PINK EYE:
A DISEASE OR A PHYSIOLOGICAL DISORDER?

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What is pink eye/corky patch?

A tuber disease/disorder characterized by an ephemeral pink discoloration around the lateral buds/eyes (i.e. pink eye) of the tuber and the periderm between the eyes. The pink color is observed at or before harvest (Nolte et al. APJ, 1993).

No foliar symptoms
Characteristic ephemeral pink discoloration around the eyes of the tuber
The pink discoloration often extends beyond the eyes of the tuber
The corky surfaces may become extensive and are sometimes referred to as “bull hide.”
These corky surfaces may become extensive and are sometimes referred to as “bull hide.”

These surfaces are difficult to remove during processing.

AND
PE/corky patch can lead to a large range of infections.
Other defining characteristics of PE/corky patch:
Durably strong autofluorescence directly beneath the surface of the affected areas of the tuber.

Brown discoloration under skin
What is the causal organism of PE/corky patch?

It has been associated with:

- *Pseudomonas fluorescens*
- *Verticillium dahliae*
- *Rhizoctonia solani*
What is the causal organism of PE/corky patch?

Has been associated with:

*Pseudomonas fluorescens*

*Verticillium dahliae*

*Rhizoctonia solani*

However, these three pathogens do not cause pink eye.

No causal organism has been identified.

Koch’s postulates have not been completed for any pathogen or combination of pathogens.
Environmental conditions associated with PE:

- Excess water late in the season along with
- Higher than normal air and soil temperatures and
- Premature vine death that reduces canopy cover.

The PE/cork patch melody occurs sporadically.

To date, efforts to reproduce PE/corky patch under controlled conditions have been unsuccessful.

No greenhouse or growth chamber conditions for inducing PE/corky patch have been reported.

The inability to specifically induce PE/corky patch for study has contributed to the intractable nature of this problem.

There has been a lack of cellular and physiological data characterizing PE/corky patch.
IN THIS RESEARCH WE ADDRESS THE CRUcial QUESTIONS?

-- What is the source of PE autofluorescence?

-- What are some of the specific physiological and cellular characteristics of tuber PE?

-- Why are various microorganisms associated with PE?
Research Approach

Tuber samples were analyzed for:

1. suberin poly(phenolics) (SPP) and suberin poly(aliphatics) (SPA) at the cellular level.
   *SPP provide protection against bacterial infection, but not fungal. Autofluoresce.
   *SPA blocks fungal infection.
   (Lulai and Corsini 1998)

2. Water vapor conductance - porometric. Indicative of wax accumulation in suberized cells.

3. Fungal hyphae - detected using specific stains and immunodetection.
RESULTS
What is the source of PE/corky patch autofluorescence?

Analysis at the cellular level
Native Periderm

Cortical Parenchyma
(not fluorescent)

Bright Fluorescent Phellem Cells.

Phellem (skin)

Phellogen & Phelloderm (not fluorescent)

HEALTHY, NON-PINK EYE TUBER
NON-PINK EYE AND PINK EYE TUBER TISSUES

NOTE THE AUTOFLUORESCENCE OF CORTICAL PARENCHYMA CELLS IN THE PINK EYE TUBER TISSUE COMPARED TO NON-PINK EYE/CONTROL TUBER TISSUE.
LIKE THAT OF A SUBERIZING CLOSING LAYER.
Note the deterioration of autofluorescence in the Native Periderm and the development of PE autofluorescence on the cell walls of the cortical parenchyma.
COMPARE SOME OF THE CHARACTERISTICS OF PINK EYE TO THOSE OF SUBERIZATION DURING WOUND HEALING
WOUND HEALED TUBER SURFACE

SUBERIN ACCUMULATION ON CELL WALLS AT THE WOUND SURFACE
SUBERIZED CLOSING LAYER OF A POTATO TUBER
FOUR DAYS AFTER CUTTING

Existing cells at the cut surface wound-heal, this includes accumulation of suberin components.
31 DAY SUBERIZED WOUND
SUBERIZED CLOSING LAYER AND SUBERIZED WOUND PERIDERM
COMPROMISED PERIDERM OF PINK EYE TUBER

**Problem**
- Brown discoloration indicates that they are dead!!

**DEVELOPING CLOSING LAYER**

**PHELLOGEN (meristematic cells)**

**PHELLEM (skin)**

**SMALL BREAKS IN SUBERIZED CELLS**
Parallel Sections Showing: (1) Suberin Poly(phenolic) And Suberin Poly(aliphatic) Accumulations, And (2) Development Of A Nascent Internal Phellogen, But No Internal Periderm.

-- Suberin Poly(phenolic)

-- Nascent Phellogen Limiting SPP Accumulation (→).  

-- Dark Area Suggests Deterioration of the Nascent Phellogen.

-- Suberin Poly(aliphatic)

-- SPA Accumulation in the Area of the Nascent Phellogen is Limited, Indicating that the Phellogen did not Produce Phellem & the Phellogen is Dead or Inactive.
PINK EYE TUBERS MAY BE CHARACTERIZED AS HAVING COMPROMIZED PERIDERM

-- Nascent PE development
-- Periderm is deteriorating
-- Phellogen is fracturing and may be dead.

-- Periderm is not present
WHAT ABOUT TUBER WATER VAPOR LOSS?

WOUNDED TUBERS LOSE SIGNIFICANT AMOUNTS OF WATER UNTIL THEY ARE HEALED OR AT LEAST PARTIALLY HEALED.

DO PINK EYE AFFLICTED TUBERS LOSE MORE WATER THAN HEALTHY, i.e. NON-PINKE EYE TUBERS?
DO PINK EYE AFFLICTED TUBERS LOSE MORE WATER THAN HEALTHY, i.e. NON-PINK EYE TUBERS? YES!!!
WHY ARE VARIOUS MICROORGANISMS ASSOCIATED WITH PE/CORKY PATCH?

THE DATA SHOWS THAT THE SUBERIN BARRIER OF THE NATIVE PERIDERM IS COMPROMISED OR ABSENT.

MICROORGANISMS ARE NOT BLOCKED FROM ENTERING.

IF THE ABOVE IS TRUE, THEN WE SHOULD HAVE EVIDENCE OF NON-ROT CAUSING MICROORGANISMS INVADING AND EXISTING IN THE TUBER PARENCHYMA (FLESH).

DO WE HAVE SUCH EVIDENCE?
A UNIQUE SAMPLE OF PINK EYE/CORKY PATCH PARENCHYMA CELLS TREATED TO VIEW SUBERIN POLY(ALIPHATICS), BUT ALSO REVEALING THE PRESENCE OF FUNGAL HYPHAE.

-- Fungal Hyphae are Visible as Dark Strands.

-- This Internal Suberin Poly(aliphatic) Barrier (White Arrow) Appears Complete.

-- Note the Concentration of Hyphae (Yellow Arrow) Above the Suberin Poly(aliphatic) Barrier.
ILLUSTRATION OF SUBERIN POLYALIPHATICS BLOCKING ADVANCEMENT OF FUNGAL HYPHAE IN TUBER TISSUE

HYPHAE

FULLY SUBERIZED BARRIER
FUNGAL HYPHAE TREATED TO INDUCE FLUORESCENCE OF HYDROPHOBIC AREAS.

Hyphae Morphology and Size Suggests that they are *Rhizoctonia solani*.
PINK EYE TUBERS SHOWING SCALY SURFACE = COMPROMISED PERIDERM

DRY OPEN WOUNDS SUSCEPTIBLE TO INFECTION

Courtesy Dr. Neil Gudmestad, NDSU
PINK ROT ORGANISMS INFECTING PINK EYE AREAS OF THE POTATO TUBER

i.e. PINK ROT INFECTING VIA THE PORTALS OF ENTRY CREATED BY THE COMPROMISED PERIDERm THAT DEFINES PINK EYE TUBERS

Courtesy of Dr. Neil Gudmestad, NDSU
PINK ROT ORGANISMS INFECT VIA THE PORTALS OF ENTRY CREATED BY THE COMPROMISED PERIDERM THAT DEFINES PINK EYE TUBERS

Courtesy of Dr. Neil Gudmestad, NDSU
A new model describing a physiological basis for pink eye is created from the following results:

1. The integrity of the native periderm is compromised or the barrier absent resulting in susceptibility to infection.

2. Widespread accumulations of Suberin poly(phenolics) on parenchyma walls are the source of PE/corky patch autofluorescence. Not *Pseudomonas fluorescens*.

3. The aberrant absence or compromised integrity of the suberin barrier includes increased erratic water vapor loss, putatively from loss of waxes. Variability matches that of PE/corky patch.
SUMMARY

A new model describing a physiological basis for pink eye is created from the following results:

4. Combined, the data provide a plausible explanation for PE/corky patch infection court and rot anomalies without ingress through a bonified wound opening.
TUBERS SAMPLES OBTAINED OVER ~ 5 Yr. PERIOD

Jeff Miller- free hand sectioning and microscopy
Shana Pederson- vapor conductance measurements
John Weiland- immuno-assays
Neil Gudmestad
Duane Preston
Gary Secor
Phil Nolte
Dale Steeves
John Nordgaard
Susie Thompson
Rob Sabba
A.J. Bussan

Published in The AMERICAN J. POTATO RES, (2006)83:409-421

“PINK EYE IS AN UNUSUAL PERIDERM DISORDER CHARACTERIZED BY...”
QUESTIONS
What is the likely bases for development of the pink eye syndrome?

- Loss of foliar canopy results in loss of shading to soil = soil temperature↑; the saturated soils remain wet and hot (Secor & Gudmestad).

- At this time, the tuber periderm is still in an active state for growth and maintenance of the suberizing cells in the phellem (skin). The phellogen cells of the periderm are still meristematically active; higher temperatures increase respiration = oxygen demand↑ and carbon dioxide production↑. (Lulai et al.)

- Water logged soils impede oxygen diffusion resulting in hypoxic soil conditions; tubers are starved of oxygen needed for meristematic activity in the periderm.

- These conditions (heat or heat and hypoxia), along with other potential variables, result in death of the periderm phellogen cells (meristem cells) and loss of the final biological maintenance needed for the tuber periderm as the tuber prepares to enter its resting state.

- Suberization is inhibited by temperatures approaching 95 °F.
ADDITIONAL QUESTIONS
The effect of pink eye (PE) on water vapor conductance (i.e. water vapor loss) during wound-healing: a comparison of non-PE (control) versus non-necrotic tissues of PE afflicted tubers. Results indicate that PE does not induce systemic effects on water loss.
Characteristics of healthy non-PE (a & e) and PE afflicted tubers (b, c, d, & f)
Parallel PE Sections Showing: (1) Suberin Poly(phenolic) And Suberin Poly(aliphatic) Accumulations On The Same Cells, And (2) Development Of An Internal Periderm.

- Autofluorescence indicating SPP
- Note organized files of internal phellem cells from new internal phellogen → internal periderm.
- Note phellogen delimits PE induced autofluorescence.

-- Cells treated to show SPA

Native Periderm

Unusual internal periderm

Fractured Phellogen
What is the tuber periderm?
NETTING OR RUSSETTING

PERIDERM
PERIDERM

PHELLEM
(Suberized)

PHELLOGEN

PHELLODERM

NATIVE PERIDERM AND CORTICAL PARENCHYMA

CORTICAL PARENCHYMA
NATURE OF PERIDERM DAMAGE FROM SKINNING
INCLUDING FRACTURE OF STRENGTH COMPONENTS

PERIDERM
- **PHELLEM (Skin)**
- **PHELLOGEN**
- **PHELLODERM**

CORTICAL CELLS

PHELLEM TENSILE STRENGTH COMPONENT

PHELLOGEN SHEAR STRENGTH COMPONENT
NATURE OF PERIDERm DAMAGE FROM SKINNING

MICROGRAPH SHOWING PERIDERm AND CORTICAL PARENCHYMA AFTER SKINNING INJURY

PHELLEmM (skin)

PHELLOGEN

PHELLODERm

CORTICAL PARENCHYMA
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Fractured Phellogen
Native Periderm
Unusual internal periderm
A UNIQUE SAMPLE OF PINK EYE/CORKY PATCH PARENCHYMA CELLS TREATED TO VIEW SUBERIN POLY(ALIPHATICS), BUT ALSO REVEALING THE PRESENCE OF FUNGAL HYPHAE.

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Immunodetection of *R. solani* in PE tuber tissues.

Extracts from PE tissue that (1) contained or (2) lacked visible hypae, and (3) healthy tuber tissue putatively free of *R. solani*. 