], T. De Gregorio[4], M. Maspero[4], and Moya-Elizondo, Ernesto[1].

[1]Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.
[2] INIA Quilamapu, Chillán, Chile.
[3]Departamento Técnico, Frutícola Agrichile S.A., Lote A, Hijuela 1, La Florida del Alto, Curicó, Chile.
[4]Agri Competence Centre, Ferrero Hazelnut Company (HCo), Route de Trèves L-2633, Senningerberg, Luxemburgo.

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ABSTRACT

Hazelnut (*Corylus avellana*) is a high-value fruit crop in the food industry worldwide. In Chile, more than 36,000 hectares are cultivated, mainly for export. However, hazelnut production is globally threatened by *Erysiphe corylacearum* and *Phyllactinia guttata*, causal agents of powdery mildew, which compromise photosynthetic efficiency, cause premature defoliation, and can reduce yield by up to 30%. This study aimed to identify the powdery mildew species present in

the main hazelnut-growing region of Chile and validate the effectiveness of spore trapping and PCR for early detection of *P. guttata*. Additionally, the efficacy of several biological and chemical fungicides, alone or in combinations, was assessed across three management programs in two commercial orchards in Chile. *Phyllactinia guttata* was the only species detected in the twelve sampled orchards. End-point PCR using the Pg2 primer proved specific for *P. guttata* detection when tested against 13 powdery mildew species and other phytopathogenic fungi commonly found in hazelnut orchards and from spore trapping. Aerial DNA of *P. guttata* was detectable on spore trap tapes up to 14 days before the appearance of visible signs and prior to fungicide application. Among the tested treatments, Tebuconazole was more effective than the combined application of Myclobutanil/Kresoxim methyl. The most effective management program was based on sulfur, Fluxapyroxad/Pyraclostrobin and Penthiopyrad. Meanwhile, the organic strategy incorporating sulfur, *Pseudomonas protegens* and *Bacillus subtilis* QS713 tended to reduce *P. guttata* incidence in hazelnut orchards.

Keywords: Powdery mildew, *Corylus avellana*, spore hunter.

INTRODUCTION

The European hazelnut (*Corylus avellana* L.) has experienced exponential commercial expansion in Chile over the past four decades, from the O'Higgins

Region to the Los Lagos Region (CIREN, 2023), positioning the country as the leading producer of this nut crop in the Southern Hemisphere (Duran et al. 2022). The greatest planted area and most significant growth are concentrated in the south-central and southern regions, where climatic conditions favor the productive potential of this crop (INIA, 2018).

Currently, approximately 36,000 hectares of hazelnut are cultivated in Chile, and this figure is projected to reach 60,000 hectares by 2030 (CIREN, 2023). This expansion is driven by increasing global demand, attributed to the beneficial nutritional properties of hazelnuts (Ellena et al. 2013), as well as the relatively low establishment and production costs associated with this nut crop (Duran et al. 2022).

The rapid expansion of crops into new agroecosystems favors the emergence and development of novel or re-emerging pests and diseases, especially under favorable environmental conditions (Gulcu, 2022). Hazelnut production in Chile has not been the exception. In fact, a wide range of bacterial and fungal pathogens affecting this crop have been reported. Bacterial diseases include *Agrobacterium tumefaciens* Smith & Townsend, *Pseudomonas syringae* pv. *syringae* van Hall, and *Xanthomonas arboricola* pv. *corylina* Halbert & Collmer (Guerrero et al. 2014). Fungal diseases are associated with species such as *Armillaria mellea* Vahl (Guerrero et al. 2014), *Botrytis cinerea* Pers. (Guerrero et al. 2014), *Chondrostereum purpureum* Pers. (Grinbergs et al. 2024), *Cylindrocarpon* sp. Tul. and C. Tul. (Guerrero et al. 2014), *Diaporthe foeniculina*

Fuckel (Guerrero et al. 2020), *Diplodia coryli* Fuckel (Guerrero and Pérez 2012), *Diplodia mutila* Fr. (Moya-Elizondo et al. 2022), *Fusarium culmorum* W.G. Smith, *F. avenaceum* Fr. (Moya-Elizondo et al. 2025), *Phomopsis* sp. Fuckel (Duran et al. 2020), *Phyllactinia guttata* Wallr., among others (Martínez et al. 2006; Acuña 2010). Among these pathogens, *P. guttata*—one of the causal agents of powdery mildew in hazelnut (Hartney et al. 2005)—was first reported in Chile in 2006, on plant material imported from Argentina (Martínez et al. 2006). Powdery mildew is characterized by the development of a whitish mycelium on the abaxial surface of the leaves, followed, in advanced stages, by the formation of yellow to black chasmothecia. When *P. guttata* infects a significant portion of the foliage, the plant weakens and undergoes premature defoliation (Houshyarfard 2022). Although traditionally not considered economically significant due to its limited impact on nut yield, increasingly aggressive infections have been observed, causing premature leaf drop and physiological stress that can delay plant growth (Sezer et al. 2017; Erper et al. 2012). *P. guttata* is an obligate parasite of hazelnut, characterized by mucilaginous penicillate cells at the tips of its ascocarps—key sites for colonization and subsequent sporulation (Dugan and Glawe 2006). These spores play a crucial role in the fungal infection cycle, as they are produced in the late stages of infection and remain dormant until favorable conditions and susceptible host tissues are present (Baykal 2020).

Recently, *Erysiphe corylacearum* Braun & Takamatsu has been identified as a new pathogen of hazelnut in Turkey (Sezer et al. 2017), Switzerland (Beenken

et al. 2020), Italy (Mezzalama et al. 2021), Hungary (Kalmár et al. 2023), and the Czech Republic (Šafránková et al. 2024). To date, this powdery mildew fungus, which negatively affects nut yield and quality and may cause premature plant death (Erper et al. 2012; Matić et al. 2024), has not been reported in Chile. However, recent observations of severe *P. guttata* infections in orchards in south-central Chile highlight the need to investigate the diversity and prevalence of powdery mildew species in the country and to develop management programs tailored to specific orchard conditions and pathogen characteristics.

Powdery mildew is commonly managed through repeated fungicide applications, which, over time, can lead to adverse environmental impacts and development of pathogen resistance to active ingredients (Vielba et al. 2020; Kunova et al. 2021; Gulcu 2022). In Chile, however, there are no fungicides officially registered or fungicide-based management programs specifically developed for the control of powdery mildew in hazelnut orchards. As a result, growers often apply fungicides excessively or rely on products that are ineffective against the disease. This can lead to serious consequences, including the emergence of fungicide-resistant strains, disruption of ecological balance, and a decline in microbial biodiversity (Yildirim 2022), highlighting the need to develop sustainable management strategies for the control of the disease. Among these, biological control methods are especially promising due to their environmentally friendly nature. In contrast, Turkey—the world's largest producer of hazelnuts—has approved only three active ingredients for powdery mildew control:

fluopyram, tebuconazole, and triadimenol, with sulfur being the primary control agent used. Meanwhile, Italy has authorized a broader range of fungicides, including those based on boscalid, myclobutanil, pyraclostrobin, and thiophanate-methyl (SAGEA 2016). Additionally, powdery mildew can be managed using organic fungicides such as sulfur, which has been extensively used for controlling *Erysiphe necator* Schwein in grapevines (Möth et al. 2023).

Early detection of plant pathogens is critical for determining the optimal timing of fungicide applications within integrated disease management programs (Forrer et al. 2021). Identifying a pathogen in a specific orchard or region before it becomes widespread is essential for the implementation of effective eradication or containment strategies (Parnell et al. 2017). For powdery mildew, fungicide applications are typically initiated upon the visual detection of fungal mycelium on plant tissues (Forrer et al. 2021). Although predictive models can estimate the potential spread of a pathogen, the only reliable method to confirm the presence of airborne plant pathogens before the onset of visible symptoms is the use of spore traps (Jackson and Bayliss 2011).

Spore trapping devices provide valuable data on the epidemiology and both daily and seasonal dynamics of airborne phytopathogen spores. For example, their use has been documented for the monitoring of *Fusarium graminearum* Schwein in wheat (Forrer et al. 2021); *Pseudoperonospora cubensis* Berk. & Curtis (downy mildew) and *Podosphaera xanthii* Braun & Takamatsu (powdery mildew) in cucumber (Gao et al. 2019; Bello et al. 2021); and *Venturia*

inaequalis Cooke in apple (Prodorutti et al. 2025). Additionally, spore monitoring enables the quantification of airborne inoculum and its correlation with disease severity, thereby supporting the establishment of action thresholds and optimal intervention timing. To date, however, no studies have reported the use of spore trapping for the early detection or prediction of *P. guttata*. Current spore trapping techniques include slides coated with petroleum jelly (Quesada et al. 2018), Petri dishes with selective culture media (Schweigkofler et al. 2004), and volumetric spore samplers using adhesive-coated rotating cylinders (Karolewski et al. 2012; Quesada et al. 2018). Other traps employ air suction mechanisms to capture airborne particles onto a collection matrix (Quesada et al. 2018). While these techniques are effective for detecting phytopathogenic spores in orchards, they require trained personnel for microscopic examination and accurate pathogen identification (Karolewski et al. 2012; Jiménez-Zapata et al. 2022). In this context, molecular techniques such as PCR, qPCR, LAMP (Loop-mediated Isothermal Amplification of DNA), and CRISPR-Cas enable the detection of target organisms even at low airborne spore concentrations, owing to their capacity to rapidly and accurately identify specific DNA sequences (Jalali et al. 2017; Aguayo et al. 2021; Shajith et al. 2021; Arumugam et al. 2024; Palanisamy et al. 2025). Recently, Bilge et al. (2023) designed species-specific PCR primers capable of differentiating *P. guttata* from *E. corylacearum*, using leaf tissue of *Corylus avellana*. Similar molecular detection approaches have also been reported in Turkey (Baykal 2020) and the United States (Shi et al.

2009). These primers could potentially be applied to detect airborne conidia of *P. guttata* and *E. corylacearum* collected through spore traps, thereby enabling accurate determination of optimal fungicide application timing. This approach could improve disease control efficacy while minimizing fungicide use.

The objectives of this study were to: (i) determine the recurrence of *P. guttata* in hazelnut orchards in south-central Chile; (ii) develop an early detection system for this pathogen using spore trapping and PCR-based molecular diagnostics; and (iii) evaluate the efficacy of selected chemical and biological fungicides, aimed at assessing their integration into disease management programs for more efficient and sustainable control of powdery mildew in hazelnut.

MATERIALS AND METHODS

Development of a molecular detection system for *Phyllactinia guttata*.

Microbiological material

Twelve isolates of powdery mildew were collected from hazelnut leaves exhibiting typical signs of infection, including the presence of mycelium and/or chasmothecia. These isolates, collected from 12 orchards across the Maule and Biobío regions, were used to identify the predominant powdery mildew species in the area (Table 1) through nested PCR and sequencing of the internal transcribed spacer (ITS) region. Additionally, samples of 13 other powdery mildew species infecting a range of diverse hosts, including vegetable crops and common weeds found within hazelnut orchards, were collected (Table 2).

Genomic DNA from these additional species was extracted and subsequently used to validate the specificity of the primers designed for *P. guttata* detection.

DNA extraction

For DNA extraction, approximately 1.5 cm² leaf sections were excised from areas exhibiting the highest concentration of powdery mildew mycelium or chasmothecia on hazelnut leaves, as well as from leaves infected with different powdery mildew species collected from various hosts. Samples were placed in sterile 1.5 mL microcentrifuge tubes, with three replicates per sample. Additionally, DNA was extracted from spore trap tapes collected weekly in the field during December and January of the 2021/2022 and 2022/2023 seasons, using a Lanzoni® VPPS 2010 spore trap (Bologna, Italy). In the laboratory, fungal material was recovered from the tape using micropipette tips, which were then transferred to sterile microcentrifuge tubes for DNA isolation.

DNA extraction was performed using a modified CTAB protocol (Doyle and Doyle 1987). Briefly, 500 µL of CTAB buffer was added to each sample, followed by vortexing for 60 seconds to detach mycelium and chasmothecia from leaf tissues. The leaf surface was then scraped to maximize recovery of fungal structures. Samples were mechanically homogenized using plastic pestles (Tissue Grinder, Axygen®) in 1.5 mL tubes. DNA precipitation was achieved using 7.5 M ammonium acetate and 100% isopropanol.

For spore trap tape samples, the same protocol was applied, starting with the addition of 500 µL of CTAB buffer, followed by vortexing, and mechanical

grinding. DNA concentration and purity were quantified via spectrophotometry using an Epoch 2TM spectrophotometer (Biotek®, Changsha, China). DNA samples were subsequently diluted to a working concentration of 10 ng μL^{-1} for downstream applications.

PCR amplification and sequencing analysis

Specific regions of fungal ribosomal DNA were amplified, focusing on the internal transcribed spacer (ITS) region for the 12 powdery mildew isolates collected from the orchards. Nested PCR reactions were conducted following the protocol described by Cunnington et al. (2003), using primers ITS5/4 (White et al. 1990) and primer PMITS1/2 (Cunnington et al. 2003). Primer sequences are detailed in Table 3. Platinum *Taq* DNA polymerase (Thermo Scientific™) was employed, and amplification was performed using a MaxyGene™ II thermal cycler (Axygen®, New York, USA).

Amplification products were resolved on 1% agarose gels stained with SYBR® Safe (Invitrogen™) and run in 0.5X TBE buffer. A volume of 5 µL of PCR product was mixed with 1 µL of 6X DNA Loading Dye and loaded alongside 5 µL of a molecular weight standard (GeneRuler™ 1 Kb Plus DNA Ladder) for size comparison.

PCR products were submitted for bidirectional sequencing using the Sanger method (Macrogen Inc., South Korea). The resulting sequences were edited and aligned employing the Needleman-Wunsch algorithm implemented in Geneious R10 software, generating a consensus sequence for each isolate. Subsequently,

local alignment searches were performed using BLASTn against the NCBI database. Sequences exhibiting the highest identity, coverage, and optimal E-value were selected for molecular fungal identification of powdery mildew species present in hazelnut orchards.

Validation of specific primers and molecular identification

Five primer pairs previously described in the literature as specific for *P. guttata* were evaluated (Table 3). These primer sets were first subjected to *in silico* analysis using the Primer-BLAST tool in the NCBI (National Center for Biotechnology Information) database, selecting those that specifically amplified regions of the *P. guttata* genome at the expected sizes. Subsequently, the selected primers were tested by PCR against a panel of phytopathogenic fungi commonly associated with hazelnut orchards, including *Alternaria alternata*, *Diaporthe foeniculina*, *Diplodia mutila*, *Fusarium culmorum*, *Neofusicoccum parvum*, *Erisiphe necator*, *Schizophyllum commune*, *Stereum armeniacum*, *Chondrostereum purpureum* and *Fusarium oxysporum*, as well as the 13 powdery mildew species listed in Table 2. As positive controls, DNA from three previously sequenced *P. guttata* isolates obtained from hazelnut orchards was used.

Development of management programs for the control of *Phyllactinia guttata*

Evaluation of the efficacy of fungicides and biological control agents (BCAs) for the control of *Phyllactinia guttata*

During the 2019/2020 and 2020/2021 seasons, two field experiments were conducted to evaluate the efficacy of various fungicides and BCAs, applied alone or in combination, within integrated treatment programs for the control of *P. guttata* in hazelnut. The trials were carried out in a hazelnut orchard of Campo San Gregorio, located in San Gregorio de Ñiquén, Ñuble Region (GPS: 36°18'33"S 71°49'44"W), owned by Frutícola AgriChile S.A. The orchard consisted of the 'Tonda Di Giffoni' cultivar, planted in 2018 with a spacing of 3 m × 5 m, and managed under standard commercial production practices. Experimental plots comprised two treated rows separated by an untreated row, with two additional border rows. The soil at the site belongs to the Tuiquilemu series (Inceptisol) (orchard in the 2019/2020 and 2020/2021 seasons), with loam and clay-loam textures (Stolpe 2006). Orchard management, including fertilization, irrigation, insect control, and weed management, was performed by Frutícola AgriChile S.A., with the exception of fungicide applications, which were excluded from standard management during the trial period.

The treatments evaluated are detailed in Table 4 for both seasons. Experimental plots consisted of five trees each, arranged in two rows, separated by one untreated row. A randomized complete block design with four replications was employed.

In the 2019/2020 season, visual monitoring began on December 5, 2019, to track the onset of powdery mildew signs to determine the optimal timing for treatment initiation. Weekly assessments continued until signs were first observed on January 2, 2020, prompting that start of treatment applications. In the 2020/2021 season, monitoring began on December 7, 2020, and molecular monitoring was initiated on December 19, 2020. Field inspections resulted in the first visual detection of *P. guttata* on December 29, 2020, while molecular detection was confirmed during the week of December 21–27, 2020. Molecular detection procedures are described later in this section.

During the 2019/2020 season, fungicides and BCA applications were initiated on January 6, 2020, followed by subsequent treatments on January 16 and 31, with a final application on February 14, 2020. In the 2020/2021 season, the first application was carried out on January 7, 2021 (all treatments), followed by applications on January 15 (biological treatments only), January 21 (all treatments), January 28 (biological treatments only), and a final application on February 4, 2021 (all treatments). In both seasons, treatments were sprayed using a nozzle sprayer at a rate of 2.2 to 2.4 L per tree per application (≈1,500 L ha^{-1}), with volumes adjusted in accordance with foliage biomass development.

Evaluation of management programs for the control of *P. guttata*

During the 2021/2022 and 2022/2023 seasons, field experiments were conducted to validate the efficacy of *P. gutatta* management programs based on the most effective treatments identified in the previously described trials, along

with the use of molecular detection via spore trapping to determine the optimal timing for applications. These experiments were conducted in two commercial orchards owned by Frutícola AgriChile S.A.: Campo San Sebastián, located in San Rafael, Maule Region (GPS: -35.2779, -71.5428), characterized by San Rafael soil series with clay loam textures; and Campo San Gregorio, located in San Gregorio de Ñiquén, Ñuble Region (GPS: 36°18'33"S, 71°49'44"W). The orchards were planted with the 'Tonda Di Giffoni' cultivar at a spacing of 3 m x 5 m and were under commercial production. The San Sebastián orchard was established in 2009, while the San Gregorio orchard in 2007. Experimental plots consisted of five trees, arranged in two rows separated by a row, with two additional border rows left untreated. Orchard management, including fertilization, irrigation, insect control, and weed control, were implemented by the company, excluding fungicide applications.

Detection using spore trapping

In the San Sebastián and San Gregorio hazelnut orchards evaluated during the 2021/2022 and 2022/2023 seasons, a spore trap (Lanzoni® VPPS 2010, Bologna, Italy) was installed to monitor and track the presence of *P. guttata* spores, as well as the subsequent appearance of powdery mildew signs in the experimental plots. The monitoring aimed to determine the optimal timing for fungicide applications within each management program under evaluation. Monitoring started on October 26, 2021 (week 43), in the first season; and on October 25, 2022 (week 43), in the second season, coinciding with the first

appearance of powdery mildew signs based on historical records. Spore trap tapes were coated with adhesive to retain spores over one-week periods and were replaced weekly. The tapes were collected and transferred to the Plant Pathology Laboratory at the University of Concepción, Chillán Campus, for evaluation. DNA was extracted from the tapes following the previously described protocol. Subsequently, an end-point PCR was performed using the most efficient primer pair for the detection of *P. guttata*, as determined earlier in this study.

Fungicide applications and management programs

Following the detection of *P. guttata* DNA on the spore trap tapes in each orchard, the respective management programs were initiated within the subsequent days. The evaluated programs consisted of different treatment schemes based on chemical fungicides, BCAs, and their combination. These programs were designed to incorporate the rotation of fungicide active ingredients to minimize the risk of resistance development and to enhance disease control efficacy. Detailed treatment schemes are provided in Table 5.

The management programs were sprayed on experimental plots consisting of five trees each at the described sites, arranged in a randomized complete block design, with four replicates per treatment and one row separating the blocks to reduce field variability. Applications were carried out as previously described in the product efficacy trials. Spraying schedules were initiated following the detection of *P. guttata* spores, based on PCR analysis of spore trap tapes using

specific primers. Treatments were applied from December 21, 2021, to February 2, 2022, for the 2021/2022 season, and from December 5, 2022, to February 15, 2023, for the 2022/2023 season.

Meteorological conditions

Temperature and relative humidity data from December to March for each orchard and season were obtained from the on-site meteorological station and are presented in supplementary, Table S1.

Evaluations

In all field experiments conducted over the four seasons, disease incidence and severity were assessed through visual observation of fungal signs on hazelnut leaves. Incidence was recorded as the presence or absence of mycelium and/or chasmothecia on the abaxial (underside) surface of the leaves. Severity was estimated as the percentage of leaf area covered by mycelium and/or chasmothecia, using a visual scale ranging from 1 to 100% (Moya et al. 2005). For both assessments, 100 leaves were evaluated around the central tree, with 25 leaves sampled from each of the four quadrants of the tree.

Initial evaluations were performed at the time of visual detection of powdery mildew (approximately 15 - 20 days after the molecular detection of *P. guttata*), followed by three additional assessments prior to the scheduled fungicide sprayings, and a final evaluation 10 - 15 days after the last spraying. Disease incidence and severity data from each assessment were used to calculate the

Area Under the Disease Progress Curve (AUDPC) for both variables over the evaluation period (Jeger and Viljanen-Rollinson 2001).

Statistical analysis

The incidence and severity data obtained from the product and program efficacy trials were tested for normality using the Shapiro-Wilk test, and for homogeneity of variance using Levene's test. Upon validation of these assumptions, an analysis of variance (ANOVA) was performed. When significant differences were detected, means were separated using Fisher's Least Significant Difference (LSD) test ($P < 0.05$). Percentage data were normalized using a square root transformation ($y = \sqrt{(x + 0.5)}$), where *x* represents the percentage value, prior to analysis. All statistical analyses were performed using InfoStat software, version 2008 (Balzarini et al. 2008).

RESULTS

Sampling of orchards in south-central Chile

Nested PCR enabled the specific amplification of the ITS region in all powdery mildew isolates analyzed, using primers PMITS1/2 designed from conserved 18S and 28S sequences (Cunnington et al. 2003). For the ITS region, some reactions exhibited lower band intensity, which was attributed to the heterogeneous quality of the extracted DNA. No nonspecific amplification products were observed in the agarose gels (data not shown). ITS sequencing identified all isolates as *P. guttata* (Supplementary, Table S2), confirming the exclusive presence of this pathogen as the causal agent of powdery mildew in

all analyzed samples. This finding allowed confirming the absence of *E. corylacearum* in hazelnut orchards within the sampled area.

Selection of specific primers

From the five primer pairs described in the literature and tested against other fungal hazelnut pathogens and powdery mildew species, four primer pairs were excluded because they were not specific for *P. guttata*. Electrophoresis of the Pg1, PG-f2/r2, and PG-f3/r3 primer sets showed amplification not only for the positive controls of *P. guttata* but also of *A. alternata*, *D. foeniculina*, *D. mutila*, *F. culmorum*, *N. parvum*, *E. necator*, *S. armeniacum* and *C. purpureum*. Similarly, the PG-F/EP-R primer set amplified both positive controls and *A. alternata*, further confirming its lack of specificity for amplifying *P. guttata* DNA from the spore trap tapes. In contrast, the Pg2 primer set demonstrated high specificity, exclusively amplifying *P. guttata* isolates (Figure 1). Moreover, this powdery mildew specific primer placed on spore trap tapes and did not amplify DNA from any of the other 13 powdery mildew species tested, which were isolated from common weed hosts typically found in hazelnut orchards (Figure 2). The Pg2 primer set described by Bilge et al. (2023) amplified DNA of powdery mildew extracted from hazelnut leaves (O01.1 and O01.2) and tested positive for the three *P. guttata* positive controls, which were identified by sequencing the product of the universal powdery mildew primers PMITS (Cunnington et al. 2023). Given the observed specificity of the Pg2 primer set in discriminating *P. guttata* from various other powdery mildew species and

common phytopathogenic or environmental fungi, it was selected for use in early detection studies. This selection supported the validation of integrated management programs aimed at controlling powdery mildew in hazelnut orchards.

Fungicide efficacy study

During the 2019/2020 season, the evaluation of fungicide efficacy against *P. guttata* in hazelnut showed that, by integrating the values obtained from each assessment over time and calculating the AUDPC, the effectiveness of treatments based on sulfur (Sulfur 80% WG), myclobutanil + kresoxim-methyl (Silver®), and tebuconazole + fluopyram (Luna® Experience) was clearly determined. Significant differences were observed between these treatments and the untreated control for both disease incidence and severity (Figures 3A and 3B).

Although *Bacillus subtilis* QST 713 (Serenade® ASO) and tebuconazole (Tebuconazole 430 SC) showed a tendency to reduce disease severity, these reductions were not statistically significant compared to the untreated controls (Figures 3B). *B. subtilis* demonstrated a noticeable effect on severity during the first evaluation, whereas tebuconazole exhibited a more marked reduction in disease signs during the fourth assessment (Supplementary material, Table S3). *Pseudomonas protegens* Ca2 reduced disease incidence by approximately 20,8% and severity by 24,6% during the first evaluation compared to the untreated dry control; however, differences were not statistically significant when

compared to both controls (Supplementary, Table S3). Potassium bicarbonate (Kaligreen) and myclobutanil (Myclobutanil 40 WP) did not show control on *P. guttata*.

In the untreated dry control, powdery mildew exhibited a rapid and short dissemination period during both evaluated seasons. In the 2019/2020 summer season, 42.3% of the leaves showed disease signs within 20 days of initial detection. Given the polycyclic nature of powdery mildew, disease spread rapidly, with a development rate of 0.58% incidence per day observed between the second and third assessments in the dry control. Thirteen days later, disease incidence had risen to 69.3%, eventually stabilizing at around 80% by day 48. A similar trend was observed for disease severity: 13.4% of the leaf surface area was affected 20 days after the initial visual detection, which doubled by the subsequent evaluation and tending to stabilize from the third assessment onward. Under the summer climatic conditions of 2019/2020, the infection cycle developed over approximately 45 days.

During the 2020/2021 season, a similar disease progression pattern was observed in the dry control. From the initial detection to the first evaluation (24 days later), 40.8% of the leaves exhibited visible signs of infection. The pathogen showed a disease development rate of approximately 0.69 percentage points per day between the second and third assessments. Over the following 17 days, disease incidence reached 61% and eventually stabilized at ~80% by day 72.

In both seasons, there were not differences between the dry and wet untreated control, then comparisons of efficacy were conducted against the natural infection of powdery mildew observed on the dry control

Based on AUDPC values, the control efficacy of the treatments relative to the untreated dry control during the 2019/2020 season indicated that the three most effective fungicide treatments (sulfur, myclobutanil + kresoxim-methyl, and tebuconazole + fluopyram) reduced disease incidence by 44.5% to 51.4%, and severity by 64.4% to 72.8%. Among these treatments, sulfur caused phytotoxic effects on hazelnut leaves, including varying degrees of wilting and/or necrosis (Supplementary, Figure S1). In contrast, none of the other fungicides induced phytotoxic symptoms.

Analysis of the integrated AUDPC values for the 2020/2021 season revealed significant differences in disease incidence and severity among the evaluated fungicide treatments and management programs (Figure 4A and 4B). A single application of sulfur (Acoidal®) did not differ significantly from the untreated control at the incidence and severity. In contrast, fluxapyroxad + pyraclostrobin (Elmus®), penthiopyrad (Fontelis®), and myclobutanil + kresoxim-methyl (Silver®) were the most effective treatments. The three fungicide programs tested reduced AUDPC values for disease incidence by an average of 55.2% (range: 44.8 to 61.9%) and for severity by 76.1% (range: 67.2 to 82.1%).

Four applications of *Swinglea glutinosa* (Ecoswing®) differ in incidence but this was not different in the severity from the dry control. The efficacy of this plant

extract was only evident in the first four assessments of incidence but diminished in the fifth evaluation (Supplementary, Table S4).

Based on AUDPC-derived control percentages, the two most effective fungicide mixtures—myclobutanil + kresoxim-methyl (Silver®), fluxapyroxad + pyraclostrobin (Elmus®)— and penthiopyrad (Fontelis®) achieved disease incidence reductions between 44.8% and 61.9%, and severity reductions between 67,2% and 82,1% over the 2020/2021 summer season. Treatments with lower efficacy included a single sulfur application (Acoidal® SC) and multiple applications of *S. glutinosa* (Ecoswing®), with incidence reductions of 20.0% and 38.1%, and severity reductions of 43.1% and 49.9%, respectively.

Regarding the management programs, the chemical program consisting of sulfur [Acoidal® SC], tebuconazole + fluopyram [Luna® Experience S450 SC], and myclobutanil + kresoxim-methyl [Silver® SC]); the biological program consisting of *Bacillus subtilis*+*Brevibacillus* sp. [Nacillus Pro®] and *P. protegens* Ca2 and ChC7 [Taniri® WP]); and the combined program consisting of sulfur [Acoidal® SC], *P. protegens* Ca6 [MaxGrowth® SC], and *B. subtilis* QST 713 [Serenade® ASO SC] reduced disease incidence by 59.5%, 48.2%, and 49.5%, and severity by 73.1%, 72.8%, and 60.0%, respectively.

Assessment of spore trap monitoring supported by PCR for *Phyllactinia guttata* and the effectiveness of fungicide-based management programs for the control of powdery mildew in hazelnut orchards

Monitoring of spore trap tapes

Monitoring of *P. guttata* in hazelnut orchards using spore trap tapes is summarized in Table 6. Weekly detections were classified as positive (+) or negative (-). In the 2021/2022 season, the first positive PCR detections were recorded in weeks 43 and 46 of the year 2021, in the San Gregorio and San Sebastián orchards, respectively, whereas the first visual signs were observed in weeks 49 and 52 in the respective experimental sites. In the 2022/2023 season, the PCR results from the tapes were negative from weeks 43 to 46 for both orchards, with the first positive result appearing in week 47 for the San Sebastián orchard and in week 49 for the San Gregorio orchard. These results remained positive until week 01 of 2023. In the same season, visual signs were first observed in weeks 49 and 52 for the San Sebastián and San Gregorio orchards, respectively.

Evaluation of disease incidence and severity of *Phyllactinia guttata* under different management programs

Analysis of AUDPC values for *P. guttata* infection across both field experiments revealed significant differences in disease incidence among management programs in the San Sebastián orchard during the 2021/2022 season ($P = 0.05$; C.V.: 10.02) (Figure 5A). The lowest incidence was recorded under phytosanitary program A, which was based on chemical fungicides. This program differed significantly from both control treatments as well as from programs B and C, which were based on sulfur and biological control agents. Phytosanitary program A reduced disease incidence by 15.9% compared to

program C, and by 20.8% compared to the dry control (Supplementary, Table S5). In contrast, during the 2022/2023 season, disease incidence in the San Sebastián orchard was markedly lower than the previous season, and no significant differences were detected among treatments (P = 0.527). Nevertheless, both chemical and organic management programs exhibited a consistent trend toward lower AUDPC values compared to untreated controls.

For the severity variable, no significant differences in AUDPC were observed among the evaluated management programs in the San Sebastián orchard during either the 2021/2022 or 2022/2023 seasons (P = 0.123; C.V. = 19.46 and P = 0.632; C.V. = 66.89, respectively) (Figure 5B). Nevertheless, a general decline in disease severity was noted from the first to the second season, primarily associated with phytosanitary program A.

In the San Gregorio orchard, the AUDPC for incidence revealed significant differences among treatments in both the 2021/2022 (P = 0.005; C.V. = 22.87) and 2022/2023 (P = 0.050; C.V. = 20.65) seasons (Figure 6A). Consistent with the results from the San Sebastián orchard, phytosanitary program A resulted in the lowest disease index, with AUDPC values of 856.0 and 975.0 for the respective season, showing statistically significant differences compared to both control treatments. During the 2021/2022 season, phytosanitary program A reduced disease incidence by 47.7% and by 38.9% in the 2022/2023 season compared to the dry control (Figure 6A). Phytosanitary programs C differed significantly from both controls in terms of powdery mildew incidence during

2021/2022 season (35.9% of disease reduction compared to dry control), but this program was not different from the untreated control in the following season (Figure 6A). No significant differences were detected among the three management programs, and phytosanitary program B did not differ significantly from the untreated controls in either season.

In the San Gregorio orchard, significant differences in disease severity among management programs were observed during the 2021/2022 season, expressed as AUDPC (P = 0.004; C.V. = 22,87) (Figure 6B). Phytosanitary program A differed significantly from both controls in terms of powdery mildew infection levels, with phytosanitary program A achieving up to 74.6% control compared to the dry control. Conversely, the control treatments did not differ significantly from phytosanitary programs B and C, which, in turn, did not significantly differ from the other two management programs. During 2022/2023, there were not differences in severity between phytosanitary programs and both controls (Figure 6B).

DISCUSSION

The development of an integrated management program for a polycyclic disease such as powdery mildew, caused by the biotrophic fungus *P. guttata* in European hazelnut, requires accurate pathogen identification, an effective monitoring and forecasting system for early detection, and the use of fungicides capable of ensuring disease control. Identification efforts conducted in this

study, across 12 orchards in the south-central region of Chile, confirmed the absence of *E. corylacearum,* in the sampled area. *Erysiphe corylacearum* is a powdery mildew species known to affect European hazelnut, which has been reported in Turkey (Sezer et al. 2017), Italy (Matić et al. 2024), Iran (Arzanlou et al. 2018), Hungary (Kalmár et al. 2023), and the Czech Republic (Šafránková et al. 2024). Nevertheless, considering the recent and rapid spread of *E. corylacearum* across nut-producing countries in Europe and the Middle East, it is imperative that Chilean growers and phytosanitary specialists remain prepared to detect the potential introduction of this powdery mildew pathogen.

Accordingly, a monitoring system based on spore trap equipment coupled with end-point PCR proved effective for the early detection of *P. guttata*, with a 7 to 21-day window between the presence of conidia on the spore trap tapes and the first visual signs observations, which serve as indicators to initiate fungicide applications to manage disease outbreaks effectively. Despite the growing importance of *P. guttata*, relatively few studies have addressed the development of species-specific primers for its detection (Baykal 2020; Shi et al. 2020; Bilge et al. 2023). Moreover, previously reported primers had not been validated against the range of phytopathogens associated with hazelnut agroecosystems, thereby limiting their reliability for aerobiological monitoring using spore trap samples. In the present study, among five primer sets available in the literature, the Pg2 primer set developed by Bilge et al. (2023) was selected based on its superior performance. This set was evaluated against DNA from 13 different

powdery mildew species, including four species infecting weeds in European hazelnut orchards (*Erysiphe convolvuli*, *E. polygoni*, *Peronospora verbenae*, and *E. aquilegiae*), as well as eight other environmental and pathogenic fungi (*A. alternata*, *D. foeniculina*, *D. mutila*, *F. culmorum*, *N. parvum, C. purpureum, B. cinerea*, and *E. necator*). Pg2 exhibited the highest specificity both in laboratory assays and when applied to samples collected from the spore traps.

Studies on the use of spore trapping for the detection of powdery mildew are relatively scarce. Balduque-Gil et al. (2024) evaluated three primer sequences previously reported as specific for *E. necator* to validate their specificity. Their results showed that only one primer pair was truly specific for *E. necator*, while the other two amplified DNA from both *E. necator* and *Plasmopara viticola*, pathogens that can co-occur in the same vineyard. A similar situation was observed in the present study, where Pg2 (Bilge et al. 2023) was the only primer set showing high specificity for *P. guttata* among the five primers evaluated. In studies monitoring the aeromycobiota and pathogenic fungi of plants affected by *Ustilago* spp. (smut fungi) and *Podosphaera* spp. (causal agents of powdery mildew), Chen et al. (2018) found that although *Podosphaera* spp. is wind-dispersed, it was collected in greater quantities using rain samplers. This is likely because precipitation facilitates its deposition from the air, especially considering that the production and release of ascospores from chasmothecia is promoted by moisture. In south-central and southern Chile, the period of spore dissemination and disease symptom development is typically marked by high

temperatures and low relative humidity, suggesting that wind is the primary dispersal mechanism for *P. guttata*. However, in spring, when rainfall is more frequent, ascospore release from *P. guttata* chasmothecia may be stimulated by precipitation. This factor should be considered in future research aimed at elucidating dispersal mechanisms and identifying primary inoculum sources, particularly since no significant rainfall events occurred during the trial period.

In the present study, spore trap equipment detected *P. guttata* spores up to 21 days prior to the appearance of visible signs under field conditions, using a single trap device and PCR detection. This finding demonstrates the efficiency of this aerobiological detection system, which integrates weekly sampling with PCR-based analysis of spore trap tapes. However, in the San Sebastián orchard during the 2021/2022 season, a considerable delay occurred between spore trap tape collection and subsequent molecular analysis, resulting in a 21-day delay in the implementation of management programs. This delay resulted in a disease incidence of 74.0% in Phytosanitary Program A, which, although high, was still the most effective treatment compared with the average incidence of 87.1% observed in Phytosanitary Programs B and C, and 90.1% in the controls. In the 2022/2023 season, the same program recorded a disease incidence of 27.3%, when the time between spore capture and the application of management programs was reduced to 14 days following the first positive PCR detection of *P. guttata*. These results suggest that management interventions should be implemented no later than 14 days after the first weekly detection of

P. guttata, as climatic and phytosanitary conditions prior to spore release strongly influence management effectiveness.

Data collected over four years indicate that *P. guttata* exhibits a rapid dissemination rate (between 0.15 and 0.20 increase of incidence per day) in the south-central zone of Chile. For example, during the 2022/2023 season, disease incidence increased from 0.0% to 27.3% in only 12 days in plots under the chemical Phytosanitary Program A.

The San Sebastián orchard, located 131 km north of the San Gregorio orchard and characterized by higher average temperatures, is a larger monoculture (940 ha) than San Gregorio, which increases the likelihood of a faster disease outbreak, particularly considering that no disease control treatments were applied by the company. Consistently, during the 2021/2022 season, the AUDPC in San Sebastián exceeded that of San Gregorio by up to 1,000 units.

Since there is no prior information regarding the use of spore traps for *P. guttata* detection by PCR, this study demonstrates that this approach enables earlier disease detection than visual inspection and provides a critical time window for timely fungicide application before infection onset. This preventive approach can reduce the number of fungicide applications required, allowing growers to implement customized management programs tailored to the specific conditions of each orchard and season.

Furthermore, local atmospheric data and the spatial-temporal dynamics of pathogen spore dispersal must be considered when designing capture systems

and detection protocols to ensure reliable detection of pathogen presence or absence (Jackson and Bayliss 2011). Valdés-Gómez et al. (2017) evaluated control strategies for powdery mildew (*E. necator*) in vineyards in central Chile with the aim of optimizing fungicide use. Their approach integrated field data, including epidemiological risk-climate models, disease incidence observations, plant phenology, fungicide characteristics, and time-based decision-making. This integrated approach reduced fungicide applications by 30 to 50% without increasing damage to grape yields. Additionally, techniques like quantitative PCR (qPCR) allow real-time detection and quantification of the target sequence (Jalali et al. 2017), providing forecasts of pathogen behavior under orchard-specific conditions. This facilitates the development of tailored and/or preventive management strategies based on current meteorological and phytosanitary conditions, allowing for more precise, timely, and cost-effective disease control compared to calendar-based or arbitrary programs (Balduque-Gil et al. 2024), which are still commonly used in Chilean hazelnut orchards.

A robust monitoring program also requires pesticides that ensure effective disease control. Therefore, during the 2019/2020 and 2020/2021 seasons, evaluations were conducted to determine the efficacy of various fungicides, BCAs, and plant extracts against powdery mildew, with the aim of incorporating these products into phytosanitary programs for hazelnut orchards. The trials tested five fungicidal products, four BCAs, and one plant extract.

The chemical fungicides myclobutanil + kresoxim methyl (Silver® SC) and fluxapyroxad + pyraclostrobin (Elmus® SC) demonstrated the highest efficacy, reducing disease incidence by 57.4% and 60.6%, respectively, compared to the untreated controls. Despite its high performance (49.4% and 57.4% disease reduction), myclobutanil (Silver® SC) was excluded from the selected management programs due to the European Commission's decision not to renew its approval. Therefore, its use is no longer legally permitted in European hazelnut production for the 2023/2025 period (EFSA et al. 2024).

Biological products based on bacteria such as *B. subtilis* QST713 (Serenade® ASO), *B. subtilis* + *Brevibacillus* parabrevis (Nacillus® Pro), and *P. protegens* Ca6 (MaxGrowth®) showed potential to reduce powdery mildew infection. However, under high infection pressure, the antimicrobial compounds produced by these bacteria, or the potential activation of plant defense responses, were insufficient to halt disease progression. Evidence indicates that *B. subtilis* QST713 (Serenade® ASO) and *P. protegens* Ca6 (MaxGrowth®) are effective in inhibiting growth of pathogenic fungi such as *Diplodia mutila* in hazelnut, as indicated by reduced necrotic lesion length on branches (Retamal et al. 2024). Meanwhile, *B. subtilis* + *Brevibacillus* parabrevis (Nacillus® Pro) has been tested for powdery mildew control in grapevines (*E. necator*), but its limited efficacy in that context suggests that higher applications rates may be required (Valdés-Gómez et al. 2017).

These results suggest that such biocontrol agents could be incorporated at different developmental stages of hazelnut trees to help reduce synthetic fungicide use within integrated management programs. Similarly, *Swinglea glutinosa* extract (Ecoswing®) demonstrated potential in reducing *P. guttata* incidence and exhibited a tendency to decrease disease severity in hazelnut orchards. In this context, Yildirium (2022) incorporated *Reynoutria* spp. extracts into management programs to partially substitute sulfur or conventional fungicide applications. Alternating these products yielded high efficacy against *E. corylacearum*, although the extract alone showed limited effectiveness (79.6% leaf incidence). These findings highlight the potential of integrated management programs that combine chemical, organic, and/or biological products as a sustainable strategy to reduce reliance on synthetic fungicides in hazelnut orchards.

This is particularly relevant considering that Europe, the primary market for Chilean hazelnut, has increasingly restricted the use of various active ingredients in fungicides and bactericides permitted for disease management. These regulatory limitations are progressively reducing the chemical control options, in turn promoting the adoption of biological alternatives.

In the management programs evaluated in this study, the combined application of sulfur with biological products reduced infection pressure by 12.1 percentage points compared to the use of sulfur alone. Sulfur, a low-cost inorganic fungicide, provided adequate control of the damage caused by *P. guttata*.

However, its application has limitations, such as phytotoxicity when applied at temperatures above 27°C (Onofre et al. 2021). Despite this drawback, sulfur is recommended due to its low environmental impact and absence of residue limit concerns (Sezer et al. 2019). In the present study, phytotoxicity issues associated with sulfur applications were observed and attributed to the high temperatures typically recorded during the period in which powdery mildew develops in hazelnut in Chile. This necessitated the application of sulfur on cooler days. Across the four seasons evaluated, temperatures exceeded 27°C on 60 days between December and January in the Southern Hemisphere summer, coinciding with the period when the first signs of *P. guttata* appear in the Maule and Ñuble regions. Therefore, sulfur-based products were applied early in the management program, as molecular detection through PCR and spore trapping provided a 7- to 14-day window before signs onset. This allows applications to be timed on cooler days, thereby reducing the risk of phytotoxicity.

Systemic fungicides followed by sulfur applications, including fluxapyroxad + pyraclostrobin (Elmus®) and penthiopyrad (Fontelis®), effectively controlled *P. guttata* infections within the chemical management program. Minimum disease incidence levels were achieved, reaching 27.3% in the San Sebastián orchard in the 2022/2023 season, and 28.3% in the San Gregorio orchard during the 2021/2022 season. These values contrast sharply with the 91.8% incidence observed in the dry control under the highest disease pressure in the San

Sebastián orchard. A similar trend was observed in disease severity, suggesting that early sulfur applications provide initial plant protection, which is then maintained by the subsequent application of synthetic fungicides.

Products based on BCAs, such as *P. protegens* Ca6 (MaxGrowth® SC), *P. protegens* Ca2 and ChC7 (Taniri® WP), *B. subtilis* + *Brevibacillus parabrevis* (Nacillus Pro® WP), and *B. subtilis* QST 713 (Serenade® ASO SC), show reduced efficacy when included in management programs and exposed to seasonal environmental conditions, such as high temperatures and intense radiation. Under high disease pressure, their performance was not significantly different from that of the untreated controls.

Therefore, the combined use of these BCAs could represent a valuable component of integrated disease management approach. Based on the results obtained, incorporating both chemical and biologically based products in management programs is essential, not only for their contribution to sustainable crop production but also in light of increasing restrictions on fungicide active ingredients in key export markets (AgriChile 2022; EFSA et al. 2024).

CONCLUSION

Phyllactinia guttata is the only powdery mildew species currently reported in Chilean hazelnut orchards. The Pg2 primer set, described by Bilge et al. (2023), demonstrated high specificity for detecting *P. guttata* from spore trap tapes, successfully discriminating it from 13 other powdery mildew species as well as

recurrent fungal pathogens associated with hazelnut. Detection through spore traps and PCR provides a time window of one to two weeks for fungicide application before visible signs of infection appear on hazelnut leaves. Under field conditions, the most effective treatments for controlling *P. guttata* included chemical fungicides such as myclobutanil + kresoximmethyl, tebuconazole + fluopyram, fluxapyroxad + pyraclostrobin, and penthiopyrad, while biological control agents based on *B. subtilis* or *P. protegens,* were less effective. Sulfur also proved effective against powdery mildew; however, its use poses a risk of phytotoxicity, particularly at temperatures above 27º C. This limitation can be mitigated by using spore trapping combined with PCR detection, enabling timely fungicide applications under the prevailing climate conditions of south-central Chile. A phytosanitary program based on initial sulfur application followed by chemical fungicides was the most effective strategy to control *P. guttata* under field conditions.

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Tables and Figures

Table 1. European hazelnut orchards from which leaves exhibiting signs of powdery mildew were collected for species-specific identification by PCR, including orchard location, variety, and planting date.

Samples	Orchard	Location	Variety	Planting date
1	Santa Sofía	Curicó, Los Niches	Barcelona, Eq3	2005
2	Santa Sofía	Curicó, Los Niches	T6DL, Eq2	1996
3	Santa Catalina	Romeral	Barcelona	Not reported
4	San Sebastián	San Rafael	Barcelona	1997
5	San Sebastián	San Rafael	T6DL	1997
6	La Cachimba	Río Claro	T. Giffoni	2007
7	La Cachimba	Río Claro	Barcelona	2007
8	Santa Olga	Los Ángeles	Giffoni	2007
9	San Gregorio	San Gregorio	T. Giffoni	2015
10	La Yaga	Pelarco	T. Giffoni	2017
11	Viña Alquihue	Villa Alegre	T. Giffoni	2016
12	Viña Alquihue	Villa Alegre	Barcelona	2016

Table 2. Sample codes for powdery mildew species, host description, and collection site for the validation of *Phyllactinia guttata*-specific primers.

Code	Powdery mildew species	Host	Place, Region, Orchard
O02	*Erysiphe convolvuli*	*Convulvulus arvensis*	San Gregorio, Ñuble – Hazelnut
O03	*Erysiphe polygoni*	*Sanguinaria canadensis*	San Gregorio, Ñuble – Hazelnut
O04	*Perenospora verbenae*	*Verbena officinalis*	San Gregorio, Ñuble – Hazelnut
O05	*Erysiphe betae*	*Beta vulgaris* subsp. *vulgaris*	Chillán, Ñuble – Vegetable
O06	*Erysiphe necator*	*Vitis vinifera*	Chillán, Ñuble – Vine
O07	*Podosphaera plantaginis*	*Plantago major*	Chillán, Ñuble - House garden
O08	*Microsphaera alphitoides*	*Quercus xalapensis*	Chillán, Ñuble – park University
O09	*Erysiphe aquilegiae*	*Ranunculus repens*	San Gregorio, Ñuble – Hazelnut
O10	*Erysiphe betae*	*Beta vulgaris* subsp. *vulgaris*	Chillán, Ñuble - Vegetable
O11	*Erysiphe betae*	*Beta vulgaris* subsp. *vulgaris*	Chillánn, Ñuble - Vegetable
O12	*Perenospora verbenae*	*Verbena bonariensis*	Caracas, Araucanía - Hazelnut
O13	*Podosphaera leucotricha*	*Malus domestica*	Chillán, Ñuble – Apple tree
O14	*Erysiphe polygoni*	*Trifolium* sp.	Caracas, Araucanía – Hazelnut

Table 3. Primer sets used for amplification and sequencing of ITS regions, and for specific detection of *Phyllactinia guttata* from fungal mycelium and spore trap tapes.

Set	Forward	Reverse	Amplicon length (pb)	References
PMITS1/2	TCGGACTGGCCCAGGGAGA	TCACTCGCCGTTACTGAGGT	779	Cunnington et al. 2003
ITS5/4	GGAAGTAAAAGTCGTAACAAGG	TCCTCCGCTTATTGATATGC	683	White et al. 1990
PG-F/EP-R	CTCGTGTGATTGATGAAGTCTGAGC	CTCGTGTGATTGATGAAGTCTGAGC	403	Baykal 2020
PG-f2/r2	ACCCGTGTCGATTGTATCGTCTGT	GAGGTCAACCATAGATAAGG	499	Shi et al. 2009
PG-f3/r3	CTGAGCGTGAAGACTCTCGGCC	AAACGTGACTACGCGGAGAG	499	Shi et al. 2009
Pg1*	GTGAAGACTCTCGGCCCC	TCAAGTTTTCTCTGGCAGGC	179	Bilge et al. 2023
Pg2	CGTGCCTGCCAGAGAAAAC	TCGTCTTTTGGCTGGATCCA	374	Bilge et al. 2023

*Specific primers were kindly provided by researcher Stuart Lucas via personal communication.

Table 4. Fungicide and biofungicide treatments evaluated for the control of *Phyllactinia guttata* in hazelnut orchards over two seasons.

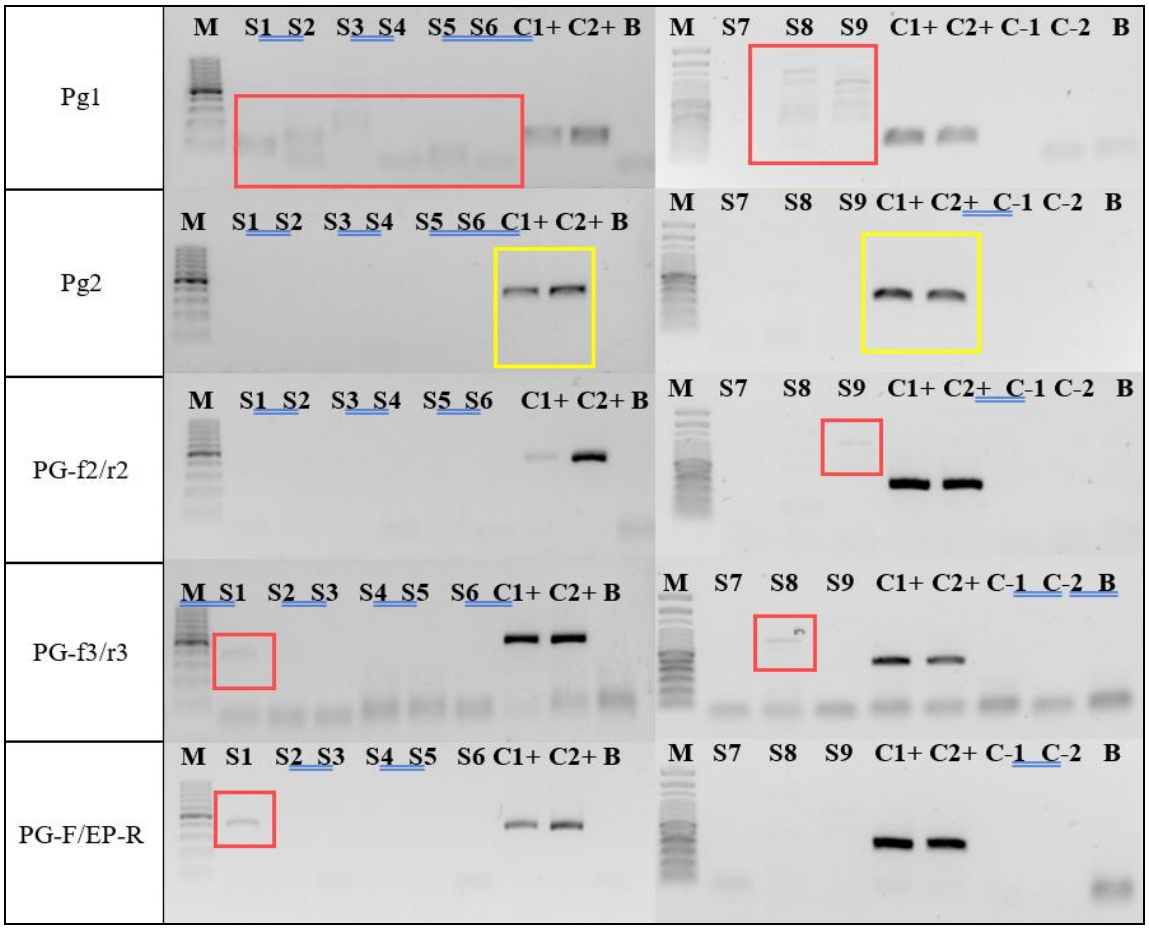
Active ingredient (a.i.)	**Product type**	**Dose p.c. (hL^{-1})**	**Commercial product (p.c) or name**	**Season**
-	-	-	Dry control	2019/2020 – 2020/2021
Water	-	-	Wet control	2019/2020 – 2020/2021
Sulfur	Organic	40 g	Sulfur 80 WG	2019/2020
Myclobutanil (4% w/v) + Kresoxim methyl (10% w/v)	Chemical	70 mL	Silver SC	2019/2020 – 2020/2021
Pseudomonas protegens Ca6	Biological	600 mL	MaxGrowth	2019/2020 – 2020/2021
Potassium hydrocarbonate (82% w/w)	Chemical	200 g	Kaligreen	2019/2020
Myclobutanil (40% w/w)	Chemical	10 mL	Myclobutanil 40 WP	2019/2020
Tebuconazole (43% w/v)	Chemical	25 mL	Tebuconazole 430 SC	2019/2020
Tebuconazole (20% w/v) + Fluopyram (20% w/v)	Chemical	40 mL	Luna Experience 450 SC	2019/2020 – 2020/2021
Bacillus subtilis QST 713	Biological	600 mL	Serenade ASO	2019/2020 – 2020/2021
Sulfur 72% w/v	Organic	300 mL	Acoidal 80 WG	2020/2021
Swinglea glutinosa (96,7 % w/w)	Biological	200 mL	Ecoswing	2020/2021
Penthiopyrad 20,0 % w/v	Chemical	40 mL	Fontelis	2020/2021
Fluxapyroxad 25% w/v + Pyraclostrobin 25% w/v	Chemical	40 mL	Elmus	2020/2021
Bacillus subtilis N5 + *B. cereus* N6 and N7 + *Brevibacillus parabrevis* N4	Biological	100 g	Nacillus Pro	2020/2021
Pseudomonas protegens Ca2 and ChC7	Biological	100 g	Taniri	2020/2021

Table 5. Management strategies and associated chemical and biological fungicides used for the control of *Phyllactinia guttata* in hazelnut orchards during the 2021/2022 and 2022/2023 seasons.

Management treatments	**Spraying and active ingredients used**			
	Spraying 1	**Spraying 2**	**Spraying 3**	**Spraying 4**
Dry control	S/A	S/A	S/A	S/A
Wet control	Water	Water	Water	Water
Program A	Sulfur 72 %[1]	Fluxapyroxad 25 % + Pyraclostrobin 25 %[2]	Penthiopyrad 20 %[3]	S/A
Program B 2021/2022	*Pseudomonas protegens* Ca2/ChC7 [4]	*Bacillus subtilis* N5 + *B. cereus* N6 y N7 + *Brevibacillus* parabrevis N4[5]	*Pseudomonas protegens* Ca2/ChC7 [4]	*Bacillus subtilis* N5 + *B. cereus* N6 y N7 + *Brevibacillus* parabrevis N4 [5]
Program B 2022/2023	Sulfur 72 %[1]	*Pseudomonas protegens* Ca2/ChC7 [4]	*Bacillus subtilis* N5 + *B. cereus* N6 y N7 + *Brevibacillus* parabrevis N4 [5]	*Pseudomonas protegens* Ca2/ChC7 [4]
Program C	Sulfur 72 %[1]	*Pseudomonas protegens* Ca6[6]	*Bacillus subtilis* QST713[7]	*Pseudomonas protegens* Ca6[6]

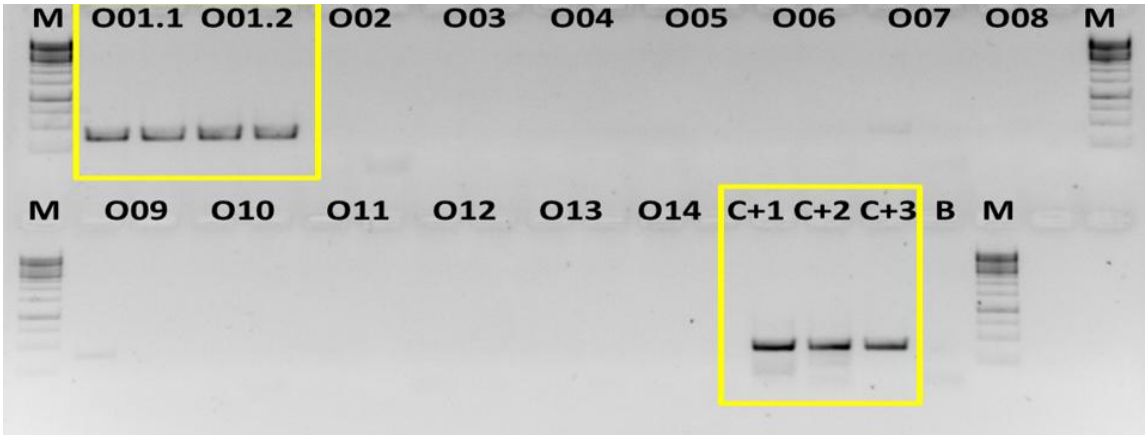
Commercial names: [1]Acoidal 80 WG (BASF) 300 mL hL^{-1}; [2]Elmus (BASF) 40 mL hL^{-1}; [3]Fontellis (Corteva) 40 mL hL^{-1}; [4]Taniri (Bionativa) 100 g hL^{-1}; [5]Nacillus Pro (Bionativa) 100 g hL^{-1}; [6]MaxGrowth (Bioprotegens) 100 mL hL^{-1}; [7]Serenade ASO (Bayer) 600 mL hL^{-1}. Spraying dates are provided in the Materials and Methods section.

Figure 1. Molecular identification with five species-specific primers for *Phyllactinia guttata* (Pg or PG) assessed on DNA of seven different fungi that affect hazelnut. Yellow boxes indicate specific amplification of *P. guttata* observed on 1.5% agarose gel electrophoresis run (80 V, 50 min), while red boxes indicate lack of specificity of the primer pair associated to presence of bands amplified from DNA of other fungi.



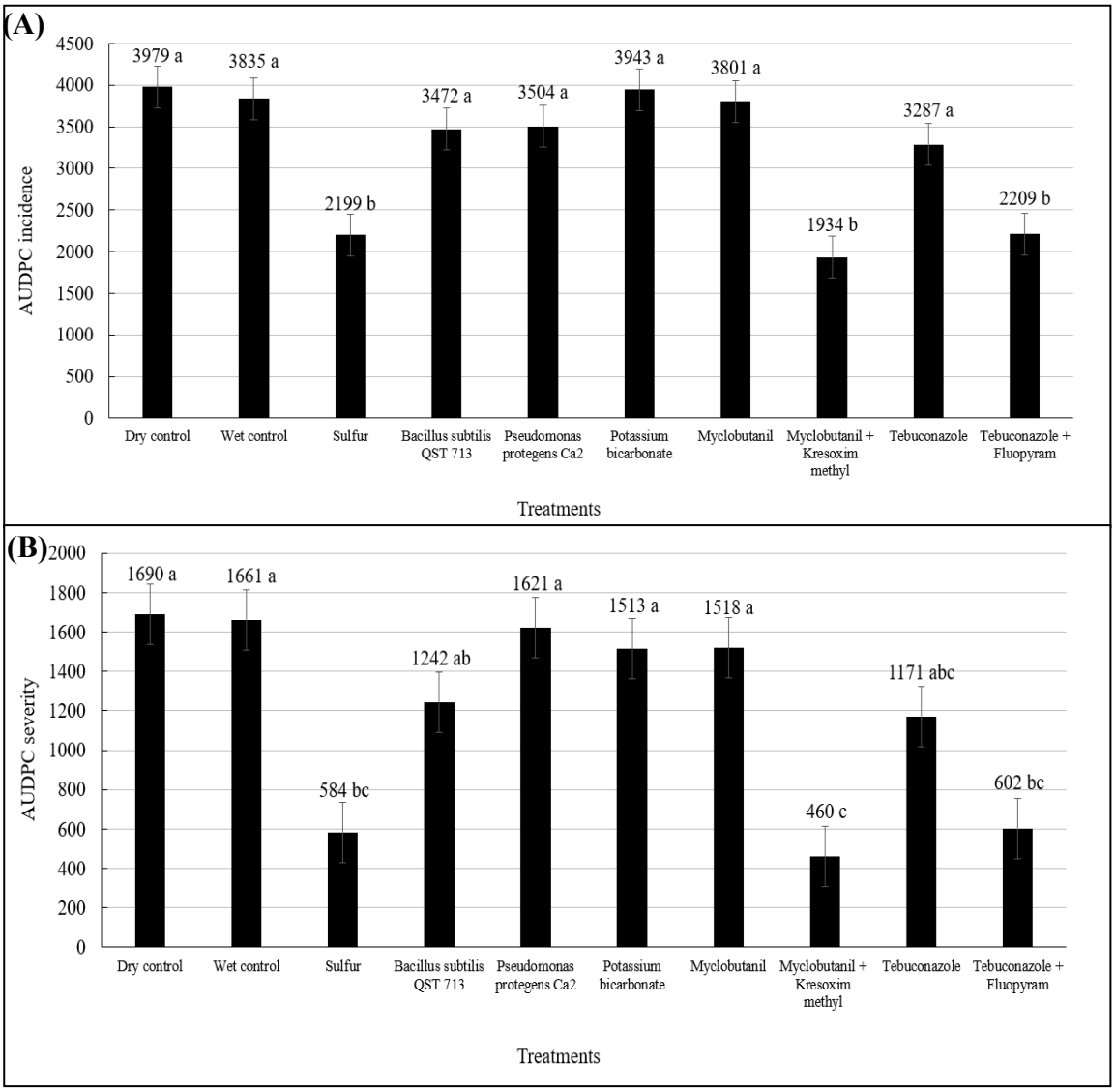
Where: M= molecular weight marker 100 bp. **S1**: DNA *Alternaria alternata*, 10 ng. **S2**: DNA *Diaporthe foeniculina*, 10 ng. **S3**: DNA *Diplodia mutila*, 10 ng. **S4**: DNA *Fusarium culmorum*, 10 ng. **S5**: DNA *Neofusicoccum parvum*, 10 ng. **S6**: DNA *Erysiphe necator*, 10 ng. **S7**: DNA *Schizophyllum commune*, 10 ng. **S8**: DNA *Stereum armeniacum*, 10 ng. **S9**: DNA *Chondrostereum purpureum*, 10 ng. **C1+**: DNA *Phyllactinia guttata* aislado "RABA1" 10 ng. **C2+**: DNA *Phyllactinia guttata* aislado "O01", 10 ng. C-1: DNA vid, 10 ng. C-2: DNA *Fusarium oxysporum*, 10 ng. **B**: Blank.

Figure 2. Electrophoresis of PCR products in a 1% agarose gel (80 V, 50 min) using the specific primer Pg 2 (Bilge et al. 2023) on DNA from various powdery mildew species, including *Phyllactinia guttata*. Yellow frames indicate positive detection of the fungal samples.



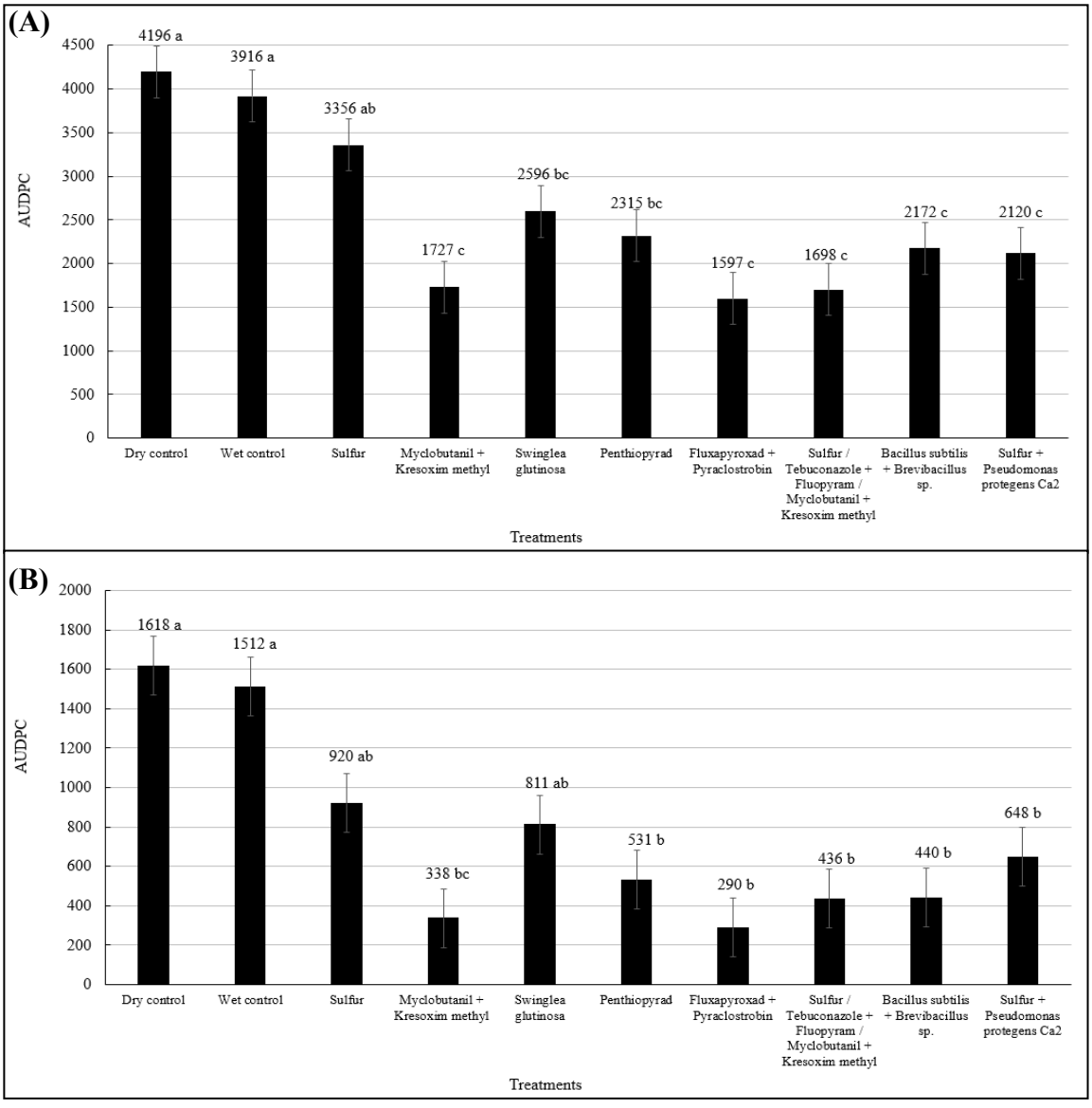
Where: M = GeneRuler™ 1 kb molecular weight marker; O01.1 and O01.2 = *P. guttata* DNA from European hazelnut; O02–O14 = DNA from powdery mildew species collected from various hosts described in Table 2; C+1 and C+2 = *P. guttata*-positive samples (2021) from San Sebastián orchard; C+3 = Sequenced *P. guttata* DNA; B = Negative control (blank).

Figure 3. Area under the disease progress curve (AUDPC) of powdery mildew (*Phyllactinia guttata*) on leaves of European hazelnut cv. Tonda Di Giffoni, based on disease incidence (A) and severity (B) under different biological and synthetic fungicide treatments. The experiment was conducted in a commercial orchard of Campo San Gregorio, San Gregorio de Ñiquén, Ñuble Region, Chile, during the 2019/2020 season.



Bars with different letters indicate statistical differences between treatments according to Fisher's LSD test (P = 0.05).

Figure 4. Area under the disease progress curve (AUDPC) of powdery mildew (*Phyllactinia guttata*) on leaves of European hazelnut cv. Tonda Di Giffoni, based on disease incidence (A) and severity (B) under different biological and synthetic fungicide treatments. The experiment was conducted in a commercial orchard of Campo San Gregorio, San Gregorio de Ñiquén, Ñuble Region, Chile, during the 2020/2021 season.



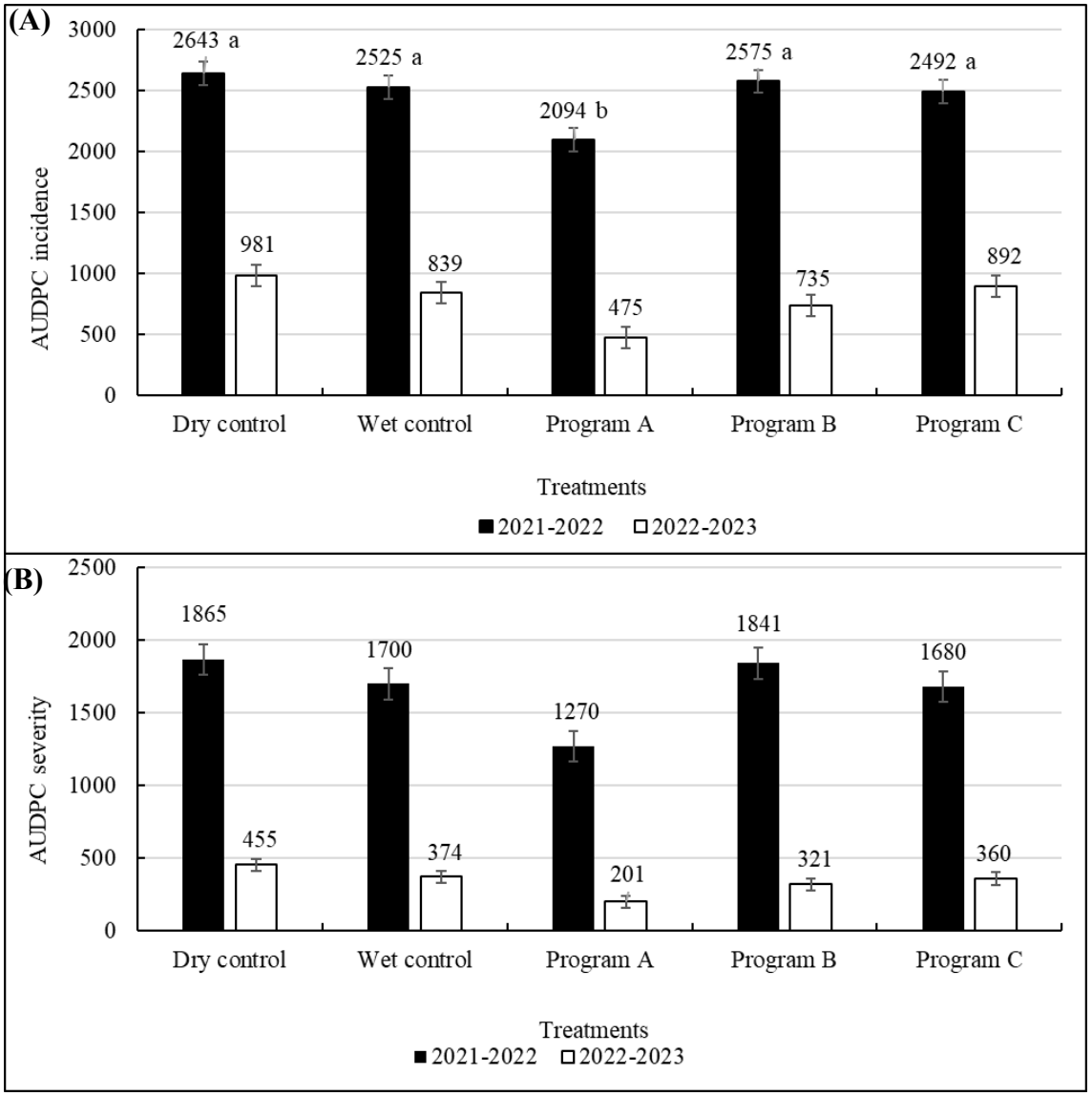
Bars with different letters indicate statistical differences between treatments according to Fisher's LSD test (P = 0.05).

Table 6. Weekly PCR detections of *Phyllactinia guttata* from spore trap tapes collected in two European hazelnut cv. Tonda di Giffoni orchards over two seasons using Pg2 primers (Bilge et al. 2023).

Week number	Week 2021/2022	Week 2022/2023	2021/2022		2022/2023	
			San Sebastián	San Gregorio	San Sebastián	San Gregorio
43	26/10 – 02/11	25/10 – 01/11	-	+	-	-
44	02/11 – 09/11	01/11 – 08/11	-		-	-
45	09/11 – 16/11	08/11 – 15/11	-	+	-	-
46	16/11 – 23/11	15/11 – 22/11	+	+	-	-
47	23/11 – 30/11	22/11 – 29/11	+	+	+	-
48	30/11 – 07/12	29/11 – 06/12	+	+	+	-
49	07/12 – 14/12	06/12 – 13/12	+*	+	+*	+
50	14/12 – 21/12	13/12- 20/12	+	+	+	+
51	21/12 – 28/12	20/12 – 27/12	+	+	+	+
52	28/12 – 04/01	27/12 – 03/12	+	+*	+	+*
01	04/01 – 11/01	03/01 - 10/01	+	+	+	+

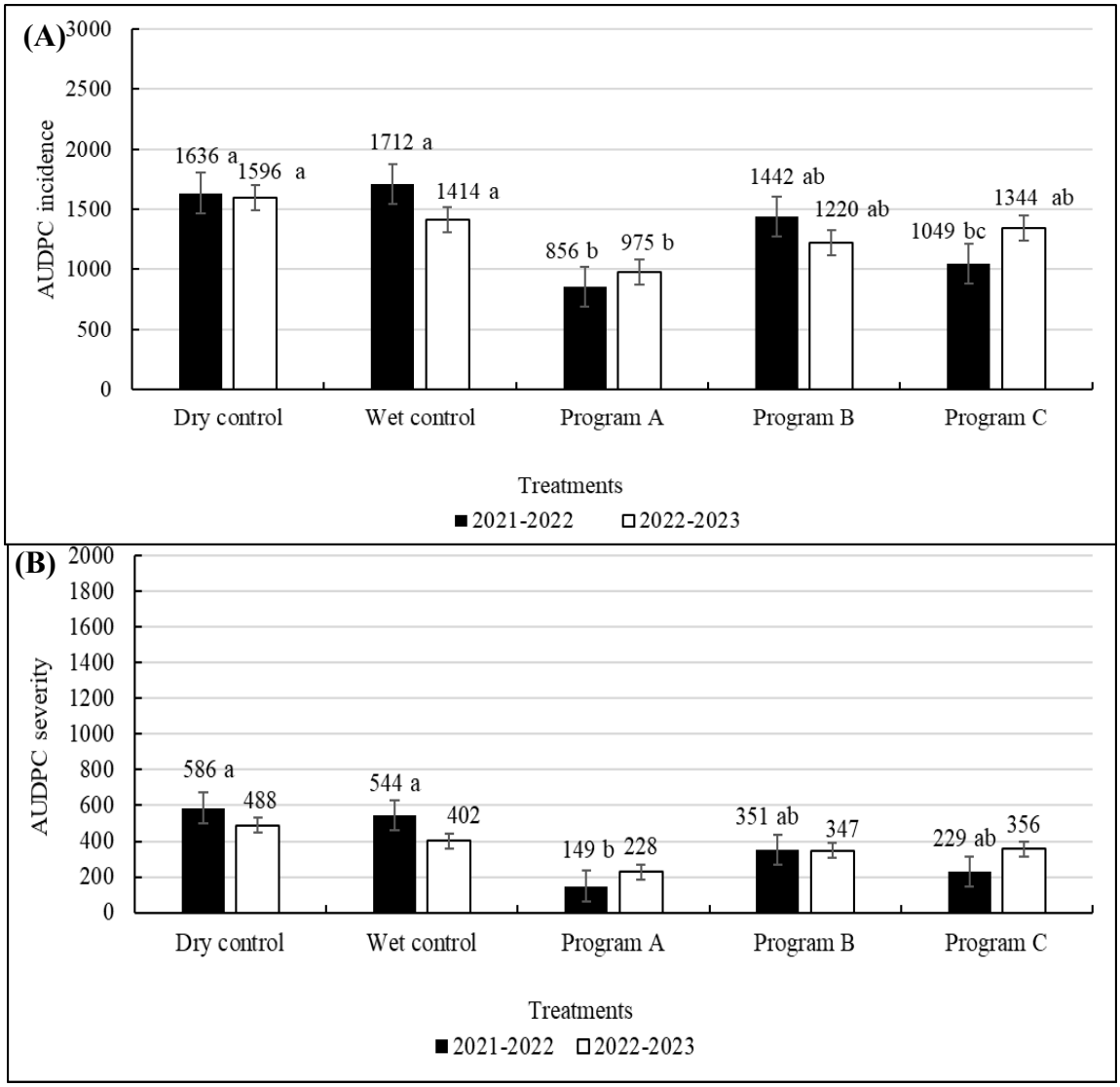
(+) indicates detection of DNA of *P. guttata* from spore trap tapes; (-) indicates no detection of *P. guttata* DNA from spore trap tapes; *Indicates the week when *P. guttata* was visually detected on hazelnut leaves in the field.

Figure 5. Area under the disease progress curve (AUDPC) of powdery mildew (*Phyllactinia guttata*) on leaves of European hazelnut cv. Tonda Di Giffoni, based on disease incidence (A) and severity (B) under different phytosanitary management programs. The experiment was conducted in a commercial orchard of Campo San Sebastián, San Rafael, Maule Region, Chile, during the 2021/2022 and 2022/2023 seasons.



Bars with different letters indicate statistical differences between treatments according to Fisher's LSD test (P = 0.05).
The description of the active ingredients used and their respective doses are found in Table 5.

Figure 6. Area under the disease progress curve (AUDPC) of powdery mildew (*Phyllactinia guttata*) on leaves of European hazelnut cv. Tonda Di Giffoni, based on disease incidence (A) and severity (B) under different management programs. The experiment was conducted in a commercial orchard of Campo San Gregorio, San Gregorio de Ñiquén, Ñuble Region, Chile, during the 2021/2022 and 2022/2023 seasons.



Bars with different letters indicate statistical differences between treatments according to Fisher's LSD test (P = 0.05).
The description of the active ingredients used and their respective doses are found in Table 5.

CONCLUSIONES GENERALES

Phyllactinia guttata es la única especie de oídio actualmente reportada en huertos de avellano europeo en Chile. El set de partidores Pg2, descrito por Bilge *et al.* (2023), mostró alta especificidad para la detección de *P. guttata* a partir de cintas de trampas de esporas, discriminándola exitosamente de otras 13 especies de oídios y de patógenos fúngicos recurrentes asociados al avellano. La detección mediante trampas de esporas y PCR permitió anticipar la presencia del patógeno con una ventana de una a dos semanas antes de la aparición de síntomas visibles en las hojas, lo que ofrece una oportunidad óptima para la aplicación de fungicidas. Bajo condiciones de campo, los tratamientos más efectivos para el manejo de *P. guttata* fueron los fungicidas químicos mIclobutanilo + kresoxim-metil, tebuconazol + fluopiram, fluxapiroxad + piraclostrobina y penthiopirad, mientras que los agentes de control biológico basados en *B. subtilis* o *P. protegens* mostraron menor efectividad. El azufre también resultó eficaz contra el oídio; sin embargo, su uso implica un riesgo de fitotoxicidad, especialmente a temperaturas superiores a 27 °C. Esta limitación puede mitigarse mediante el uso combinado de trampas de esporas y detección por PCR, lo que permite realizar aplicaciones oportunas de fungicidas bajo las condiciones climáticas predominantes del sur-centro de Chile. Un programa fitosanitario basado en una aplicación inicial de azufre seguida de fungicidas químicos constituyó la estrategia más efectiva para el control de *P. guttata* en condiciones de campo