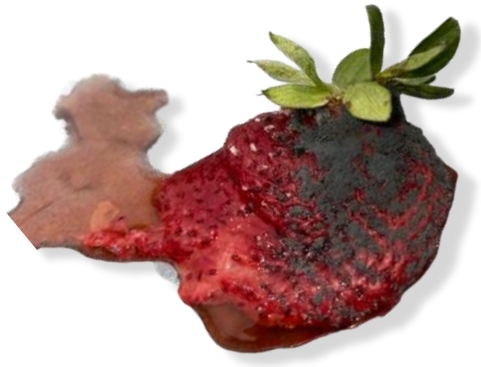


Unraveling Leak Rot

Gerald Holmes – Director

Gabriela Torres – Graduate Student

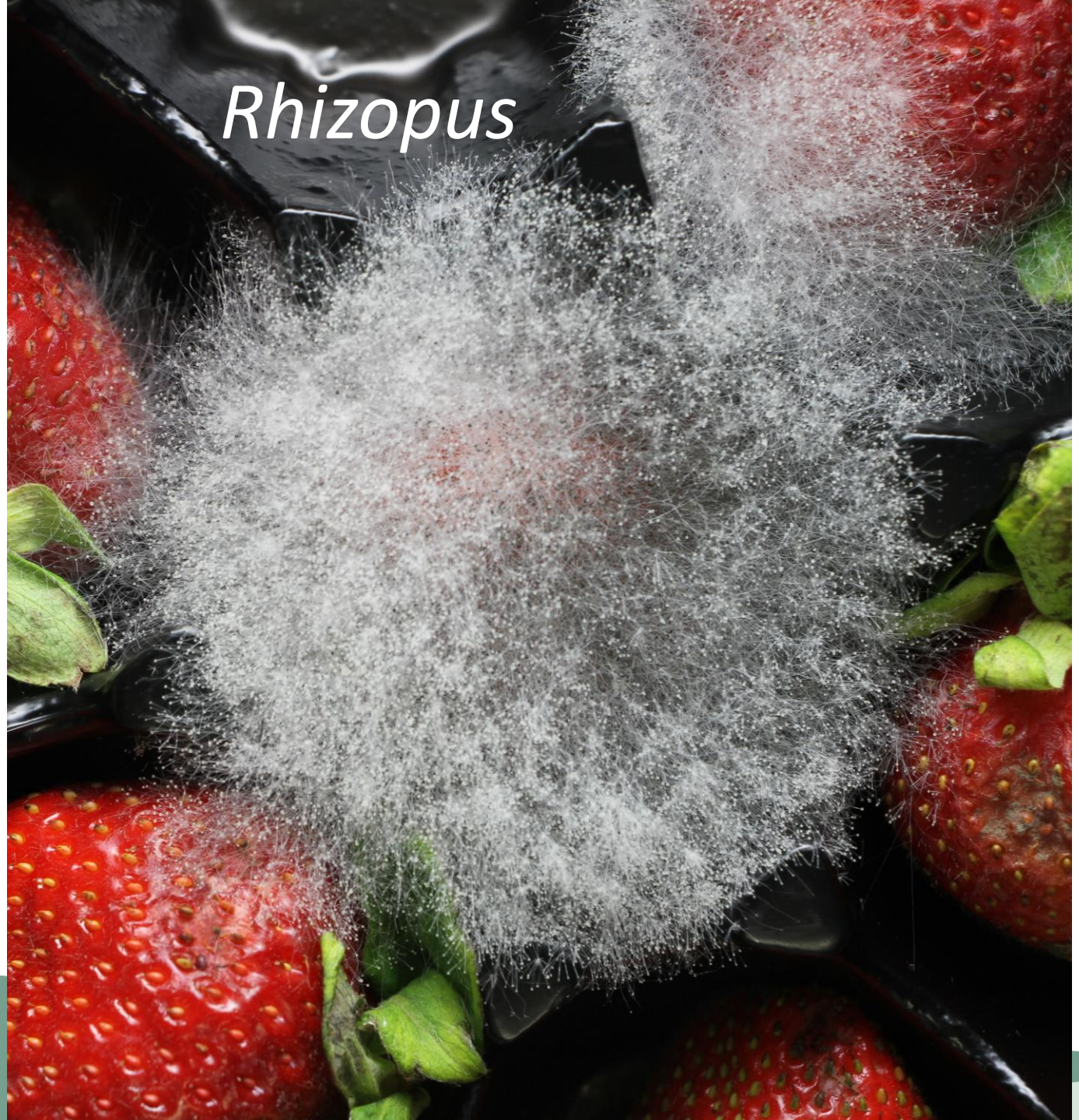

Botrytis
Cool & wet


Rhizopus
Warm & wet


Mucor
??

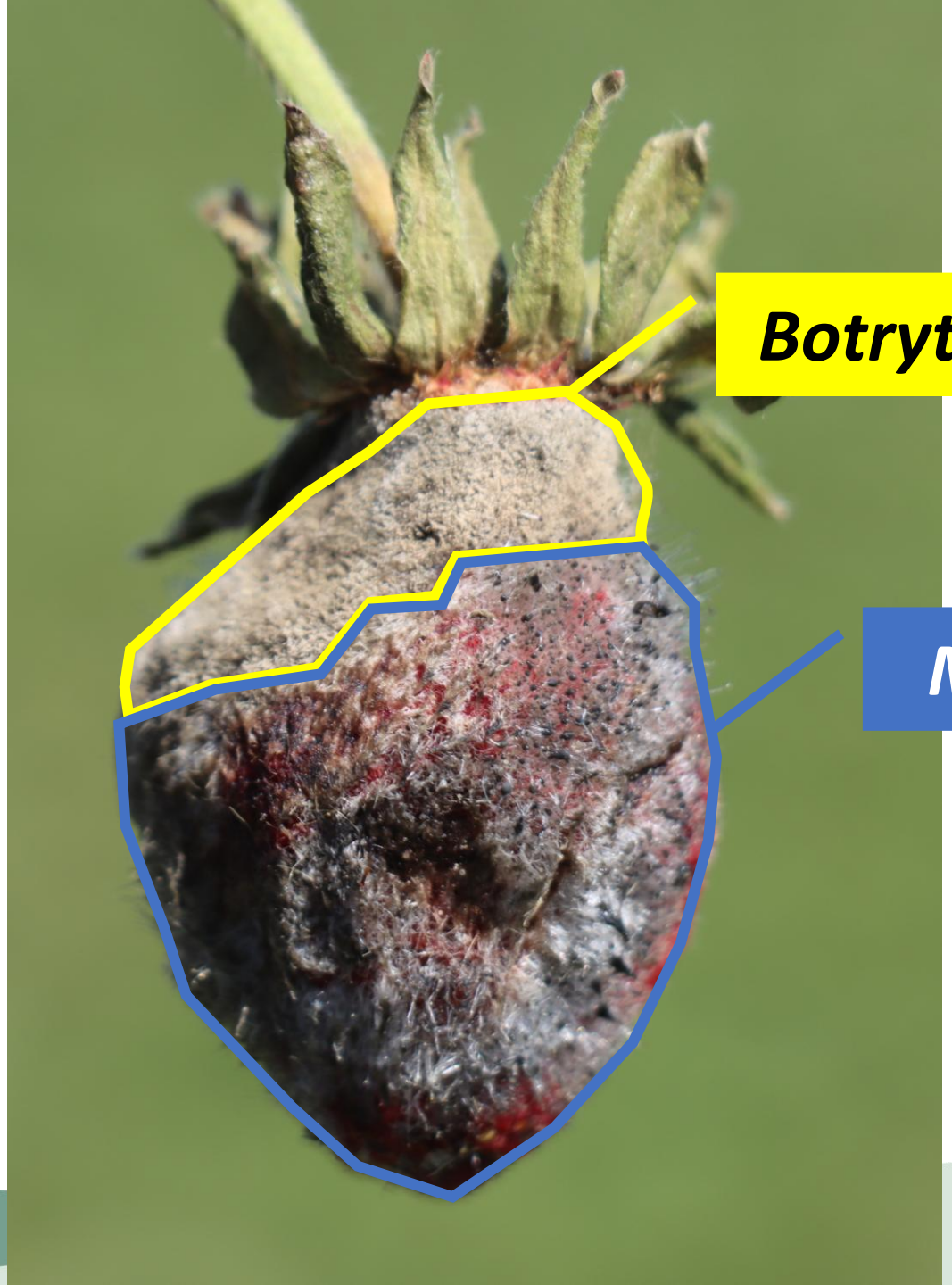

Botrytis


Botrytis

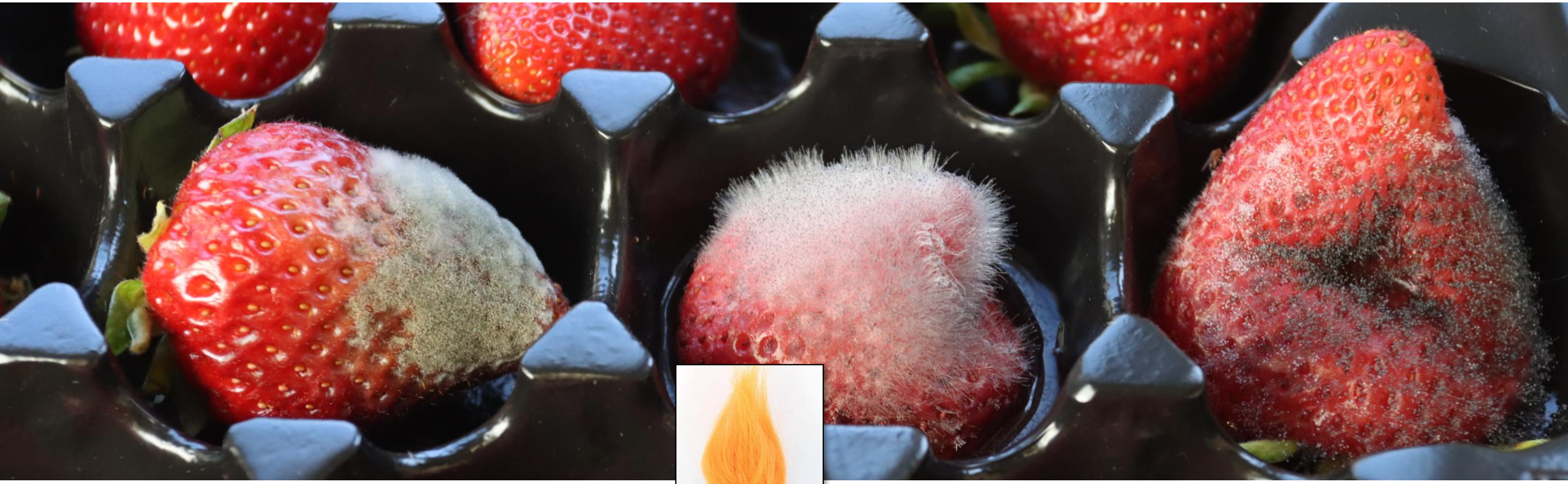

Rhizopus


Rhizopus

Mucor



Botrytis *Mucor* *Rhizopus*



Leak

- Responsible for high losses
- Very rapid decay (liquify fruit in 12 hours)
- Few products effective against leak

Project objectives

- How to distinguish *Rhizopus* from *Mucor*?
- Which one is most prevalent?
- What species are involved?
- Under what conditions does each thrive?
- Which fungicides are most effective?
- Fact sheet creation

Leak Rot Causal Agents

Rhizopus

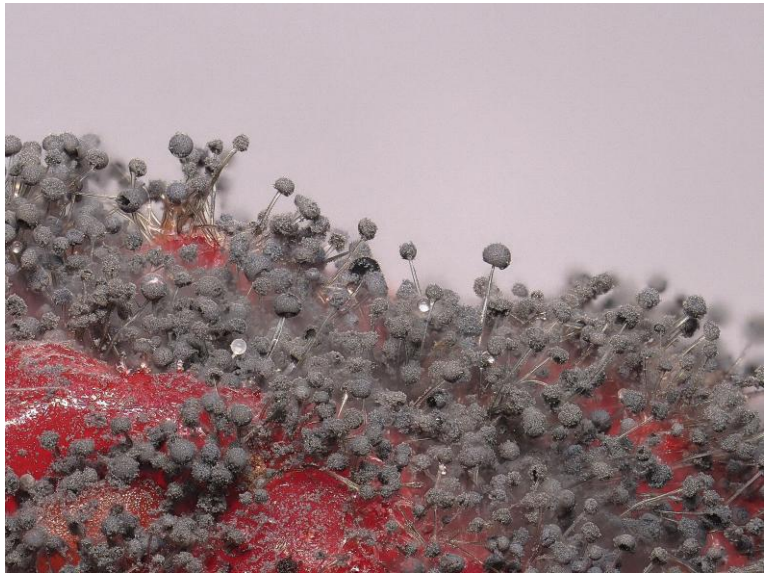
Mucor

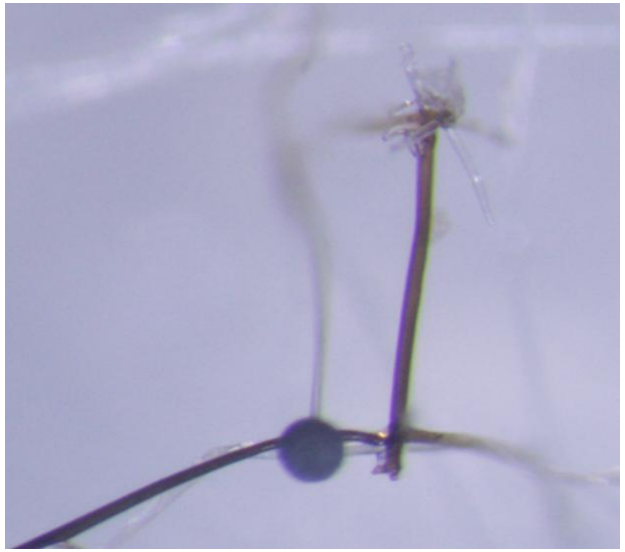
Species Currently Reported in Strawberries

- Rhizopus rot (leak)
  - *R. stolonifer*
  - *R. sexualis*
- Mucor fruit rot
  - *M. piriformis*
  - *M. mucedo*
  - *M. hiemalis* f. sp. *hiemalis*
  - *M. hiemalis* f. sp. *silvaticus*

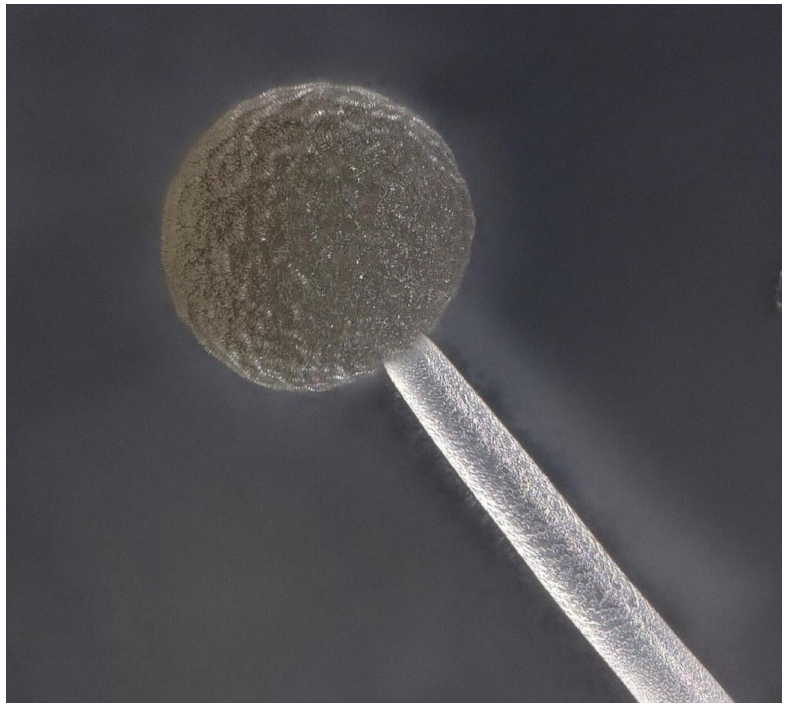
Distinctions Between Causal Agents

Rhizopus





Mucor



Rhizopus

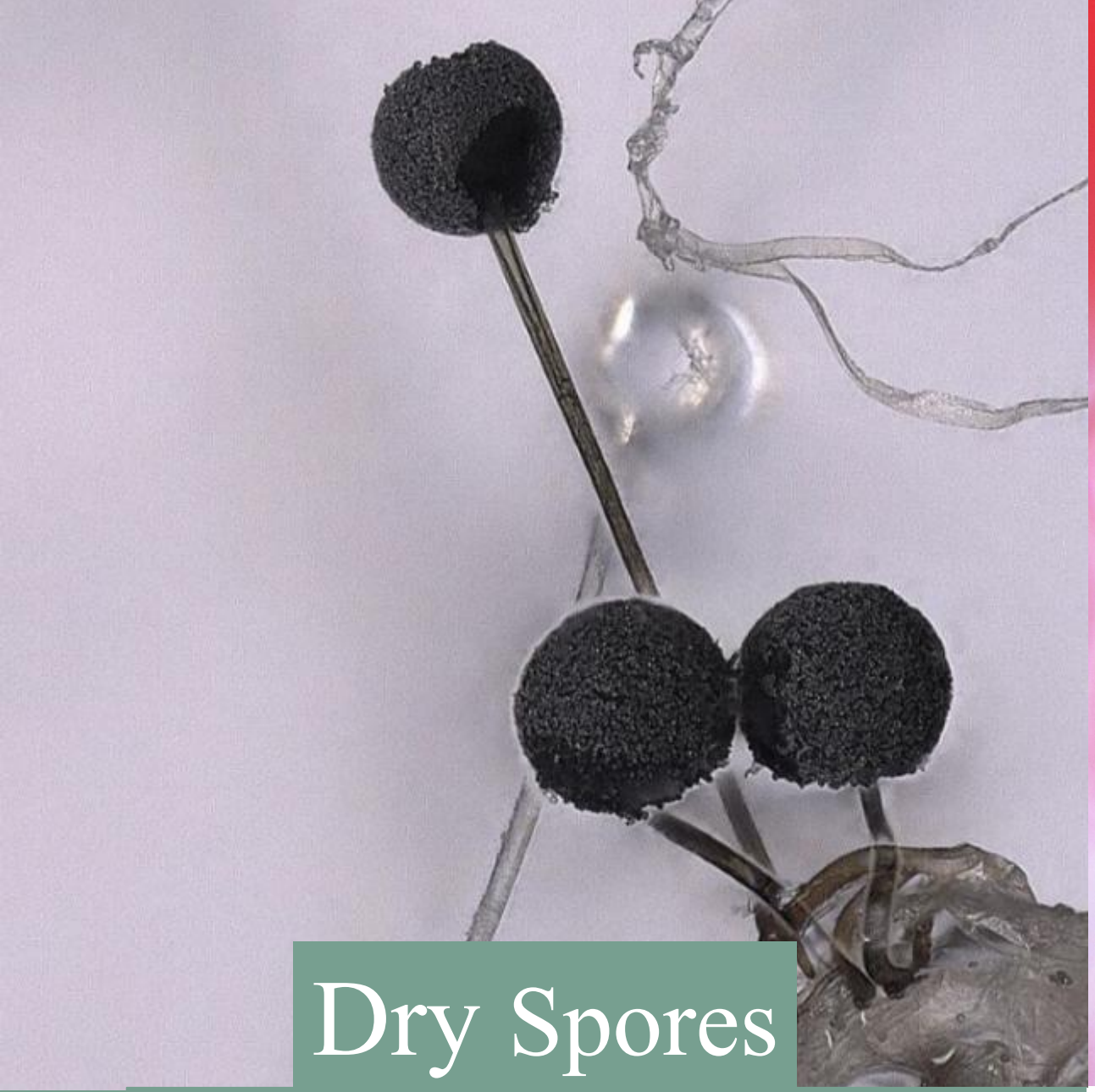
Mucor

Dry Spores

Rhizopus

Wet Spores

Mucor



Dry Spores

Rhizopus

Wet Spores

Mucor

Feature	*Rhizopus*	*Mucor*
Species reported	***R. stolonifer***, *R. sexualis*	*M. mucedo*, ***M. piriformis***, *M. hiemalis* f. sp. *hiemalis*; *M. hiemalis* f. sp. *silvaticus*
Infect fruit at 0°C	**No**	**Yes (some species)**
Minimum temp		
Maximum temp		
Optimum temp		
Sporangia production inhibited below…		
Identifying features		
- Texture	**Dry Spores**	**Wet Spores**
- Color		
- Odor		

Fill in the blanks

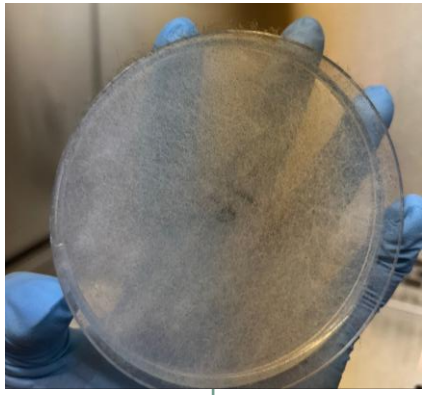
Methodology for Identification

Field Identification

Or

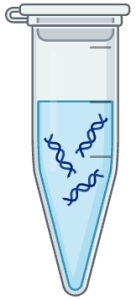
Incubation

Swab Collection

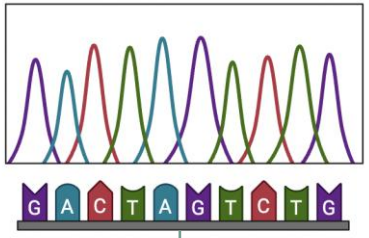


Swab Collection

Broth Culture



DNA Extraction and Amplification



Sanger Sequencing

NCBI Library

National Library of Medicine
National Center for Biotechnology Information

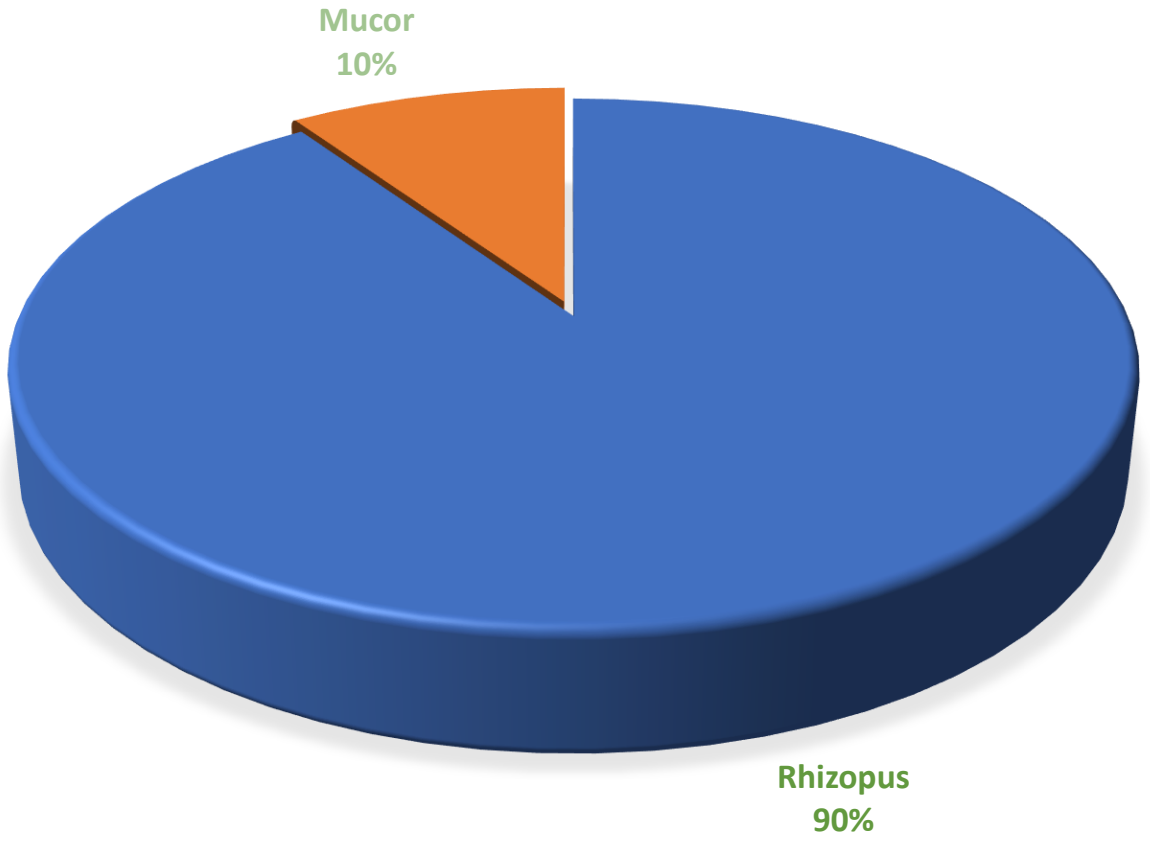
Sequences producing significant alignments Download Select columns Show 100

select all *100 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Rhizopus stolonifer isolate GN18 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Rhizopus stolon...	106	106	99%	7e-19	88.89%	887	MN511321.1
☑	Rhizopus stolonifer isolate AFTOL-ID 632 internal transcribed spacer 1, partial sequence; 5.8S ribosomal...	Rhizopus stolon...	104	104	99%	2e-18	89.02%	787	AY997085.1
☑	Rhizopus stolonifer 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), s...	Rhizopus stolon...	104	104	99%	2e-18	89.02%	877	AM933544.1
☑	Rhizopus stolonifer strain F213032 18S ribosomal RNA gene, partial sequence; internal transcribed spac...	Rhizopus stolon...	100	100	99%	3e-17	87.80%	857	KM203865.1
☑	Rhizopus stolonifer strain KACC 45890 18S ribosomal RNA gene, partial sequence; internal transcribed s...	Rhizopus stolon...	100	100	99%	3e-17	87.80%	839	JN315028.1

Prevalence in California (n=62)

- ***Rhizopus***
  - *Rhizopus stolonifer*
  - 56 isolates
- ***Mucor***
  - *Mucor piriformis*
  - 6 isolates



Fungicide Efficacy

FUNGICIDES, BACTERICIDES, BIOCONTROLS, AND NATURAL PRODUCTS FOR DECIDUOUS TREE FRUIT AND NUT, CITRUS, STRAWBERRY, AND VINE CROPS IN CALIFORNIA

ALMOND
APPLE
APRICOT
CHERRY
CITRUS

GRAPE
KIWIFRUIT
PEACH
NECTARINE
PEAR
PISTACHIO

PLUM
POMEGRANATE
PRUNE (DRIED PLUM)
STRAWBERRY
WALNUT

James E. Adaskaveg, Professor
University of California, Riverside

Themis Michailides, Plant Pathologist
University of California, Davis/Kearney Agricultural Research and Extension Center

Akif Eskalen, Professor of Cooperative Extension in Plant Pathology
University of California, Davis

Special thanks to Larry Bettiga, Farm Advisor, UCCE Monterey Co., for his review of grape fungicides and Gerald Holmes, Director of the Strawberry Center, CalPoly, for his review of strawberry fungicides

UC Davis, Dept. of Plant Pathology plantpathology.ucdavis.edu	**UC Kearney Agricultural Research and Extension Center** kare.ucanr.edu/programs/Plant_Pathology
UC Statewide IPM Program ipm.ucanr.edu	**UC Riverside, Dept. of Microbiology and Plant Pathology** cfn-fungicides.ucr.edu

FUNGICIDES AND BACTERICIDES - EFFICACY AND TIMING

STRAWBERRY: FUNGICIDE EFFICACY - CONVENTIONAL

Fungicide[1]	Resistance risk (FRAC)[2]	Gray mold	Powdery mildew	Anthrac - nose	Rhizopus/ Mucor rot	Phytophthora diseases[3]	Common leaf spot	Angular leaf spot
Miravis Prime	medium (7/12)	5	5	3	NL (4)	NL	NL	NL
Merivon	medium (7/11)	5R	5R	ND	NL (4)	NL	0	NL
Kenja	high (7)	5	3	ND	NL (2)	NL	ND	NL
Pristine	medium (7/11)	5R	4R	ND	NL	NL	0	NL
Switch[5]	medium (9/12)	5R	2	4	4	NL	NL	NL
Cannonball[5]	high (12)	5R	NL	4	4	NL	NL	NL
Elevate	high (17)	5RR	NL (0)	NL (0)	NL	NL	NL (0)	NL
Inspire Super	medium (3/9)	5R	5	4	5	NL	ND	NL
Protocol	medium (1/3)	4R	4R	3	NL	NL	4	NL
Captevate**	medium (M4/17)	4R	NL	4	NL	NL	NL	NL
Rovral, Iprodione, Nevado, etc.[7]	high (2)	4R	NL (0)	0	NL	NL	0	NL
Thiram	low (M3)	4	NL (0)	3	NL	NL	0	NL
Captan	low (M4)	4	NL (0)	NL (2)	NL (2)	NL	NL (0)	NL
PH-D, Oso	high (19)	3	4	3	NL	NL	NL	NL
Regev	medium (3/BM 01)	3	5	4	5	NL	ND	NL
Scala	high (9)	3	NL (2)	NL	NL	NL	NL	NL
Fontelis	high (7)	2R	4R	NL	NL	NL	NL	NL
Luna Sensation	medium (7/11)	1R	5	3R	2	NL	ND	NL
Luna Privilege **(foliar)/ Velum One (soil)[4]	high (7)	1/NL	5/3	NL	ND	ND	ND	NL
Luna Tranquility	medium (7/9)	1	5	NL	1	NL	ND	NL
Tesaris	high (7)	1	ND	NL	NL	NL	NL	NL
Topsin-M, T-Methyl, Incognito, etc.[6]	high (1)	1RR	4	0	NL	NL	NL (3)	NL
Intuity	high (11)	1RR	2R	NL	NL	NL	NL (0)	NL
Quadris, Abound, Acadia LFC, Arius, etc.	high (11)	1RR	3R	4R	NL (2)	NL	NL	NL
Evito*	high (11)	1RR	3R	2R	NL	NL	NL	NL
Flint Extra	high (11)	1RR	4R	2R	NL	NL	NL	NL
Cabrio	high (11)	1RR	2R	3R	NL (2)	NL	0	NL
Quilt Xcel, Avaris 2XS, etc.	medium (3/11)	NL (3)R	5R	0R	NL (0)	NL	NL	NL
Quintec	high (13)	NL (3)	5R	NL (4R)	NL (0)	NL	NL (0)	NL
Quadris Top, Acadia ESQ*.[8], etc.	medium (3/11)	NL (2)R	5R	4R	NL	NL	3	NL
Bumper, Tilt, etc.	high (3)	NL (0)	5R	NL (3)	NL (0)	NL	4	NL
Mettle, Perissim, etc.	high (3)	NL	5R	NL	NL	NL	ND	NL
Procure	high (3)	NL	5R	NL (2)	NL	NL	NL (0)	NL
Rally	high (3)	NL (0)	5R	NL (3)	NL	NL	4	NL
Rhyme[9]	high (3)	NL (0)	5R	NL	NL	NL	NL	NL
Torino	high (U6)	NL	5R	NL	NL	NL	NL	NL
Gatten*	high (U13)	NL	5	NL	NL	NL	NL	NL
Sulfur	low (M2)	NL	4	NL	NL	NL	NL	NL
Cevya	high (3)	NL	3R	NL	NL	NL	NL	NL
Zivion S[5]	low (48)	NL (0)	NL	4	NL	NL	NL	NL
Fungi-Phite, K-Phite, ProPhyt, etc.	high (P07,33)	NL	0	0	NL	4	NL	NL (2)
Orondis Gold	high (4/49)	NL	NL	NL	NL	4	NL	NL
Aliette[3,9], Legion**	high (P07,33)	NL	NL	NL	NL	4	NL	NL
Ridomil Gold SL, Ultra Flourish, etc.[9]	high (4)	NL	NL	NL	NL	4	NL	NL
Copper, etc.[10]	low (M1)	0	0	0	0	0	0	4[10]
Actigard	high (P01)	NL	NL	NL	NL	NL	NL	3

STRAWBERRY - FUNGICIDE EFFICACY - BIOCONTROLS AND NATURAL PRODUCTS

Fungicide trade names	Active ingredient	Resistance risk (FRAC)[1]	Gray mold	Powdery mildew	Anthrac - nose	Rhizopus/ Mucor rot	Phytophthora diseases[2]	Common leaf spot	Angular leaf spot
Oso	Polyoxin D zinc salt	medium (19)	3	4	3	NL	NL	ND	NL
Microthiol Dispersss, etc.[3]	sulfur	low/ (M2)	NL	4	NL	NL	NL	NL	NL
All Phase	*potassium sorbate; sodium lauryl sulfate*	low (NC)	ND	4	ND	NL	NL	ND	ND
Serenade ASO, etc.[3]	*Bacillus subtilis* QST 713	low (BM 02)	0	3	0	NL (0)	NL (0)	NL (0)	NL
Sonata	*Bacillus pumilis* QST 2808	low (BM 02)	NL (0)	3	0	NL	NL	NL	NL
Timorex Act	tea tree oil	low (BM 01)	0	3	ND	0	ND	NL	ND
ProBlad Verde, Fracture[3]	Banda de *Lupinus albus* doce	low/(NC)	0	3	ND	ND	NL	ND	NL
Aviv, BACIX, etc.	*Bacillus subtilis* IAB/BS03	low (BM 02)	0	3	ND	NL	ND	ND	NL
Kaligreen, MilStop, etc.	potassium bicarbonate	low (/NC)	0	3	NL	NL	NL	NL	NL
M-Pede, Des-X, etc.[3]	potassium salts of fatty acids	medium (28)	NL	2	NL	NL	NL	NL	NL
Double Nickel	*Bacillus amylo-liquefaciens* D747	low (BM 02)	0	2	0	NL	0	NL	1
Actinovate	*Streptomyces lydicus* WYEC 108	low (BM 02)	0	2	NL	NL	0	NL	1
Serifel	*Bacillus amylo-liquefaciens* MBI 600	low (BM 02)	0	2	0	NL	0	ND	2
Taegro	*Bacillus amylo-liquefaciens* FZB24	low (BM 02)	0	2	0	NL	ND	NL	NL (2)
Theia	*Bacillus subtilis* AFS032321	low (BM 02)	0	2	0	NL	0	NL	ND
Regalia	*Reynoutria sachalinensis* extract	low (P5)	0	2	ND	NL	ND	NL	NL
Stargus	*Bacillus amylo-liquefaciens* F727	low (BM 02)	0	1	ND	NL	NL (1)	NL (1)	NL (1)
Copper, etc.[4]	Copper	low (M 01)	0	0	0	0	0	0	4[5]
Cinnerate	cinnamon oil	low (BM 01)	0	ND	NL	ND	NL	NL	NL
Lalstop G46	*Clonostachys rosea* J1446	low (BM 02)	0	0	NL	NL	ND	ND	ND
Oxidate, Jet-Ag, etc.	Hydrogen peroxide; peroxyacetic acid	low (NC)	0	0	NL	NL	NL	NL	2
Julietta	*Saccharomyces cerevisiae* LAS02	low (BM 02)	0	NL	NL	NL	NL	NL	NL
Procidic, etc.	citric acid	low (NC)	0	NL	NL	NL	0	NL	NL
Rango	cold pressed neem oil	low (NC)	2	ND	ND	NL	0	0	NL
BotryStop	*Ulocladium oudemansii* U3	low (BM 02)	0	NL	NL	NL	NL	NL	NL
Botector	*Aureobasidium pullulans* DSM 14940; DSM 14941	low (BM 02)	0	NL	ND	NL (1)	NL	NL	NL
Howler	*Pseudomonas chlororaphis* AFS009	low (BM 02)	0	NL (2)	NL	NL	ND	ND	0
Veg'Lys	garlic oil	Unknown/ (NC)	0	NL	0	NL	0	0	ND

Fungicide efficacy against Rhizopus rot

Conventional

- Miravis Prime (4)
- Merivon (4)
- Kenja (2)
- Switch (4)
- Cannonball (4)
- Inspire Super (5)
- Regev (5)
- Luna Sensation (2)
- Luna Tranquility (1)
- Abound (2)
- Cabrio (2)

a.i. = fludioxonil

Organic

- Not labeled (NL)

Fungicides for Rhizopus rot (field trial data)

YEAR	Non-treated	Switch	% Reduction	Merivon	% Reduction	Kenja	% Reduction
2015	54	8	85.2%	4	92.6%		
	32	21	34.4%	21	34.4%		
	29	17	41.4%	15	48.3%		
2017	5.1	0.8	84.3%				
2018	41	20	51.2%	8	80.5%	8	80.5%
2018 L	78	46	41.0%				
	21	9	57.1%				
2020	35.9	14.8	58.8%	4.7	86.9%		
AVG	**37**	**17.1**	**56.7%**	**10.5**	**68.5%**	**8**	**80.5%**
			n=8		n=5		n=1

Rhizopus and Mucor fruit rots of Strawberry

Issue 13 July 2016

The California Strawberry Commission Production Guidelines are produced in cooperation with scientists who conduct research related to strawberry production. These guidelines are a tool for growers, providing critical scientific background information on diseases and pests common to strawberry production in California. For copies of this guideline or others in the series, visit www.calstrawberry.com.

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Introduction and significance

Of the numerous pathogens that cause fruit diseases of strawberry, the gray mold fungus (*Botrytis cinerea*) is the most widespread and important in California and other strawberry growing regions in the world. The disease is called Botrytis fruit rot or gray mold. Botrytis fruit rot is found on strawberry throughout the state and can cause significant loss of pre- and post-harvest fruit because the disease can develop both in the field and during storage and transit. If environmental conditions favor the fungus, it remains one of the most difficult pathogens to control.

Figure 1. Brown, discolored flower tissues infected with *Botrytis cinerea*.

Figure 2. Brown, discolored flower tissues infected with *Botrytis cinerea*.

Symptoms and Signs

Manifestation of gray mold depends on which part of the strawberry is infected and the physiological status of that tissue. Young, newly expanding leaves can be infected but do not show symptoms because the fungus remains dormant (quiescent or latent) in these tissues. When such leaves are mature and beginning to senesce and decline, the fungus can become active and produce the characteristic gray, velvety growth on the dead portions of the leaf. *B. cinerea* can also infect flowers and cause a blossom blight disease. Symptomatic flowers exhibit brown, discolored lesions on petals, receptacle (the small, green central part of the flower that eventually becomes the fruit), and sepals (Figures 1 and 2). If gray mold continues to develop in the flower, the pathogen will kill the pedicel (the stem attached to the fruit) (Figure 1), causing the entire flower and undeveloped fruit to wither and die.

In many cases, the infected flower will not show symptoms because *B. cinerea* can colonize internal flower tissues but remain dormant. Once these fruits begin to expand and develop, the pathogen becomes active and causes a firm, light brown decay on the calyx end of young fruit (Figure 3) as well as a similar firm decay on white, pink, and red stage fruit. Fully mature red fruit are especially susceptible to *Botrytis* infections following physical damage (Figure 4), in which case the pathogen can rapidly colonize the injured tissue and spread throughout the entire fruit. If environmental conditions favor the fungus, any part of the strawberry plant that is colonized by *B. cinerea* can be covered with the gray, fuzzy growth of the pathogen. On mature red fruit in the field, advanced cases of gray mold will result in the entire fruit covered with a gray carpet of the spores (Figure 5). *Botrytis* that is growing on one diseased fruit will readily grow onto other fruit that are in contact with the infected one; this "nesting" habit results in clusters of diseased fruit (Figure 5). If left on the plant, infected fruit will eventually become shriveled, dried, and hard. On post-harvest fruit in cold storage, gray mold growth may be whiter in color because the pathogen usually needs light to fully develop the gray spores.

Figure 3. Light brown decay of fruit and pedicel resulting from gray mold infection of the strawberry flower.

Figure 4. Early stages of gray mold on fruit showing sunken lesions and the initial growth of the gray mycelium and spore.

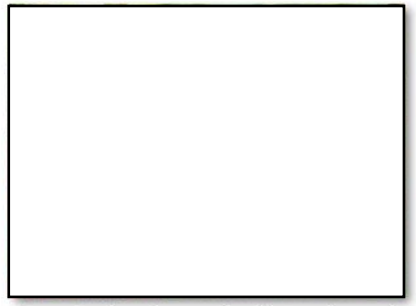

Figure 5. In advanced stages, infected fruit can be covered entirely with *Botrytis* growth that spreads to adjacent fruit by contact (i.e., nesting).

Causal Organism

Botrytis cinerea is one of the most common fungal plant pathogens and infects well over 200 different plant hosts. In addition to being an aggressive primary pathogen, *B. cinerea* is a versatile organism, able to grow and reproduce on damaged, senescing, and dead tissues of strawberry, vegetables, and many other plants. *B. cinerea* reproduces primarily by making asexual spores, or conidia. These gray masses of conidia are readily spread by wind, splashing water, and physical/mechanical activity. Under magnification one can see the distinctive "botryose" (from the Greek for "bunch of grapes" and hence is the root word for "Botrytis") clusters of spores at the ends of the spore-bearing branches (Figure 6). Like many fungi, *B. cinerea* also has a second, sexual form named *Botryotinia fuckeliana* that consists of a tiny mushroom-like structure (apothecium) that contains a different spore type (ascospore). However, this phase apparently has not been found in strawberry fields in California or elsewhere.

Under some conditions, *B. cinerea* can produce an overwintering structure, the sclerotium, which is a hard, black, oblong to spherical structure up to ½ inch in length. Sclerotia can withstand dry, warm, and cold conditions. Under more conducive conditions, sclerotia will germinate to form mycelium that can colonize a host and produce spores. In California, sclerotia are not commonly observed in strawberry fields, though in other countries sclerotia appear to be plentiful and are an important source of primary inoculum.

Recent research has demonstrated that gray mold may be caused by more than one species of *Botrytis*. Some researchers have molecular evidence that *Botrytis cinerea* is actually a complex entity consisting of different but closely related species (*B. cinerea* and *B. pseudocinerea*). While these different species can be separated on the basis of DNA analysis, they are indistinguishable when growing on host plants or in culture. The presence of more than one *Botrytis* species has not yet been documented on strawberry in California but has been found on blueberry in the San Joaquin Valley. Future studies could investigate if more than one *Botrytis* species is present on California strawberry and whether this has any practical implications.

Conclusions

- *Rhizopus stolonifer* predominates
- *Mucor piriformis* is much less common
- Differentiated by growth habit, color and wet vs. dry spores
- Remaining work
  - More isolates from more places
  - Are there additional species??
  - Fungicide efficacy under lab and field conditions
  - Optimum temperature for growth
  - Implications for disease management
  - Fact Sheet

Acknowledgment

- Growers and PCAs
- Cooler managers
- Strawberry Center students and staff
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- Strawberry Commission (funding)