# Cleaning and Disinfesting Machinery and Equipment Contaminated with Clubroot Spores

Clubroot Summit

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# Introduction

- *Plasmodiophora brassicae* is primarily a soil-borne pathogen
- Soil movement has been implicated in the spread of clubroot in Alberta
- Infested soil could be moved by various means:
  - Agricultural machinery and farm vehicles
  - Custom operators (seeding, harvesting, fertilizing)
  - Oil and gas operators (drilling, pipelining, servicing)
  - Contractors (road building, excavating, trenching)
  - Recreational users (quadding, bogging, hunting)
  - Wind and water erosion
- Both local and long-distance spread of infested soil is possible, especially when fields are accessed by multiple users

# Introduction

- Sanitation of machinery, vehicles, tools and equipment has been used by vegetable producers as a means of clubroot prevention for many years
- The Alberta Clubroot Management Plan (2008) recommends:
  - Removing soil and plant debris from farm equipment
  - Cleaning contaminated surfaces by pressure washing
  - Applying a disinfectant (1-2% bleach) to clean surfaces
- Oil and gas companies have adopted similar practices (http://www.capp.ca)
- However, questions have been asked about the practicality of sanitation measures, their potential adverse environmental consequences, and the relative effectiveness of available disinfectants

# Project Objectives

1. To compare the effectiveness of various cleaning methods for use on machinery and equipment, e.g. scraping, compressed air & power washing
2. To assess the ability of various physical and chemical treatments to clean soil residues from hard surfaces and to kill clubroot spores
   - Physical methods – Dry heat, hot water, steam, freezing
   - Cleaners – Industrial detergents and related products
   - Disinfectants – sodium hypochlorite, hydrogen peroxide, quaternary ammonia, electrolyzed water, acetic acid, peracetic acid, potassium peroxomonosulphate, chlorine dioxide, oxidized silver, essential oils, etc.
3. Evaluate promising sanitation methods on a pilot scale and advance the most promising ones to commercial-scale field testing

# Previous Studies

Donald, E.C., J.M. Lawrence and I.J. Porter. 2002. Evaluation of a fluorescent staining technique as an indicator of pathogenicity of resting spores of *Plasmodiophora brassicae*. Australasian Plant Pathology 31: 373-379.

- Adapted a fluorescent staining method to indicate the viability of resting spores
- Tested 3 physical methods (dry heat, pressurized heat & ultraviolet light), 3 ionic stresses (calcium, sodium & potassium salts) and 9 commercial disinfectants (quat ammonias, phenols, peroxides, halogens & alcohols) for sporocidal activity
- Spore viability and pathogenicity were assessed
- They found that physical treatments were the most effective, while ionic and chemical treatments were either marginally effective or ineffective

# Optimization of Experimental Methods

- Inoculum – Dried canola roots with mature galls were ground in a Wiley to a powder consistency
- Stains – Evan's Blue and a mixture of Calcofluor White M2R and Ethidium Bromide were evaluated for their ability to differentiate living from dead resting spores
- Disinfectants – 10 commercial products tested at 0.5, 1, 2 and 5 times the general use label rate
- Stock Spore Concentration: 10 million spores/mL
- Reaction Vials - 4 mL spore suspension + test chemical to achieve the desired concentration
- Reaction Times – 10, 20 and 30 minutes

# Differential Staining of Clubroot Spores with Calcofluor White and Ethidium Bromide

(Takahashi & Yamaguchi 1988)

A = Resting spores unheated

B = Resting spores heated at 50°C for 16 hr

C = Resting spores from clubroot galls stored in a freezer (-20°C) for 3 months

D = Resting spores from clubroot galls stored in a freezer (-20°C) for 8 years

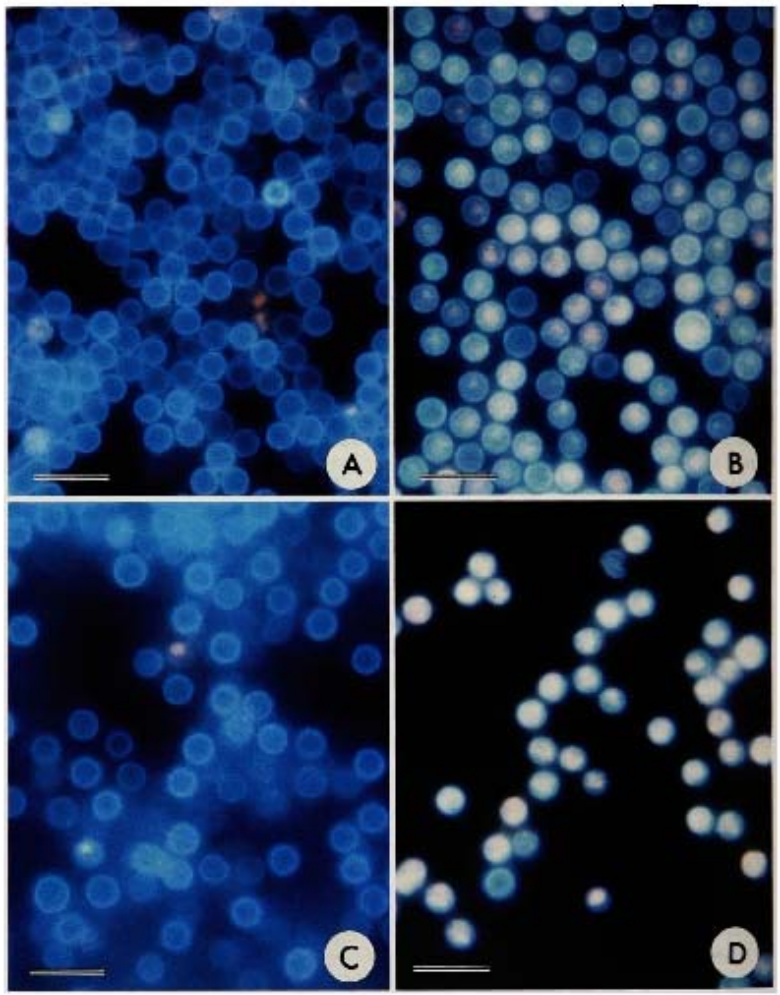



Plate I. Fluorescence micrographs of resting spores of *Plasmodiophora brassicae* differentially stained with a mixture solution of calcofluor white M2R and ethidium bromide. Bars represent 10 μm.

A. Resting spores unheated.
B. Resting spores heated at 50 C for 16 hr.
C. Resting spores prepared from clubroot galls stored for 3 months in a freezer.
D. Resting spores prepared from clubroot galls stored for 8 years in a freezer.

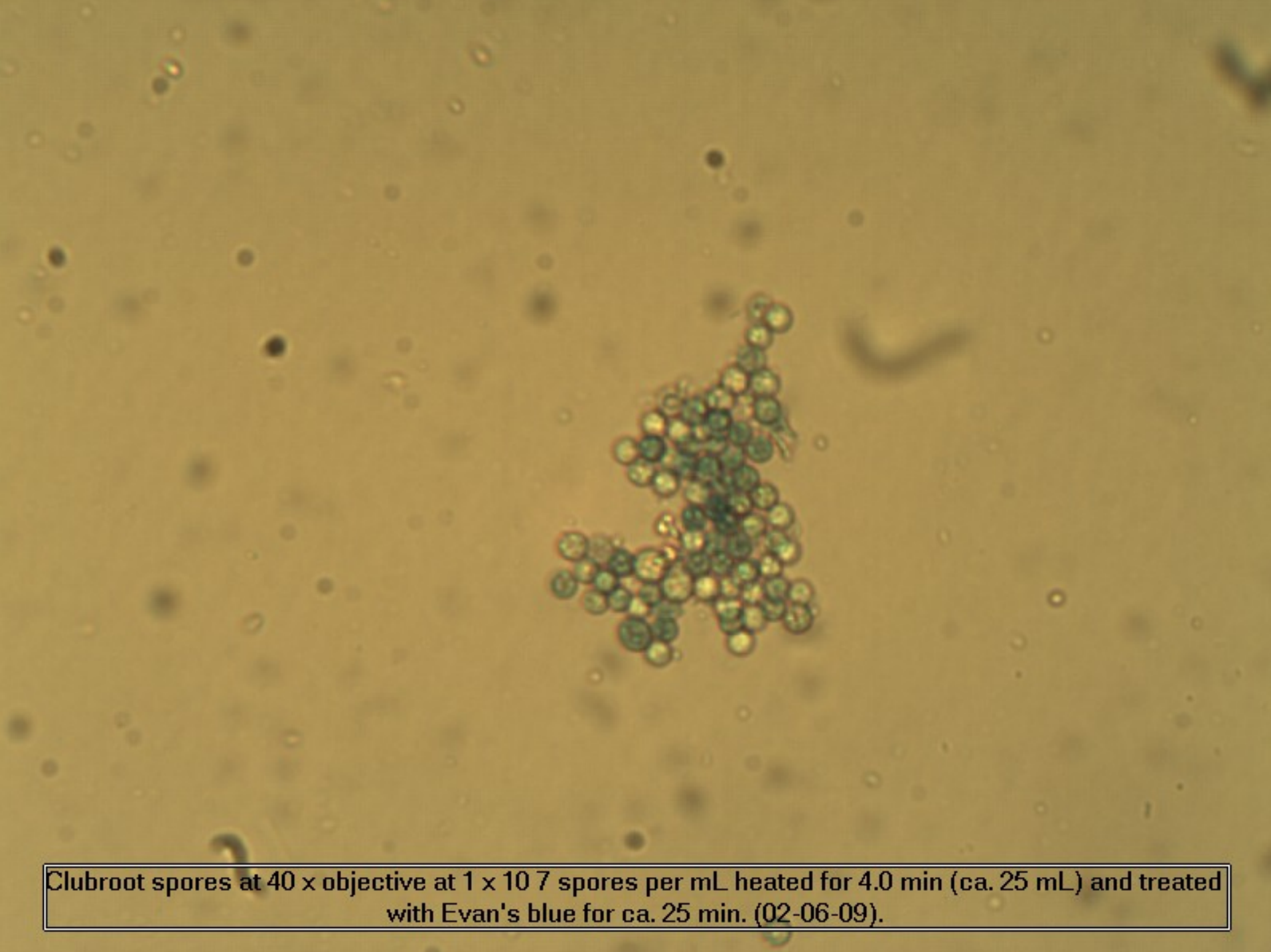
# Disinfectants for Equipment Sanitation

| Product Names | Active Ingredients |
|---|---|
| General Storage Disinfectant | Quaternary ammonia |
| Industrial Bleach | Sodium hypochlorite |
| SaniDate | Hyd. peroxide + peracetic acid |
| Virkon | Potassium peroxomonosulphate |
| Twin Oxide | Chlorine dioxide |
| ECA anolyte | Mixture of disinfectant ions |
| KleenGrow | Quat. amm. + isopropyl alcohol |
| HyperOx | Hyd. peroxide + peracetic acid |
| Vinegar | Acetic acid |
| Thymox | Thymol oil |

# Optimization of Experimental Methods

- Filtration - 0.22 micron Millipore filter
- Spore Suspension – Re-suspend treated spores in sodium phosphate buffer and withdraw aliquots for staining and inoculation onto plants
- Inoculation – Roots of ‘Granaat’ Chinese cabbage seedlings were dipped in a suspension of treated spores for 10 sec and transplanted to trays; residual suspension was pipetted onto seedlings
- Disease Ratings – Roots were washed and rated for gall symptoms (0-3 scale) after 3-4 weeks
- Statistical Analyses – Regression of disinfectant concentrations, exposure times and estimated spore viabilities onto disease ratings


Rec'd Dec 8/98
BULK SIZE
BROWN SUGAR
Brown Sugar
PLEASE KEEP DOORS CLOSED
PLEASE KEEP DOORS CLOSED
Spill-X-S
Spill-X-C
Spill-X-A
AnalaR
CHITIN
OFF!
LENS PLUS
DEIONIZED
Pipettes
Fisher Scientific
UTENSILS-STIR STIKS\SPOONS\KNIVES
PIPETTING MACHINE ACCES.
GRAM STAINING/ CHEMICAL PAN
SCREENING STACKS
UTENSILS-TAPE
TWEEZERS\STRING
BLADES\HANDLES\PINS



Clubroot spores at 40 x objective at 1 x 10 7 spores per mL heated for 4.0 min (ca. 25 mL) and treated with Evan's blue for ca. 25 min. (02-06-09).

Granaat Chinese Cabbage Seedlings Inoculated with Clubroot Spores

**Sanitation of Farm Machinery and Equipment**
**Air Seeder**


# Air Compressor for Cleaning Soil and Dust from Farm Machinery and Equipment


Water Wagon for Cleaning Soil and Dust
from Farm Machinery and Equipment

Air Seeder after Cleaning with Compressed Air and Water

# Sanitation of Oilfield Equipment

Setting up to wash a drilling rig. >>





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