The effectiveness of wash water treatments to prevent or reduce the spread of plant pathogens in the Holland Marsh.

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Importance

- Vegetables from different fields are being washed in the same facility and the wash water can contain potential plant pathogens
- Infested irrigation water is not only an important source of pathogens inocula, but also an efficient means of spreading potential pathogens from a single site to an entire farm
- These potential pathogens cause important diseases in the “Holland Marsh” but there is no effective control yet
- This will increase the use of pesticides to control disease outbreaks and increasing production costs

Partial list of plant pathogens found in different water sources and associated crops

Fungal Species	Water Source			Associated crops	Country
	Pond	Lake	canal		
Botrytis cinerea	+			Tomato	USA
Fusarium moniliforme	+			Vegetables	USA
Fusarium oxysporum	+			Vegetables	USA
Fusarium solani	+			Vegetables	USA
Plasmodiphora brassica	+			Cabbage	USA
Rhizoctonia solani	+			Vegetables	USA
Sclerotinia sclerotiorum	+		+	Vegetables	USA
Phytophthora spp	+	+	+	Vegetables	USA
Pythium aphanidermatum	+		+	Vegetables	USA
Pythium irregulare	+		+	Vegetables	USA
Pythium sylvicatum	+			Vegetables	USA

Objectives

- To determine if the treatment techniques used in the Holland Marsh are effectively eliminating potential plant pathogens by:
  - Detecting, identifying and quantifying plant pathogens in water samples
  - Therefore we can determine the best method to treat vegetables wash water

Objectives (continue)

- The main plant pathogens of concern in the Holland Marsh are:
  - *Fusarium* spp (Fusarium root rot of carrot),
  - *Pythium* spp (Cavity spot of carrot),
  - *Stemphylium vesicarium* (Leaf blight of onion)
  - *Rhizcotonia crocorum* (Violet root rot of carrot),
  - *Sclerotinia sclerotiorum* (Sclerotinia rot of carrots, pink rot of celery and sclerotinia drop of lettuce)
  - *Sclerotium cepivorum* (White rot of onion)
  - *Agrobacterium tumefaciens* (Crown gall of carrot)

Methods

- Isolating, identifying and quantifying potential plant pathogens in the vegetables wash water at different levels:
    - From water at the facility (source)
    - After vegetables being washed
    - Water after treatment

Available Methods

Filters & Baiting

- **Filters or sieves** used to retain pathogens and placed onto a growth medium.
- **Baiting**: Whole or selected plant parts (Trap) pathogens and colonized tissue plated on suitable medium.
- Time consuming

Immunological

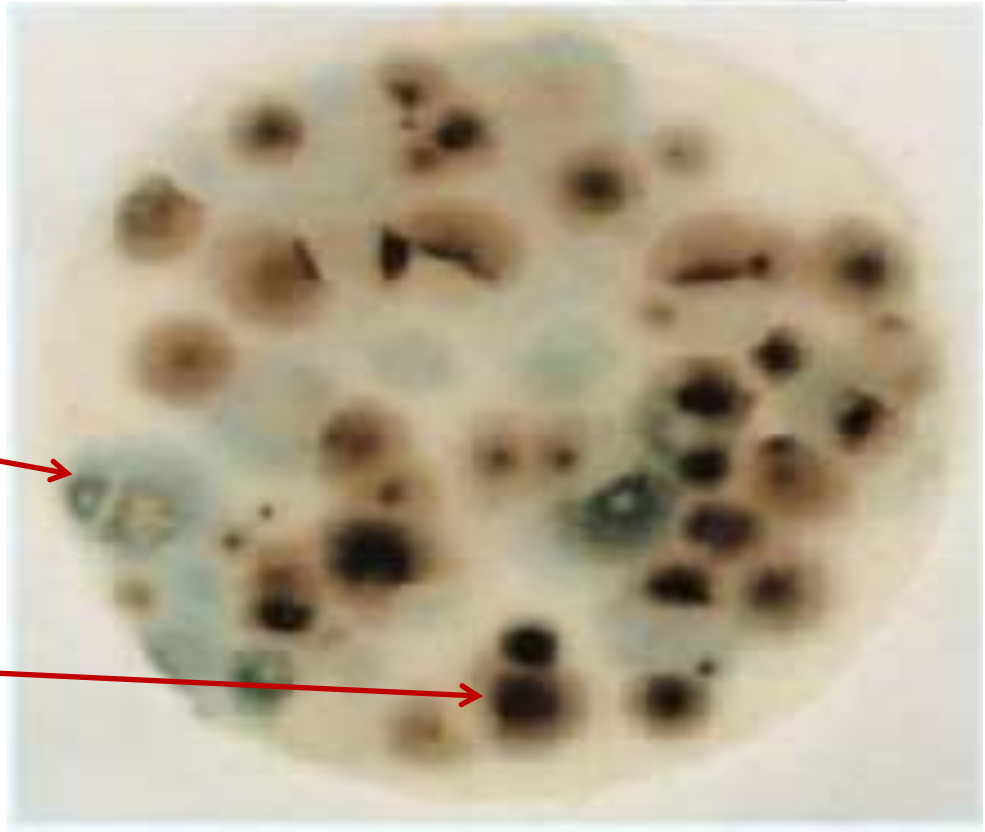
- Based on the ability of antibodies to recognize specific proteins or polysaccharides.
- Qualitative data
- Expensive
- Availability of antibodies

Molecular

- Based on the detection of specific segments of DNA or RNA.
- Qualitative and quantitative data.
- More specific (species level).
- Highly sensitive.
- Results in few hours.
- Can be expensive

A New Method Evaluation

- The 3M Petrifilm™ Yeast and Mold Count Plate:
  - Designed to identify food borne yeast and mold in food products
  - Yeast are indicated by small, blue-green colonies
  - Molds are indicated by large, variably colored colonies



A New Method Evaluation (continue)

- Petrifilms tested to confirm that the common plant pathogenic fungi can be detected
- Serial dilutions used to determine the lowest concentration that can be detected.
- 1mL of each dilution was inoculated on the petrifilms (4 petrifilms/ dilution).
- Results recorded after 48 hours.

Results based on pure fungal cultures

Spores Concentration	3M petrifilm	PDA	3M Petrifilm	PDA
	Fusarium (CFU/ml)		Stemphylium (CFU/ml)	
≥ 10000/ml	Colonies diffused and merged		Colonies diffused and merged	
1000/ml	750	860	876	1017
100/ml	245	315	360	476
10/ml	85	130	95	175
Control	0	0	0	0

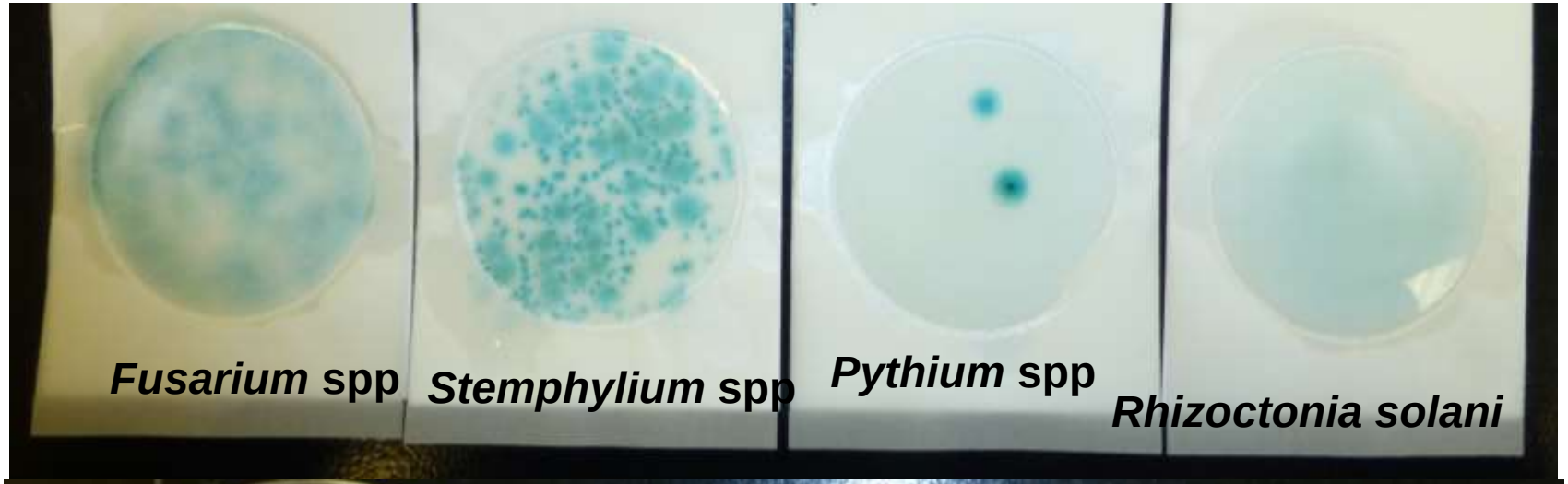
Each number represents the average of 4 plates
Results taken after 48 hours
PDA = potato dextrose agar medium

Results based on pure fungal cultures

Spores concentration	3M Petrifilm	PDA
	Pythium (CFU/ml)	
≥100,000/ml	Colonies diffused and merged	
10000/ml	8	9
1000/ml	1	3
100/ml	1	2
10/ml	0	0
Control	0	0

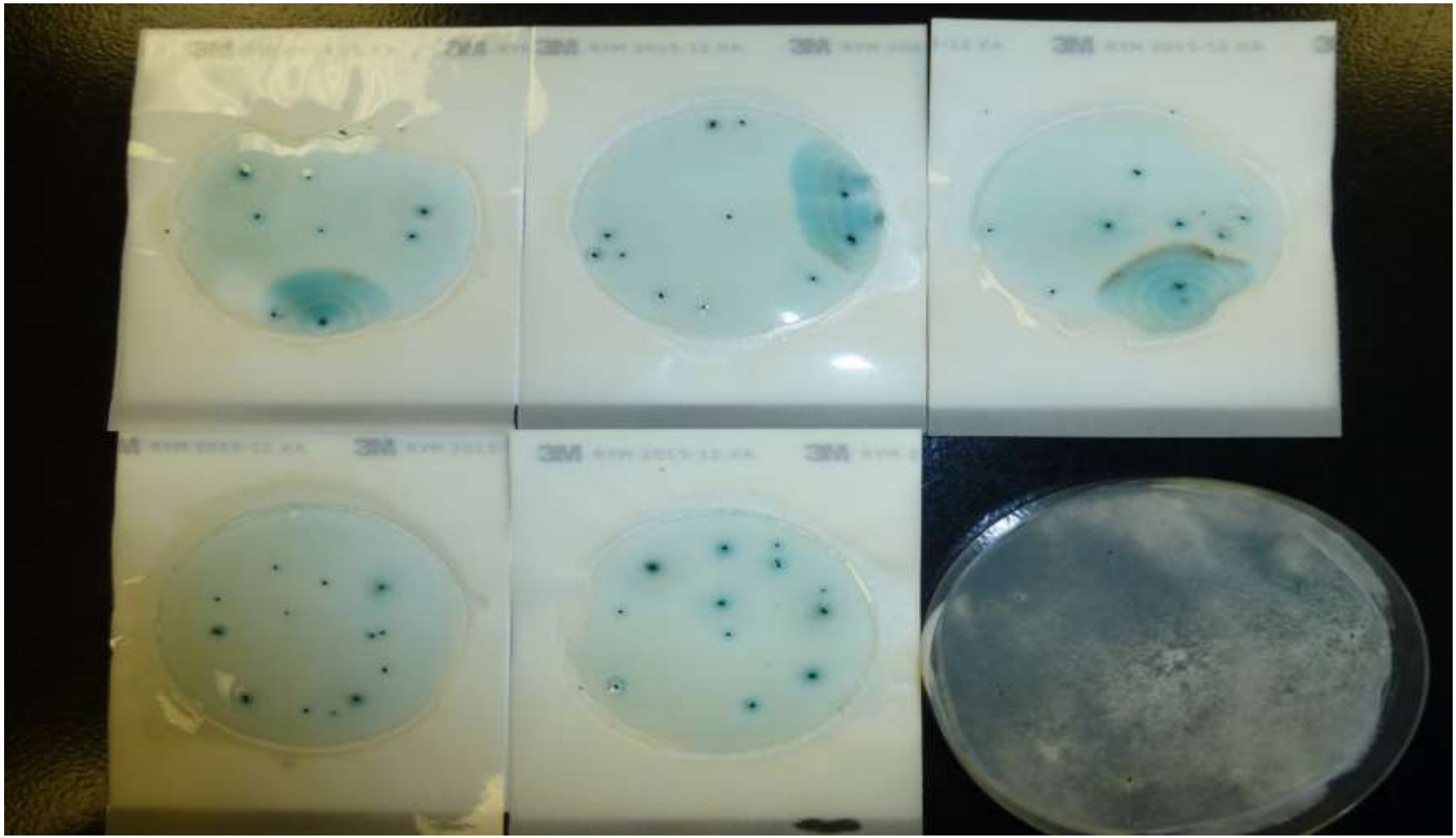
Each number represents the average of 4 plates
Results are taken after 48 hours
PDA = potato dextrose agar medium

Results (continue)



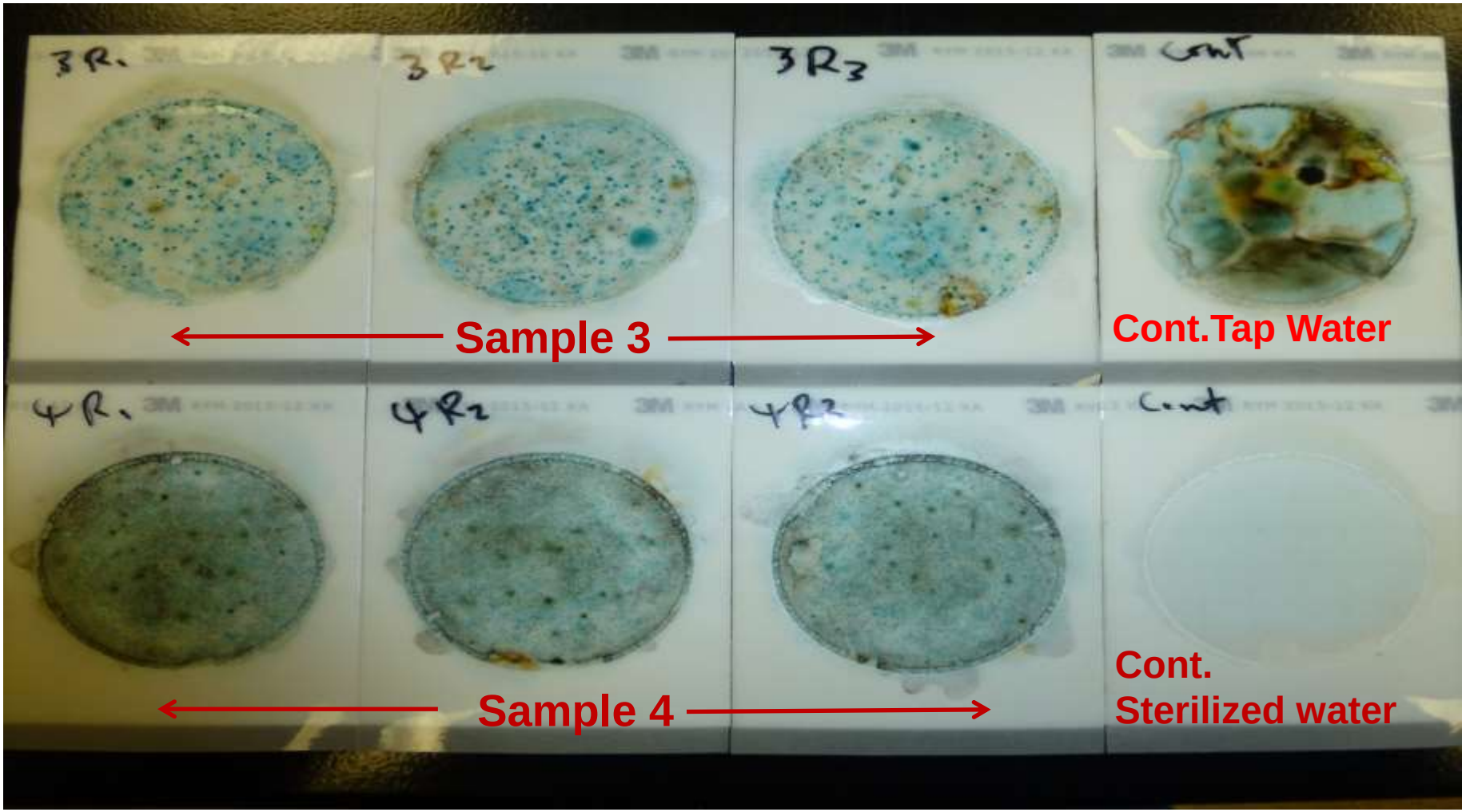
Fusarium* spp** ***Pythium* spp** ***Stemphylium vesicarium

Results (continue)



Sclerotia of *Sclerotium cepivorum*, the causal agent of onion white rot disease

Results from carrot wash water



Sample "3" is taken from "carrot wash pit".
Sample "4" is taken from a "settling pond" after washing carrots

Conclusion

- **There is no available information on the plant pathogens in carrot wash water before and after treatment and there may be a risk of spreading plant pathogens in the Holland Marsh.**
- **The 3M petrifilms can detect mold and yeast in water but can not identify if they are plant pathogens.**
- **Plating methods are needed to detect and identify plant pathogens in water samples.**
- **Initial results show that there are high numbers of yeast and molds in water sampled going into and out of settling ponds.**
- **Research will assess the effectiveness of treatments on important plant pathogens in wash water in the Holland Marsh and enable producers to evaluate the effectiveness of their wash water treatment system.**

Acknowledgments

- The Holland Marsh Growers' Association

Thank you
Questions?