Proyecto: **Identificación de Manejos de Huerto que Inciden en la Susceptibilidad a Pudriciones de Cerezas a Través de Análisis Exploratorios y Análisis de Sensibilidad a Fungicidas de Principales Patógenos**

Informe Final

Semana 26- 24 Jun 2021

Temp 2020-21

Objetivos

- Determinar a través de análisis exploratorios que factores de manejo inciden en la susceptibilidad a desarrollo de pudriciones en pre y postcosecha en frutos de cerezo
- Determinar grado de sensibilidad a fungicidas de mayor uso en pre y postcosecha, para el control los patógenos de mayor relevancia, causantes de pudriciones en cerezas.

Objetivo I Actividades

- Selección de Unidades de monitoreo (mín 5 por Exportadora)
- Muestreos a cosecha de fruta para analizar carga a través de qPCR
- Muestreo a cosecha para análisis de Nitrógeno en fruto
- Guarda de fruta (0ºC- 30 d- Bolsa Traslapada sin Aplicación de Fungicidas).
- Revisión de Cajas estableciendo frecuencias de pudriciones (48 hrs 20ºC)
- Generación de Base de datos
- Análisis Multivariados

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Objetivo I

Tres problemas se presentaron durante el desarrollo del proyecto:

- No todas las exportadoras comprometidas participaron del envío de muestras
- No Todas las exportadoras almacenaron fruta
- No todas las exportadoras enviaron fichas de manejos y recepciones

Propuesta de Análisis de Datos

- Dos niveles para generación de Análisis Multivariados

1) Análisis Exploratorio General: Todas las Unidades Monitoreadas (n=20), considerando fichas de manejo completas + qPCR + N
2) Análisis Exploratorio Particular: Todos las Unidades Monitoreadas (n=10, Frusan y Ranco Cherries), considerando fichas de manejo completas + qPCR + N + Almacenaje.

Resultados Generales

- 20 Unidades de Muestreo
- Frusan, Ranco Cherries, Fruttita, Prize y Quelén.
- Ficha Completa de Manejos
- Análisis de Nitrógeno
- Análisis de qPCR Multipatógeno

Exportadora	Predio
Frutitta	Agr Cafena
Frutitta	Agr Lautaro
Frutitta	Agrícola Invec
Frutitta	Claudio Azocar
Frutitta	E. Zegers (Sta Olga)
Prize	Agr Los Coigues
Prize	Agr Los Encinos
Quelen	Agr Mercedario/Fundo El Bo..
Quelen	Agr Mercedario/Fundo El Bo..
Quelen	Agr Mercedario/Fundo El Bo..
Frusan	Agr El Coigue
Frusan	Agr El Hualle
Frusan	Agr Los Campos 2
Frusan	Suc Sergio Valenzuela
Ranco	Agr San Francisco
Ranco	Frutal Quilvo SPA/Fundo Hu..
Ranco	La Soledad/Huerto Angostur..
Ranco	Nueva San Francisco
Ranco	Santa Teresa
Frusan	Puente Negro

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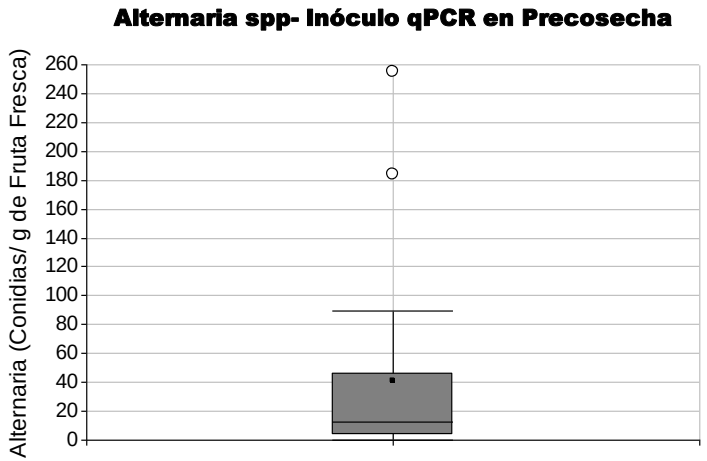

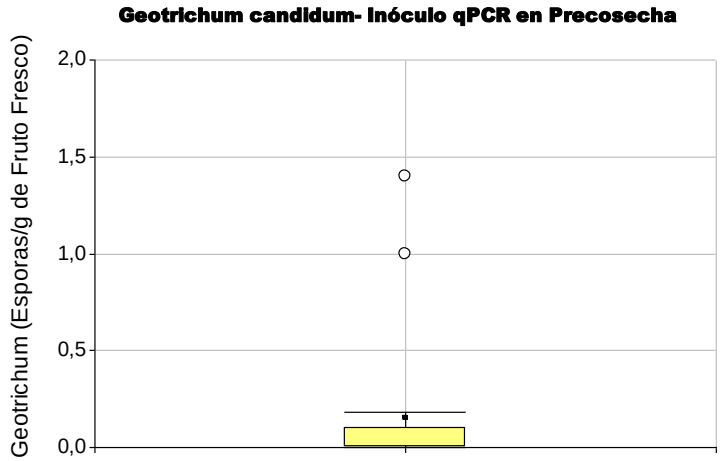

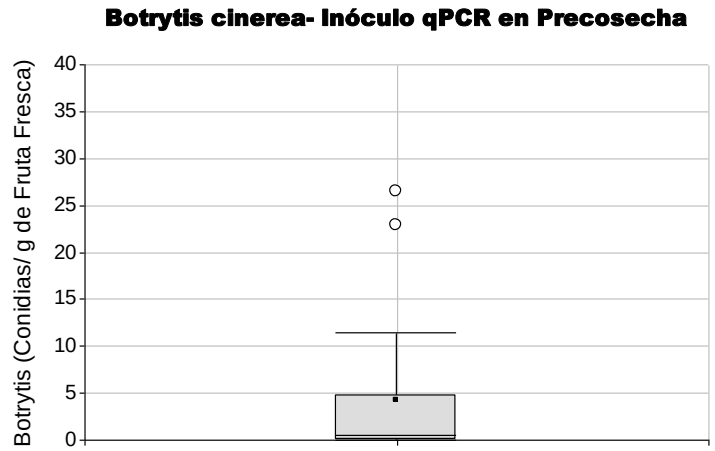

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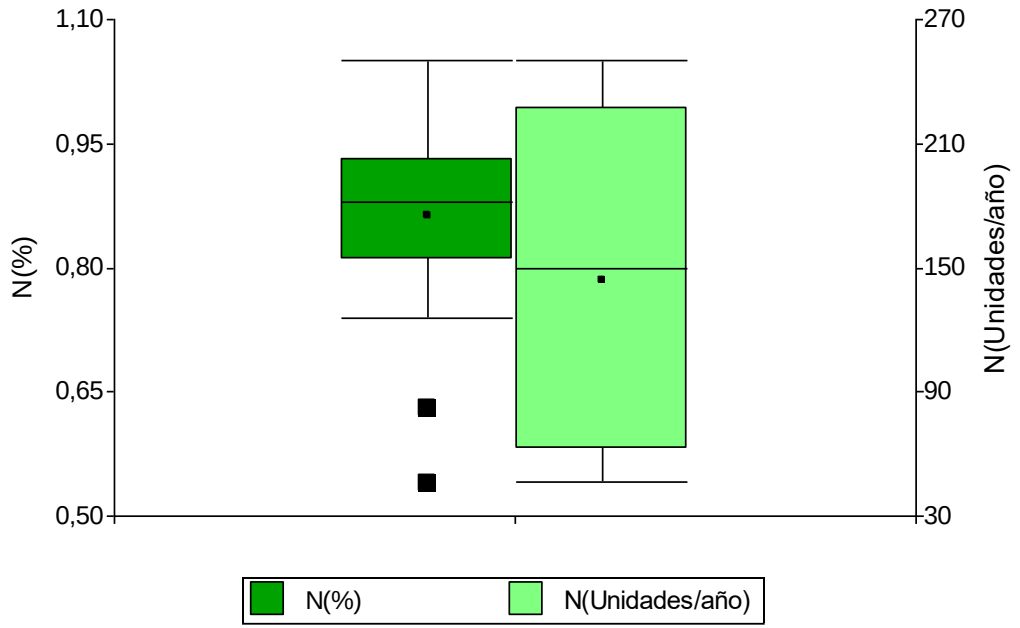
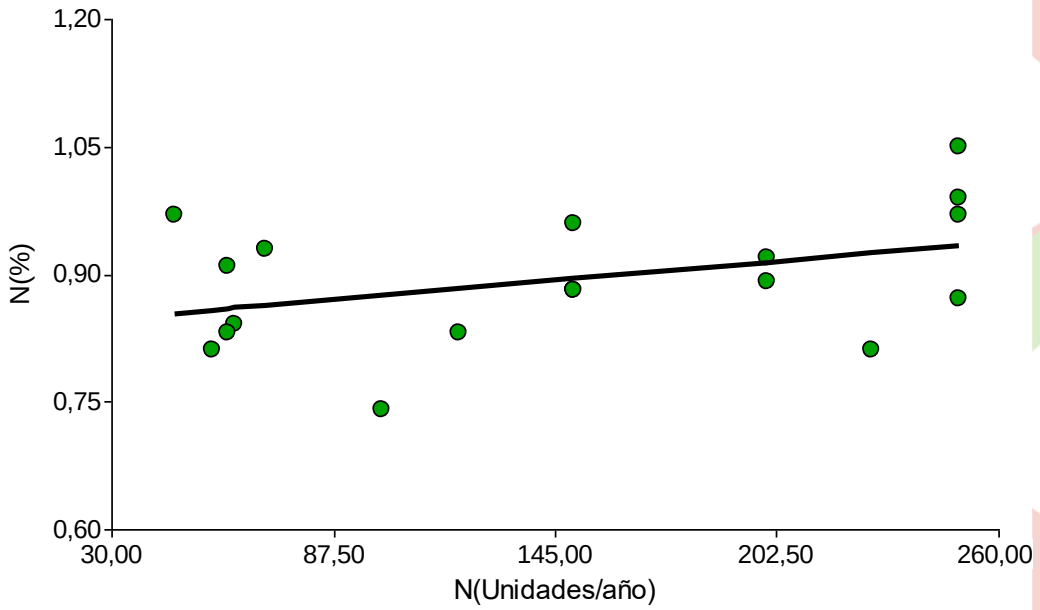
Análisis Nitrógeno en Fruto y Unidades Aplicadas por Año



Regresión N (%) Fruto vs Unidades de N Aplicadas



Variable	N	R^2	R^2 Aj	ECMP	AIC	BIC
N(%)	18	0,16	0,10	0,01	-39,19	-36,52

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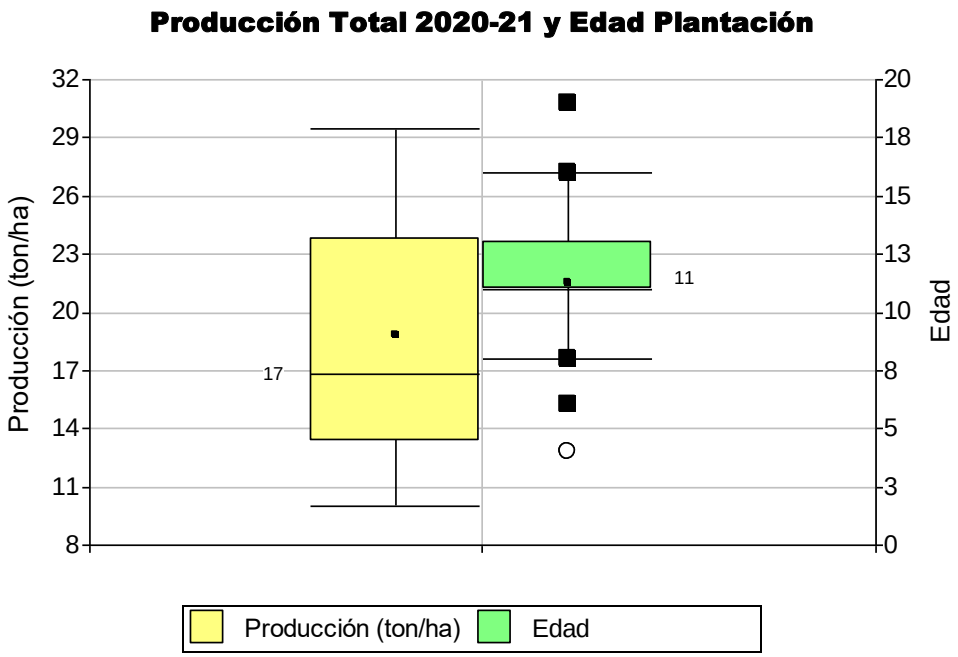

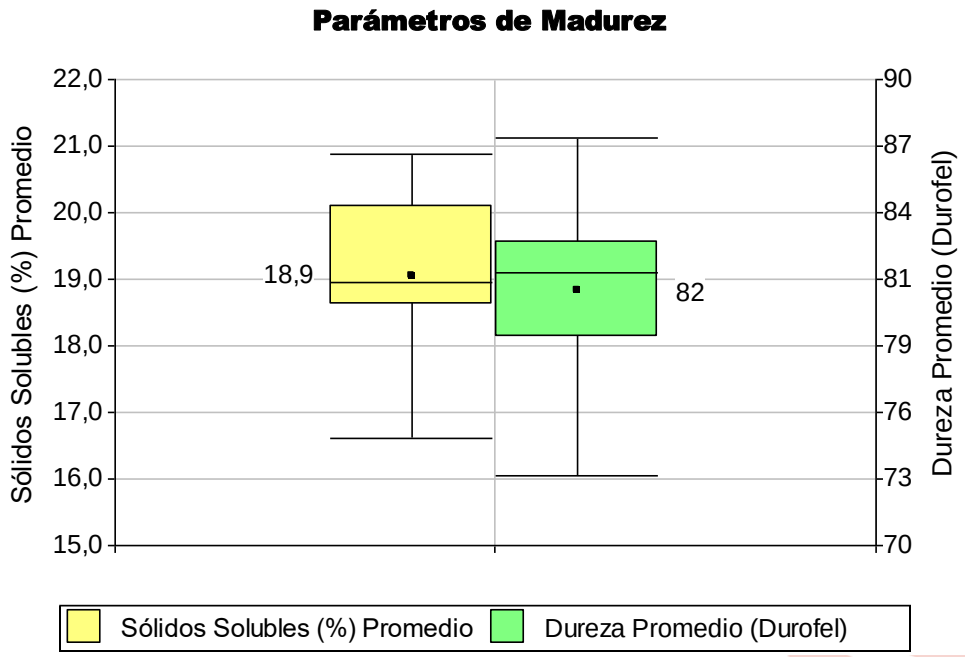

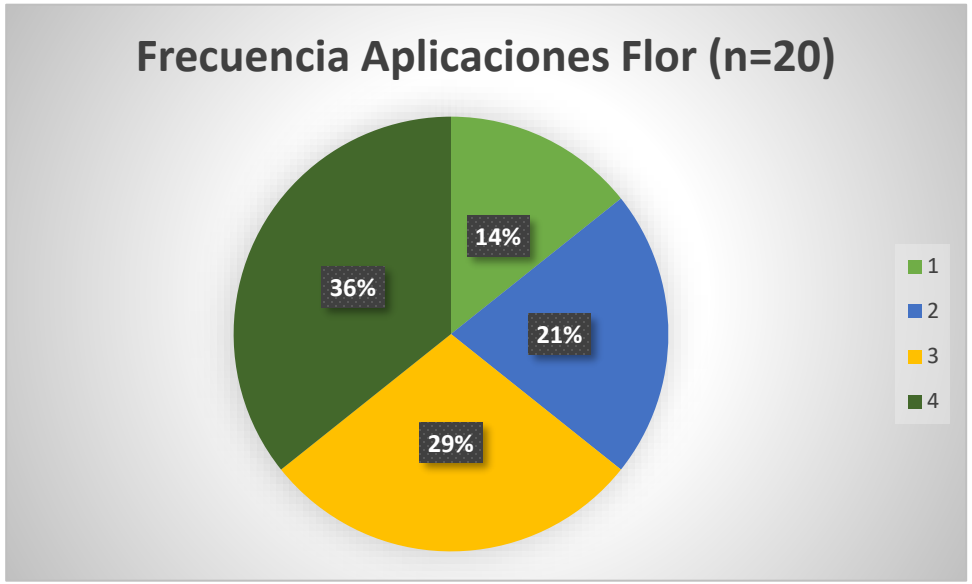

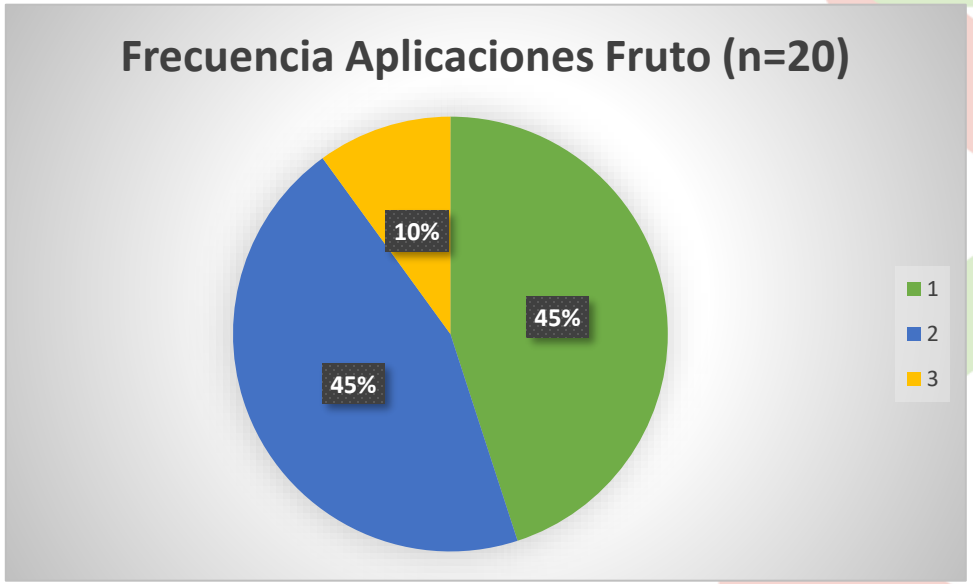

I. Frecuencia de Pudrición Total Promedio a Recepción = 1,4 %
II. Tiadizuron Brote: 5%
III. Tiadizuron Fruto Recién Cuajado y más tarde: 35%
IV. GAs: 100%

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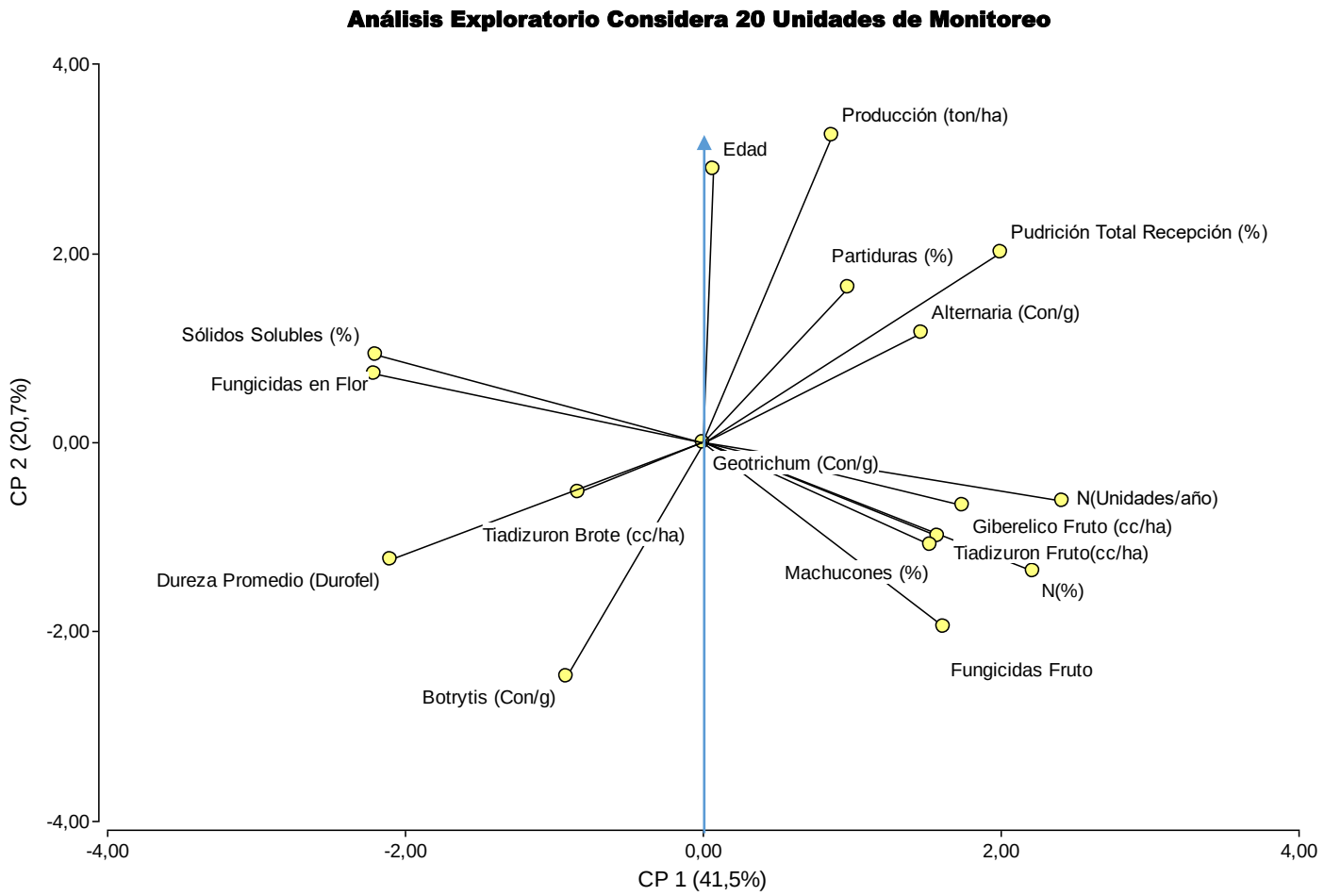
Resultados

Análisis General



Considerar n es muy inferior a lo inicialmente programado, pérdida de representatividad.

Tendencias Generales:

Relaciones Inversas Más claras:

1) A mas N aplicado menos %SS
2) A más GAs y TDZ aplicados desde cuaja menos %SS
3) A más N% en fruto menos %SS
4) A más N% en fruto menos Dureza
5) A mayor Producción menos Dureza
6) A mayor número de aplicaciones en flor tendría un impacto negativo en inóculo de *Alternaria* a Cosecha y Pudrición Total a Recepción

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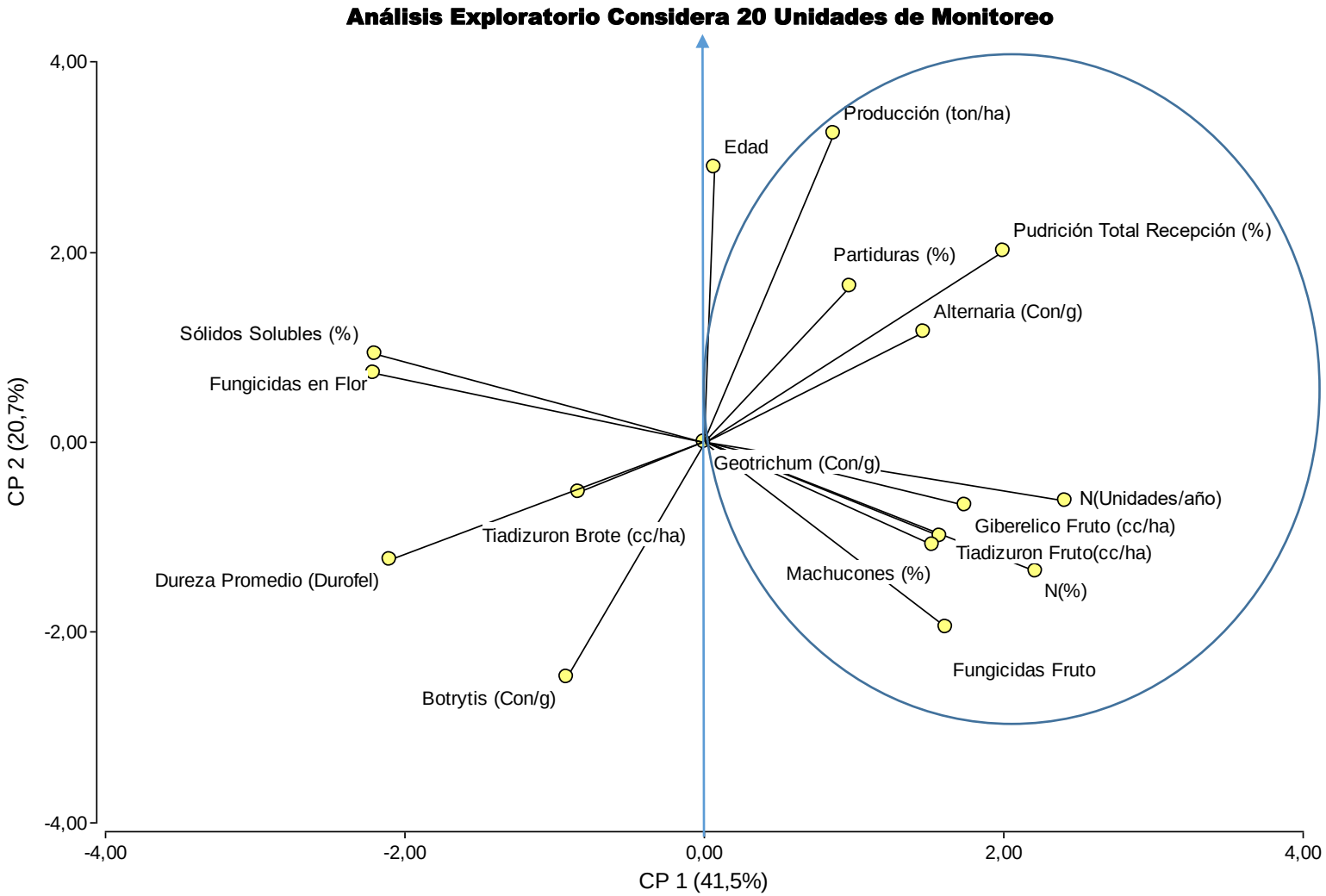

Considerar n es muy inferior a lo inicialmente programado, pérdida de representatividad.

Tendencias Generales:

Relaciones Directas Más Potentes:

1) A más N aplicado más N% en fruto
2) A más N fruto más Frecuencia de Machucones
3) Existe una relación entre utilizar más fungicidas en precosecha y más N% en fruto, Uso de TDZ y GAs.
4) A más producción, más Pudrición Total en recepción, Más Partiduras, Más Inóculo de Alternaria.
5) Pudrición Total a Recepción, Inóculo de Alternaria a Cosecha, Tendrían Relación directa con N aplicado y en Fruto, Uso de TDZ en Cuaja y GAs.

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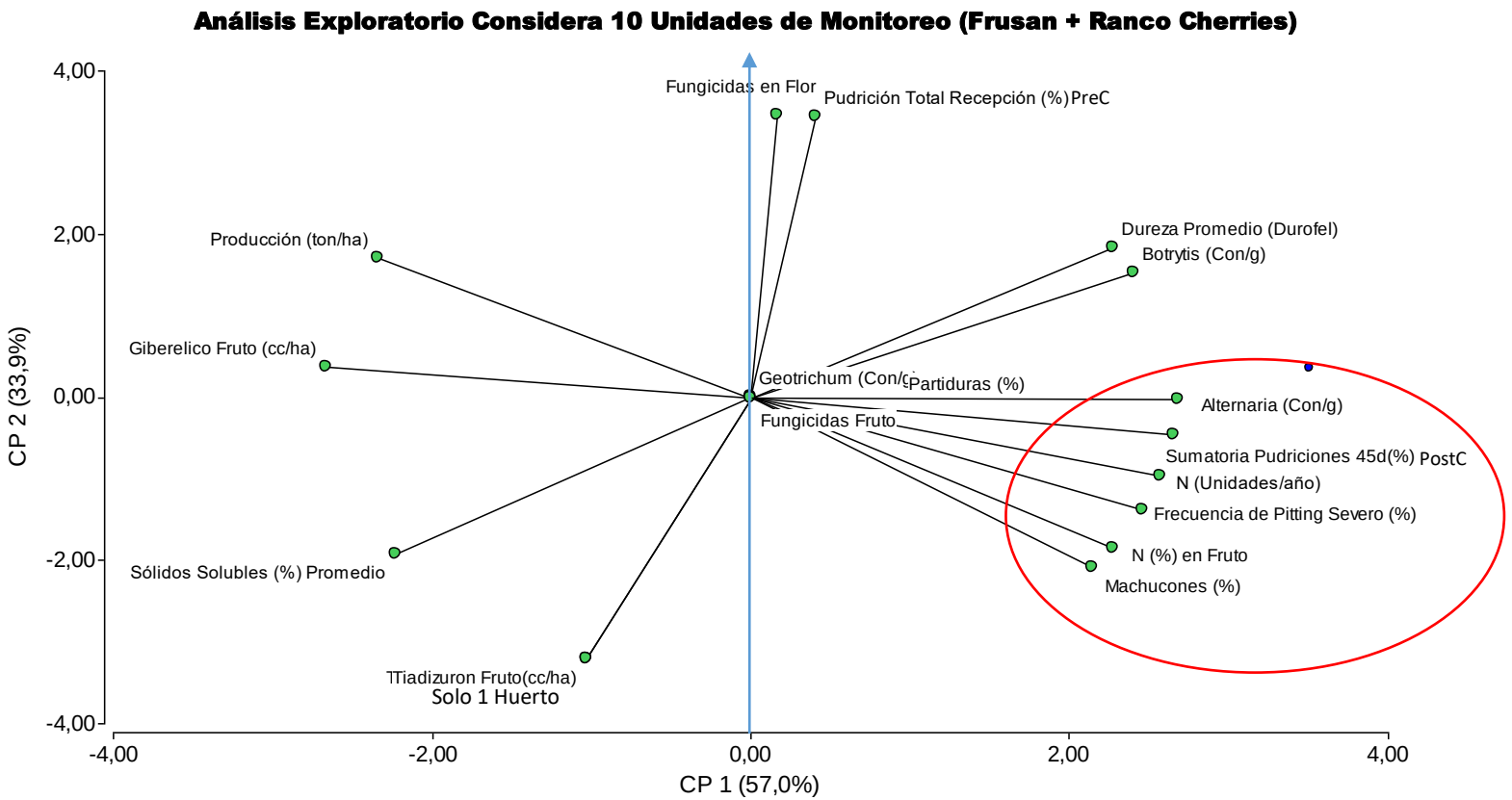
Resultados

Análisis General



Pérdida de Resolución:
N Aplicado: 46-150 UN
Producción: 11-24 Ton/ha
Durofel:78-87
Grupo sin Detecciones de Partiduras

Relaciones Directas más claras:

A mayor N (aplicado y en fruto) mayor Sumatoria de Pudriciones en Postcosecha (30d 0ºC), Mayor Pitting Severo, Mayor frecuencia de Machucones, ¿Partiduras?

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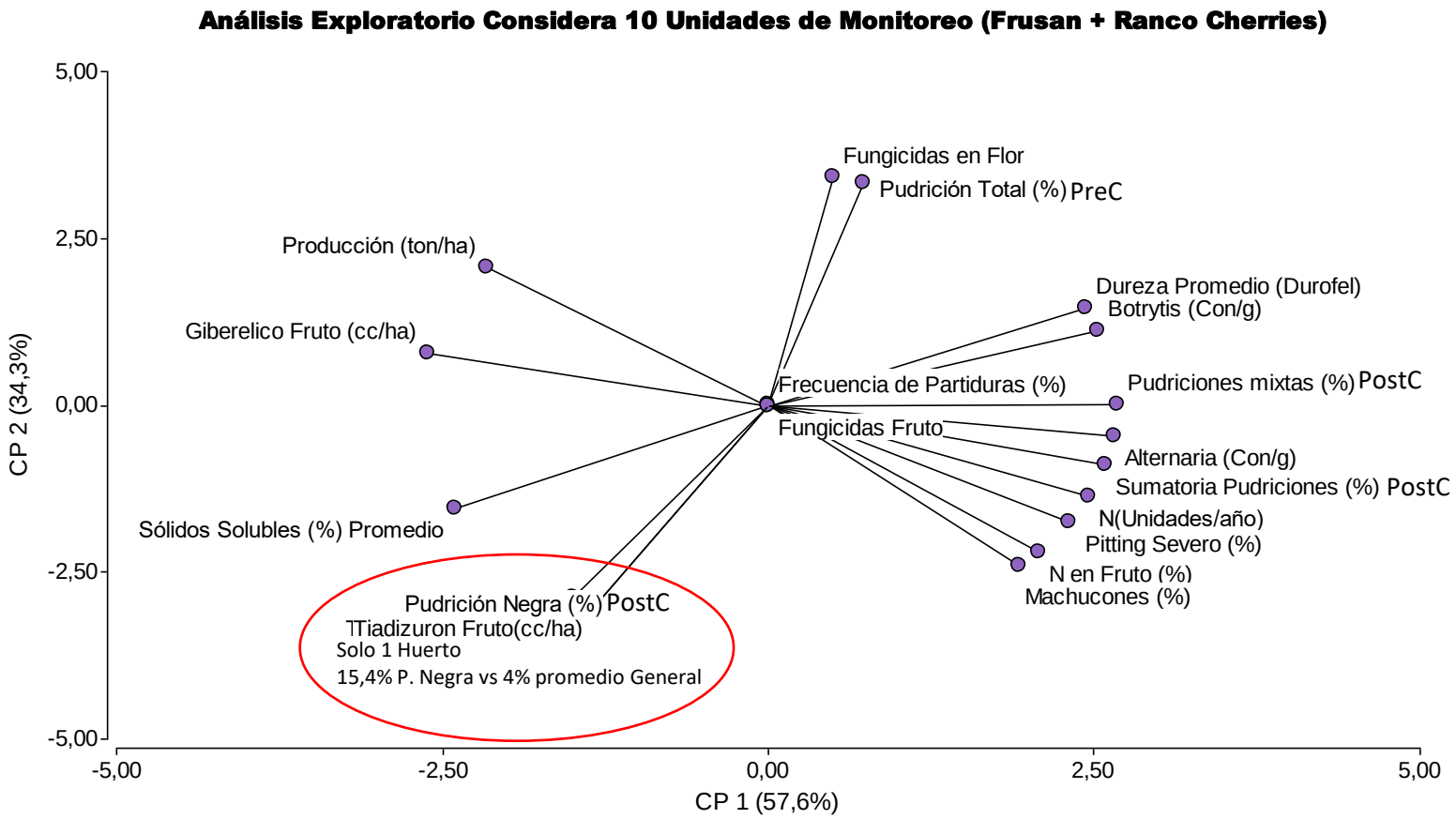

TDZ, ¿Podría ser un *Susceptibilizador* a Pudrición Negra (Alternaria)?

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Hallazgos más Relevantes Estudio Exploratorio

- El **Nitrógeno**, tanto en cantidad aplicada en la temporada y como en concentración en fruto, sería un factor de *susceptibilización* a pudriciones. Desafío: Generación de Estándares- Exploratorios y/o Ensayos Dirigidos
- Huertos de **mayor producción** serían más susceptibles a infecciones, acumulan más inóculo.
- Uso de **Reguladores de Crecimiento** tendrían un efecto susceptibilizador (¿TDZ de Cuaja?) Desafío: Comprobar Efecto/ Relación Dosis – Fenología / Ensayos de Campo

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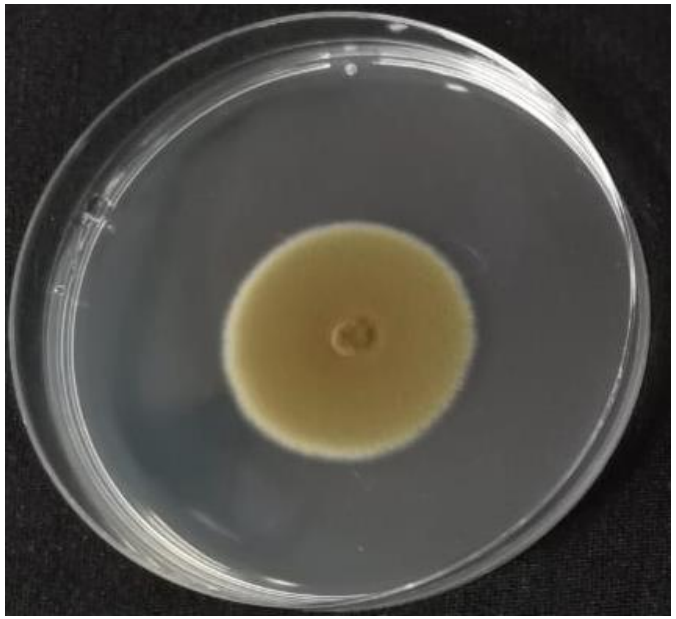
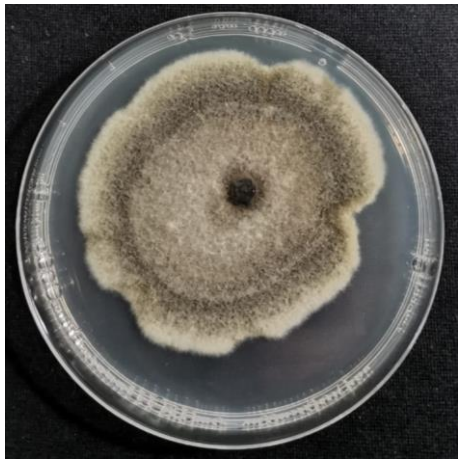
Resultados

Estudios de Sensibilidad

Antecedentes generales

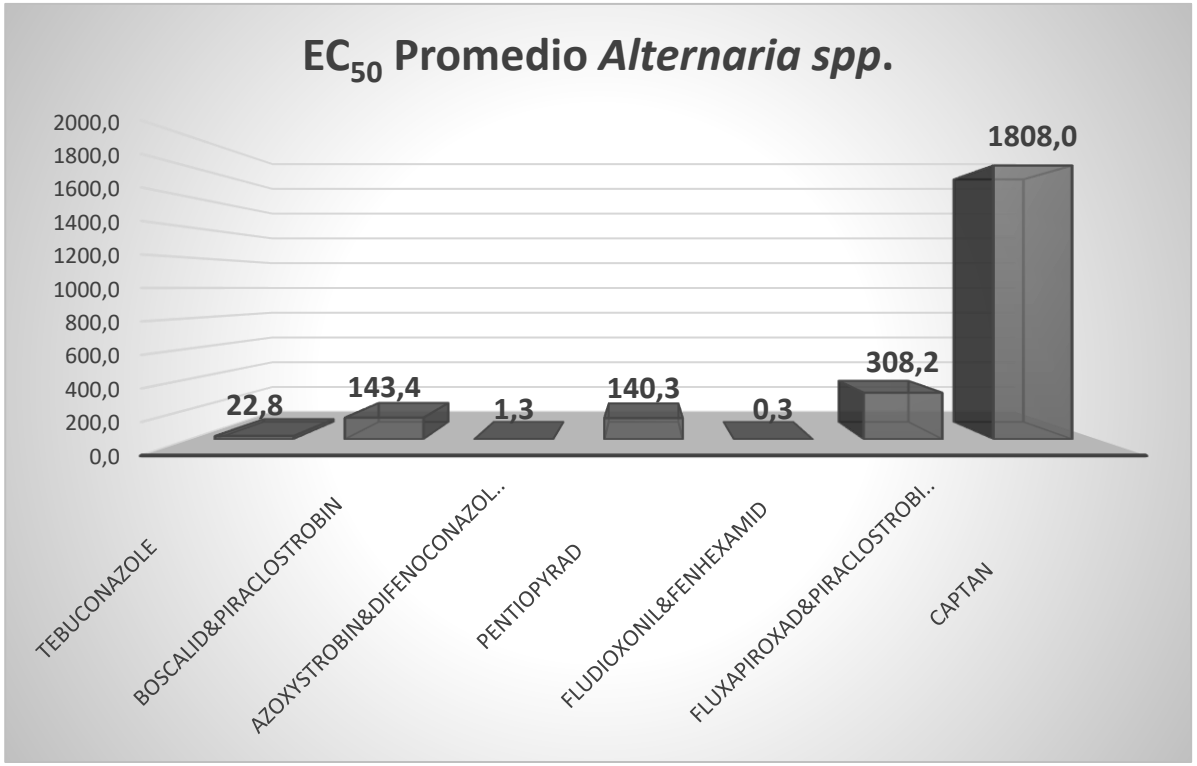
- 13 Aislados *Alternaria* spp.
- 6 Aislados *Cladosporium spp*.
- 7 Aislados *Botrytis cinerea*.
- 8 Aislados *Geotrichum candidum* (2 huertos)
- 10 Aislados *Aureobasidium sp*.
- 7 formulaciones comerciales
- Pruebas de Crecimiento Miceliar y Cálculo de EC50

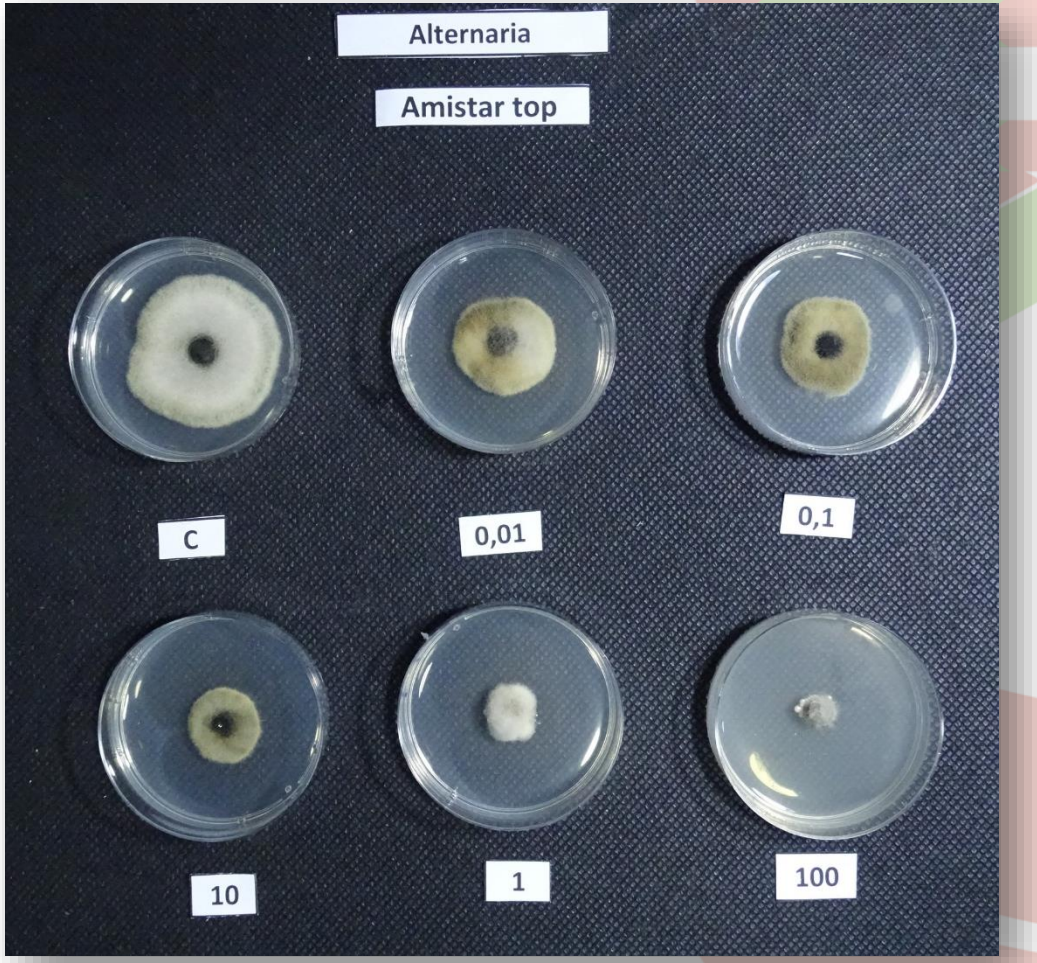




Formulaciones Utilizadas

- Tebuconazole/Apolo®
- Boscalid&Piraclostrobin/Bellis®
- Azoxystrobin&Difenoconazole/Amistar Top®
- Pentiopyrad/Fontelis®
- Fludioxonil&Fenhexamid / Frontal®
- Fluxapiroxad&Piraclostrobin /Elmus®
- Captan/ Captan Anasac®





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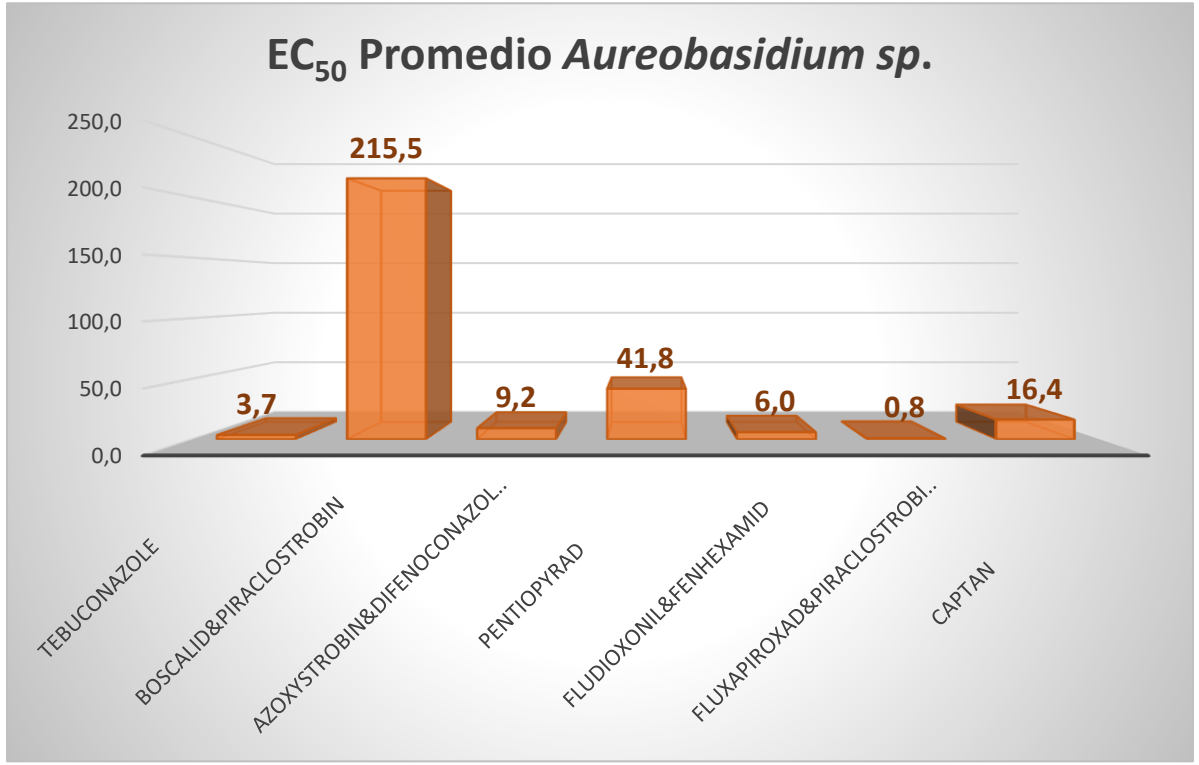

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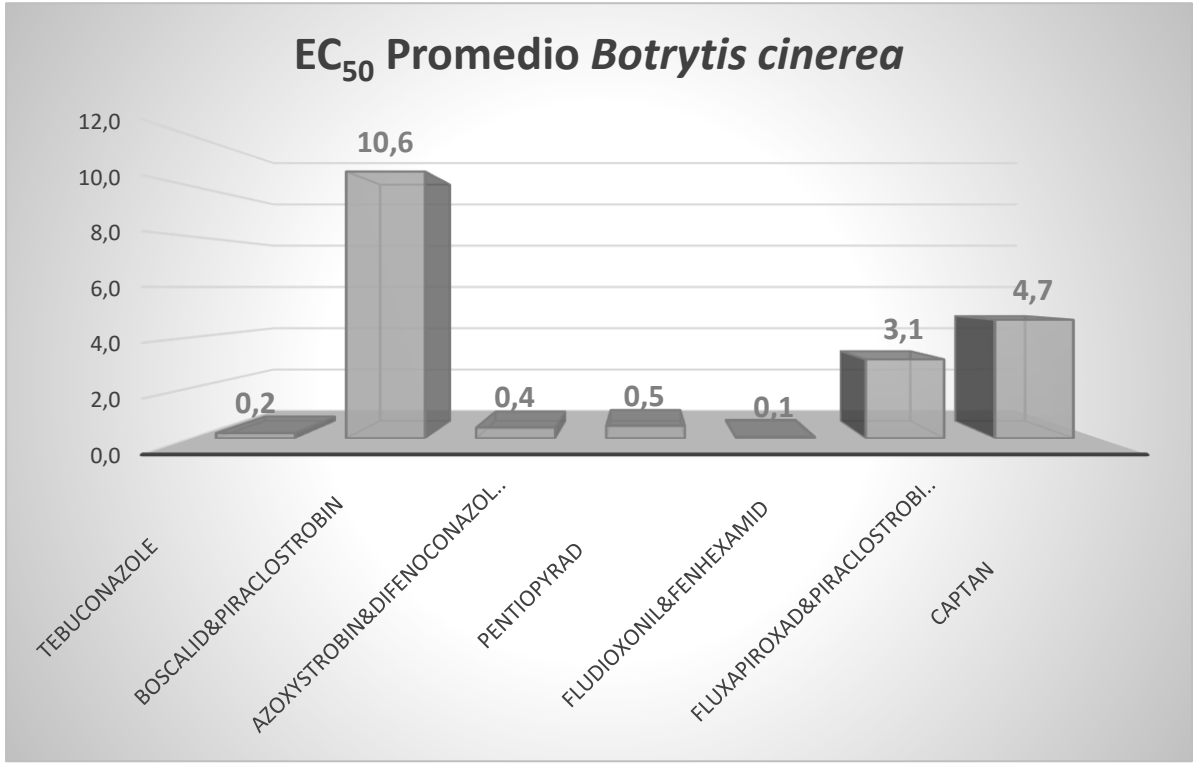

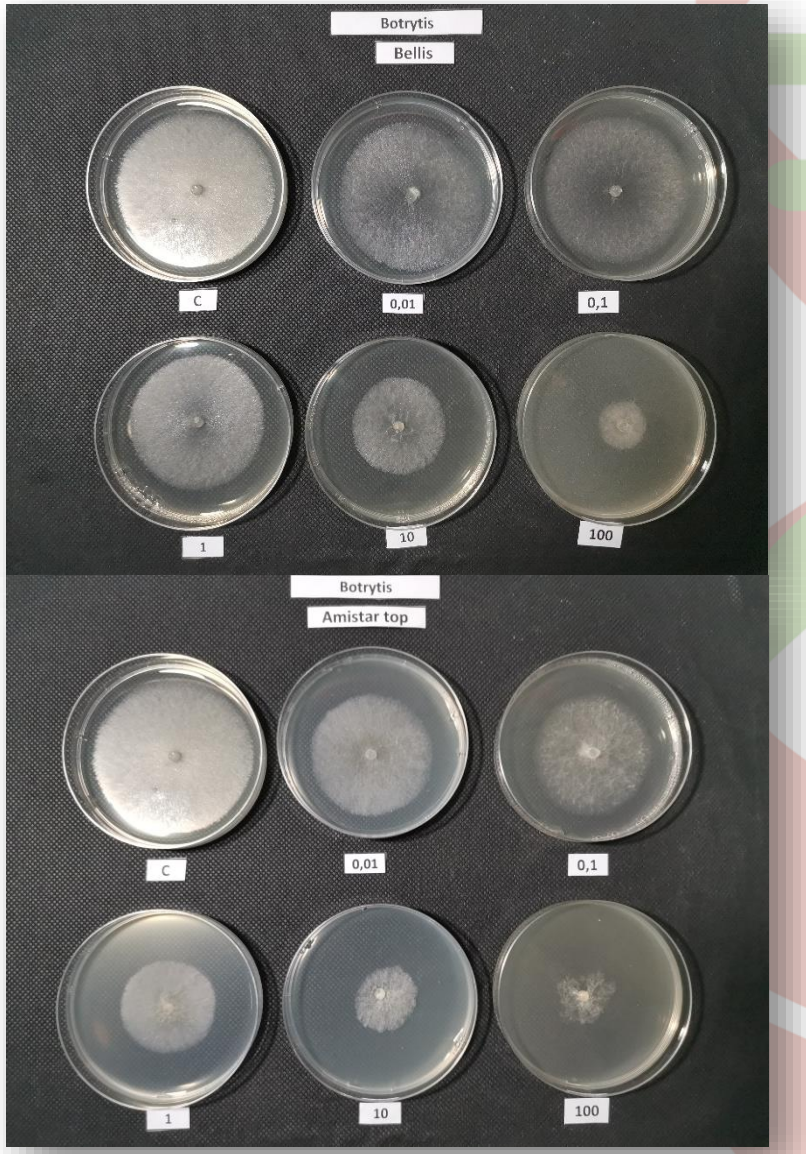

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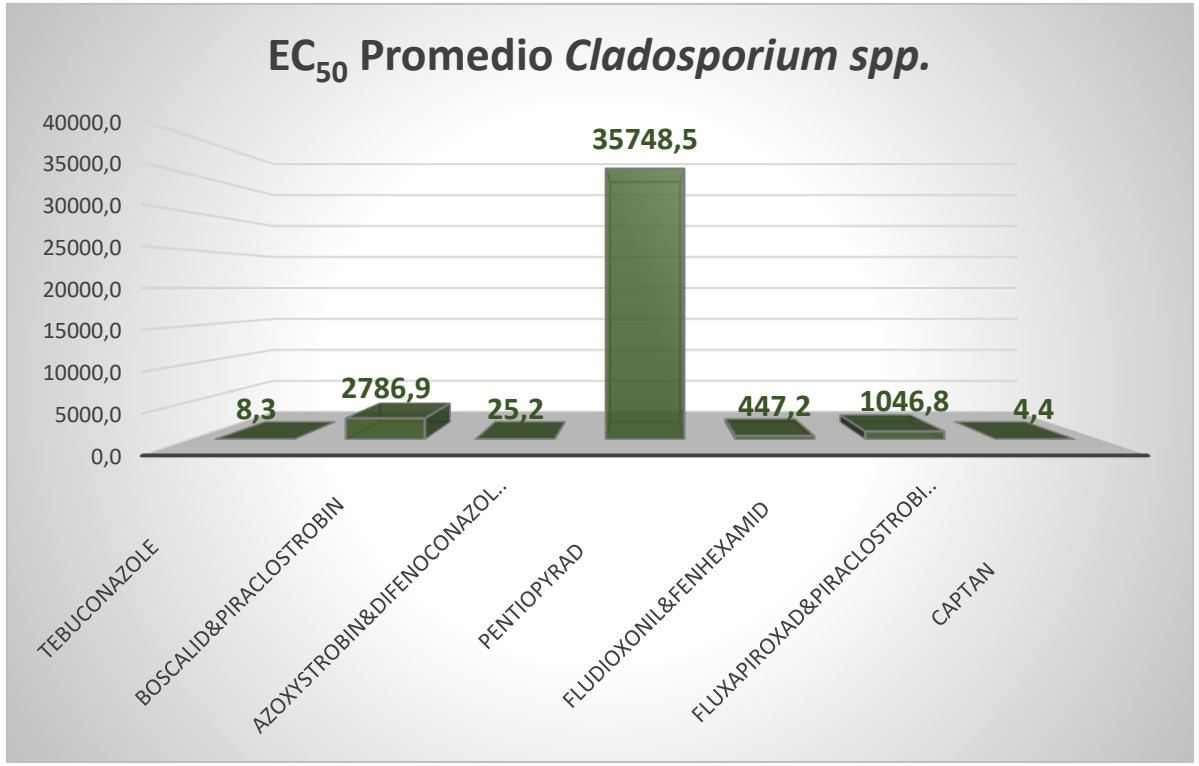

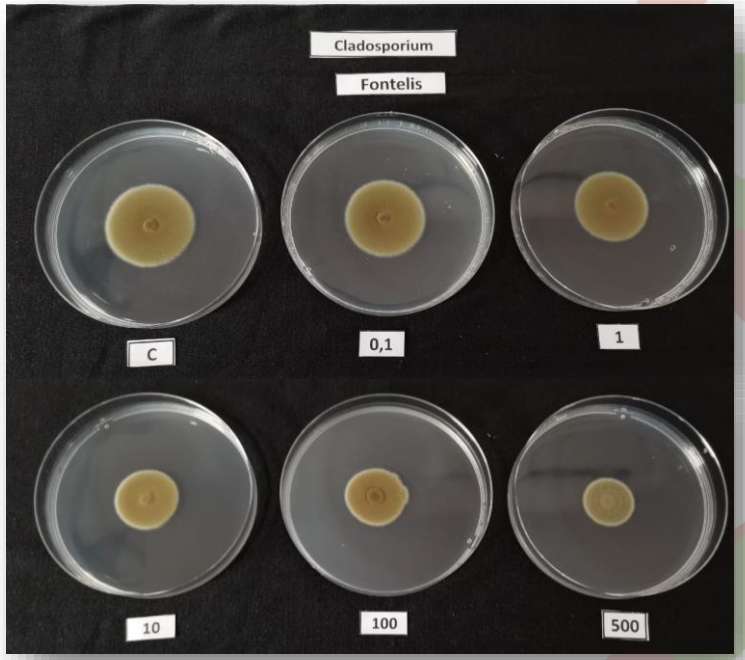

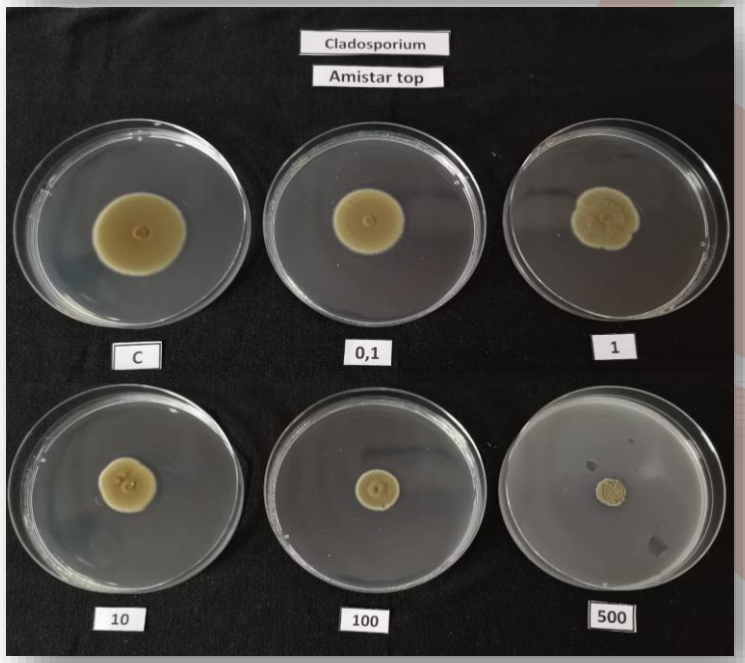

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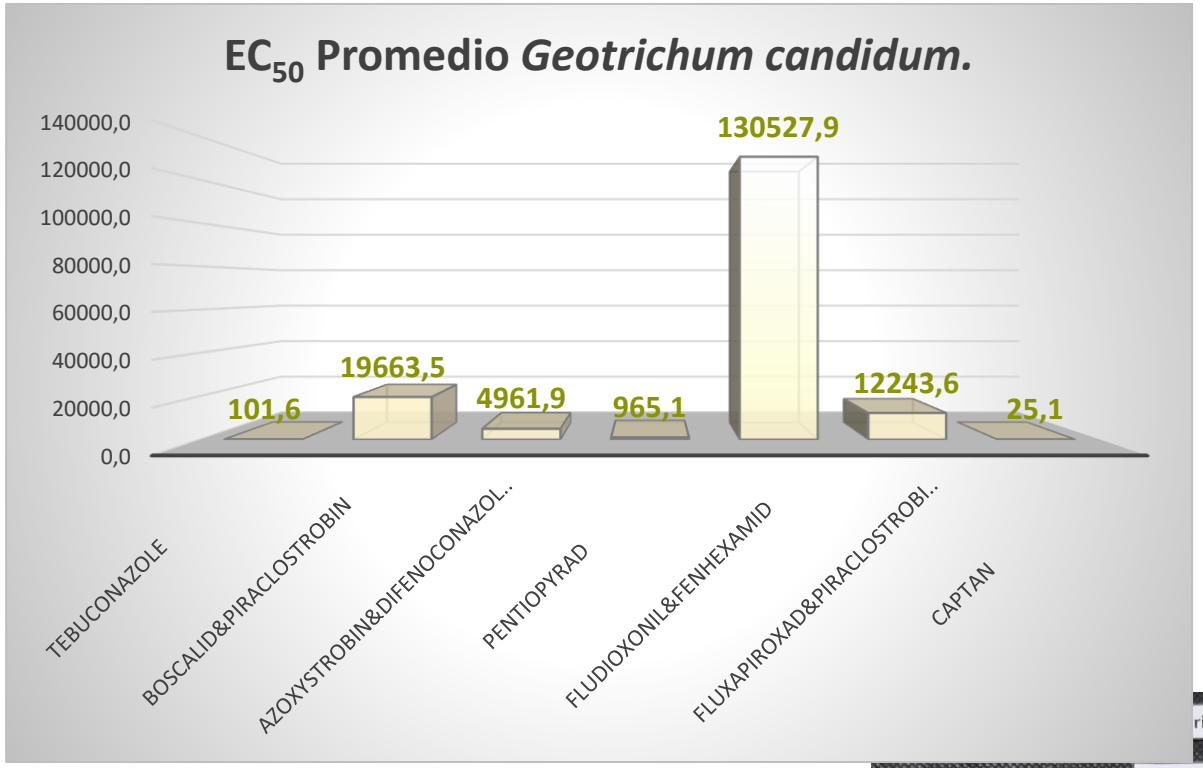

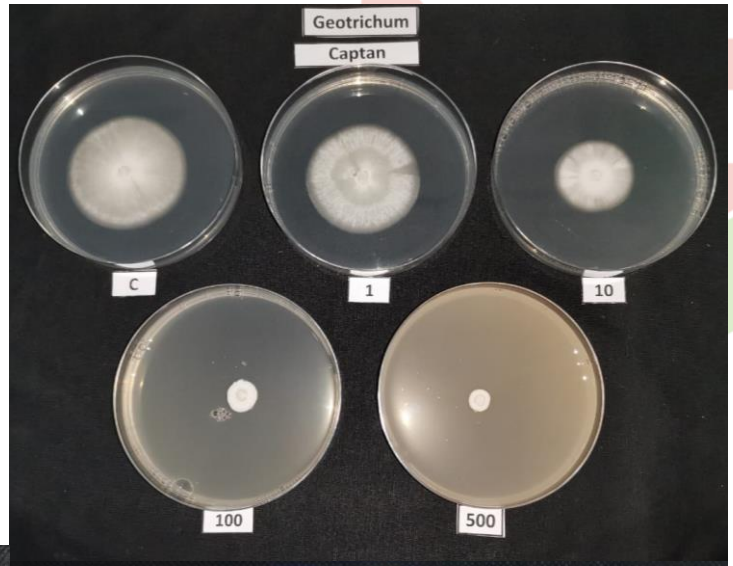

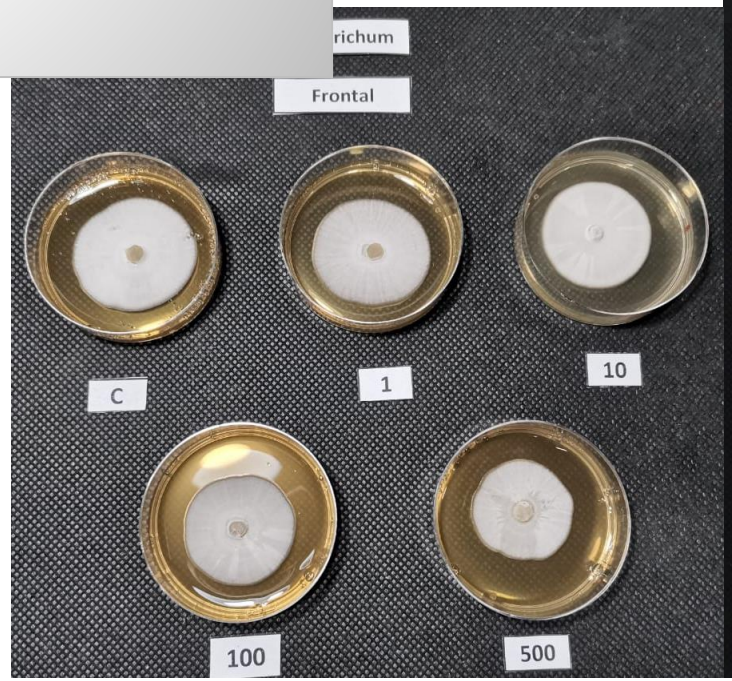

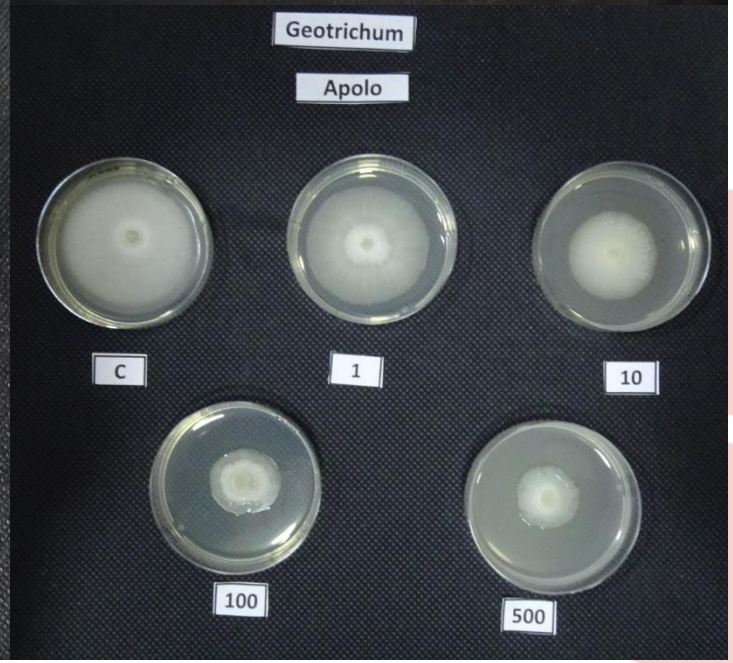

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Interpretación de Resultados

- En virtud de cada formulación, Población Analizada fue categorizada en un rango:
- Desde **+++** Más Sensible a **---** Menos Sensible

Patógeno	Tebuconazole	Boscalid&Piraclostrobin	Azoxystrobin&Difenoconazole	Pentiopyrad	Fludioxonil&Fenhexamid	Fluxapiroxad&Piraclostrobin	Captan
Alternaria	+	- (7/13)	++	- (7/13)	+++	-- (9/10)	---
Aureobasidium	+	--	++	--	+	++	+
Botrytis	+++	+	+++	+++	+++	++	++
Cladosporium	+++	---	++	---	-	--	+++
Geotrichum	+	---	---	--	---	--	++

Dato Importante, en la población de *Alternaria* analizada:

Hay dos tipos de Manejos

1) Una Aplicación de Boscalid, Única Carboxamida en la temporada.

Más Complicaciones

2) 1 a 3 aplicaciones de Carboxamidasen la temporada, siempre en mezcla

¿Qué dice la literatura?

Sensitivities of Baseline Isolates and Boscalid-Resistant Mutants of *Alternaria alternata* from Pistachio to Fluopyram, Penthiopyrad, and Fluxapyroxad

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Abstract

Avenot, H. F., van den Biggelaar, H., Morgan, D. P., Moral, J., Joosten, M., and Michailides, T. J. 2014. Sensitivities of baseline isolates and boscalid-resistant mutants of *Alternaria alternata* from pistachio to fluopyram, penthiopyrad, and fluxapyroxad. Plant Dis. 98:197-205.

Resistance of *Alternaria alternata* to boscalid, the first succinate dehydrogenase inhibitor (SDHI) fungicide labeled on pistachio, has become a common occurrence in California pistachio orchards and affects the performance of this fungicide. In this study, we established the baseline sensitivities of *A. alternata* to the new SDHIs fluopyram, fluxapyroxad, and penthiopyrad and assessed their cross resistance patterns with boscalid. Examination of the effective fungicide concentration that inhibits mycelial growth to 50% relative to the control (EC_{50}) for 50 baseline isolates revealed that the majority were sensitive to boscalid, penthiopyrad, fluopyram, and fluxapyroxad. Analysis of EC_{50} values for boscalid for 117 *A. alternata* isolates originating from boscalid-exposed orchards showed that 44, 3, 1, and 69 isolates had sensitive, reduced sensitivity, moderately resistant, and highly resistant boscalid phenotypes, respectively. Molecular investigation of the occurrence of known *SDH* mutations showed that, among the 69 isolates highly resistant to boscalid, 44, 2, 14, and 1 isolates possessed the mutations leading to the H277Y, H277R, H134R, and H133R amino acid substitutions in AaSDHB, AaSDHB, AaSDHC, and AaSDHD subunits, respectively. Some *SDHB* or *SDHC* mutants displayed highly sensitive, sensitive, or reduced sensitivity phenotypes toward penthiopyrad or fluxapyroxad, whereas other had low, moderate, or high levels of resistance to these fungicides. In contrast, all the *SDHB* mutants were sensitive to fluopyram, while 10, 5, and 1 *SDHC* mutants had sensitive, reduced sensitivity, and moderately resistant fluopyram phenotypes, respectively. The *SDHD* mutant had reduced sensitivity to fluopyram and penthiopyrad but was highly resistant to fluxapyroxad. The discrepancies of cross-resistance patterns between SDHIs suggest that their binding sites in complex II may differ slightly and that additional mechanisms of resistance to these compounds are likely involved. Ultimately, the findings of this study should lead to the rational and sustained deployment of new SDHIs in Alternaria late blight spray programs.

Resistance to Boscalid Fungicide in *Alternaria alternata* Isolates from Pistachio in California

Herve F. Avenot and **Themis J. Michailides,** Department of Plant Pathology, University of California Davis, Kearney Agricultural Center, Parlier 93648

ABSTRACT

Avenot, H. F., and Michailides, T. J. 2007. Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachio in California. Plant Dis. 91:1345-1350.

Boscalid is a new carboxamide fungicide recently introduced in a mixture with pyraclostrobin in the product Pristine for the control of Alternaria late blight of pistachio. In all, 108 isolates of *Alternaria alternata* were collected from pistachio orchards with (59 isolates) and without (49 isolates) prior exposure to boscalid. The sensitivity to boscalid was determined in conidial germination assays. The majority of isolates from two orchards without a prior history of boscalid usage had effective fungicide concentration to inhibit 50% of spore germination (EC_{50}) values ranging from 0.089 to 3.435 µg/ml, and the mean EC_{50} was 1.515 µg/ml. Out of 59 isolates collected from an orchard with a history of boscalid usage, 52 isolates had EC_{50} values ranging from 0.055 to 4.222 µg/ml, and the mean EC_{50} was 1.214 µg/ml. However, in vitro tests for conidial germination and mycelial growth also revealed that seven *A. alternata* isolates, originating from the orchard exposed to boscalid were highly resistant (EC_{50} > 100 µg/ml) to this fungicide. Furthermore, in vitro tests showed no significant differences between wild-type and boscalid-resistant mutants in some fitness parameters such as spore germination, hyphal growth, sporulation, or virulence on pistachio leaves. Experiments on the stability of the boscalid-resistant phenotype showed no reduction of the resistance after the mutants were grown on fungicide-free medium. Preventative applications of a commercial formulation of boscalid (Endura) at a concentration which is effective against naturally sensitive isolates failed to control disease caused by the boscalid-resistant isolates in laboratory tests. To our knowledge, this is first report of field isolates of fungi resistant to boscalid.

Additional keywords: boscalid resistance, carboxamides, predicted fitness

plex succinate ubiquinone reductase is a functional part of the tricarboxylic cycle and the mitochondrial electron transport chain and catalyzes both the oxidation of succinate to fumarate and the reduction of quinine. The Sdh complex consists of four subunits, a flavoprotein (Fp) subunit (SdhA), an iron sulfur protein (Ip) subunit (SdhB), and two membrane-anchored protein subunits (SdhC and SdhD). The Fp and Ip subunits form the soluble part of the complex and carry the Sdh activity, whereas the SdhC and SdhD subunits anchor Fp and Ip to the membrane and carry the quinine reduction activity (15,18). Then, due to its novel mode of action, boscalid can control pathogens which have developed resistance to other chemical classes of fungicides. Through the inhibition of complex II, it inhibits spore germination, germ tube elongation, mycelial growth, and sporulation and is registered for use against *Botrytis cinerea* and *Sclerotinia*, *Alternaria*, and *Monilinia* spp., powdery mildews, and other pathogens in fruit, vegetables, and vines. In pistachio,

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