Evaluation of Oospores on Spinach Seed

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Production of spinach

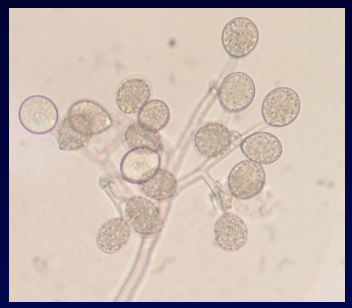
- 275,000 tons of spinach in the U.S. (USDA NASS, 2016)
- 50% fresh spinach in California is organic
- Organic production valued $150 million dollars

Symptom of downy mildew (*Peronospora farinosa* f. sp. *Spinaciae*)

Disease cycle

Healthy spinach

Sporangia



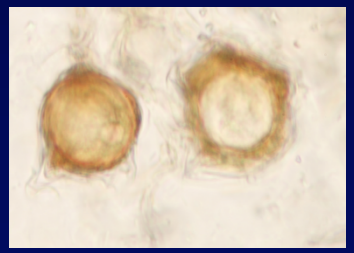
Under wet & cool condition

Germination and Infection

Wind
Splash water

6 – 8 days

Oospores on seeds?



Diseased leaves

Introduction

- Seed transmission may result from oospore on seed surface (Inaba et al., 1983)
- Oospores were detected on seed surface (Feng et al., 2014; Kunjeti et al., 2016)
- Same or similar races were identified in different places, countries at the same time (Feng et al., 2014)

Questions of interests

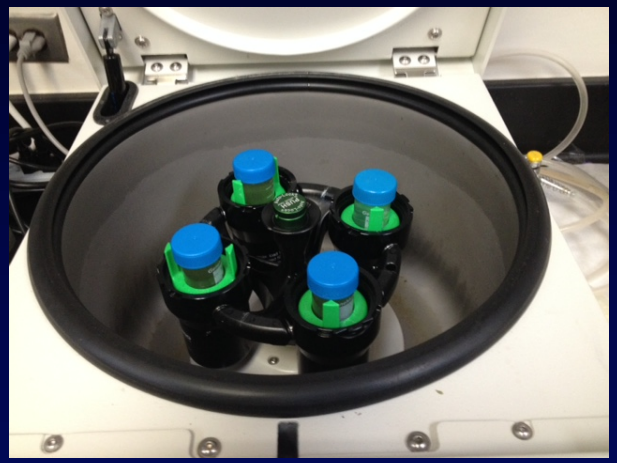
- Determine whether commercial seeds contain oospores
- Testing different methods for vitality of Oospores

Seed wash assay

2% Tergitol 25 ml +
1, 5, and 10 g seeds

Shake at 200 rpm for 2h

Seed wash assay



Centrifuge at 3,000 rpm for 2 min.

Re-suspend pelleted debris in 1 ml H_2O

Oospore observation

The number of Oospores on 1 g, 5 g, and 10 g of seeds

Lot#	Cultivars	Weight	Number of Oospores (in 20 µl)				Oospores / 1 g seed
			Test 1	Test 2	Test 3	Mean	
1006	cultivar-1	1 g	3.0	6.7	4.3	4.7	233.3 a
		5 g	25.0	24.3	27.8	25.7	257.0 a
		10 g	23.3	65.7	49.3	46.1	230.5 a

Numbers of Oospores on commercial seeds.

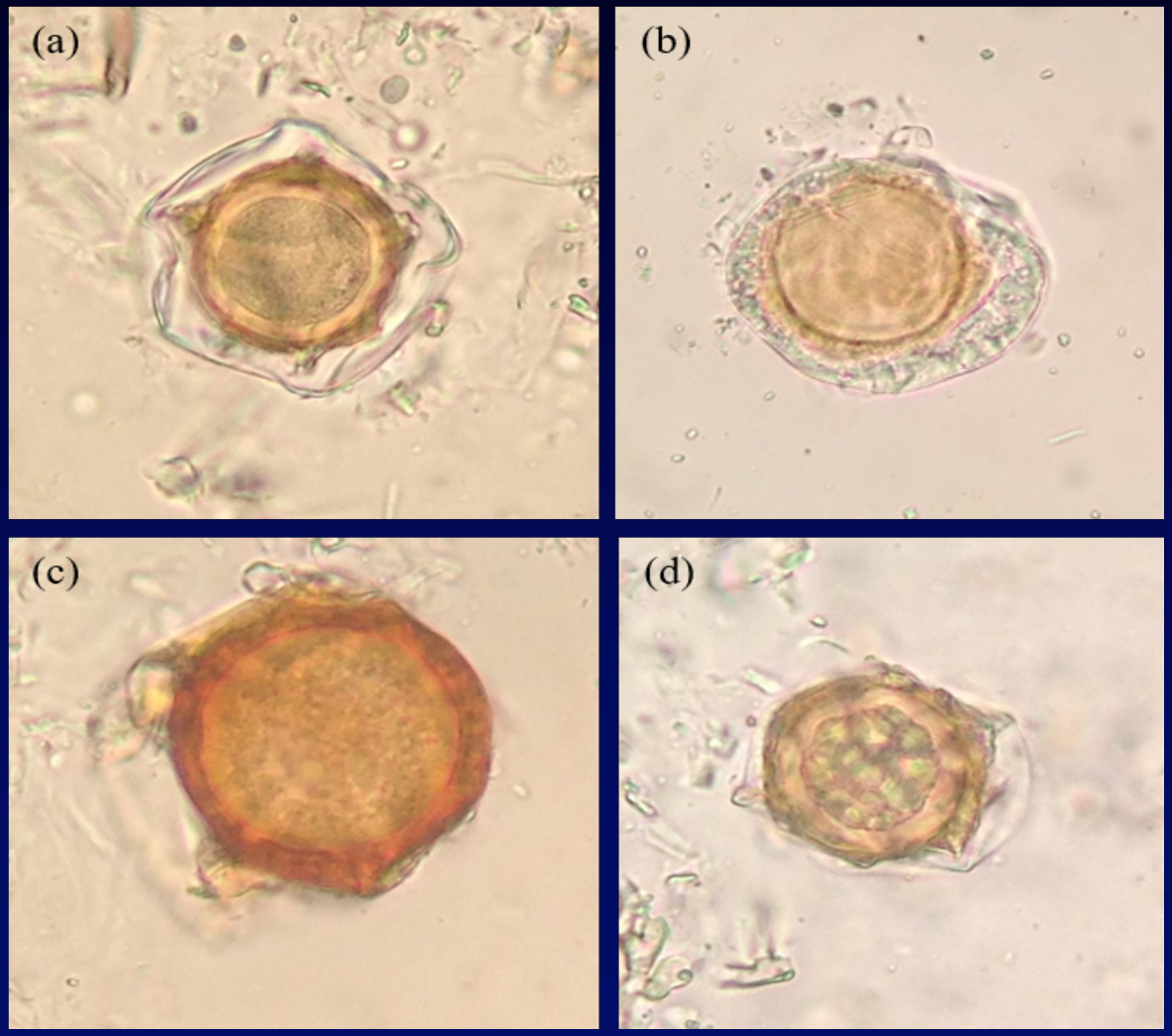
Lot#	Cultivars	Oospores / 1 g seed	Oospores / ha
1006	Cultivar-1	230.5	25,824,580
1007	Cultivar-1	144.4	16,178,175
-	Cultivar-2	2.8	313,704
1022	Cultivar-8	74.7	8,369,181
1023	Cultivar-8	14.5	1,624,540
1024	Cultivar-8	12.2	1,366,854
1026	Cultivar-8	57.7	6,464,548

Numbers of Oospores on commercial seeds

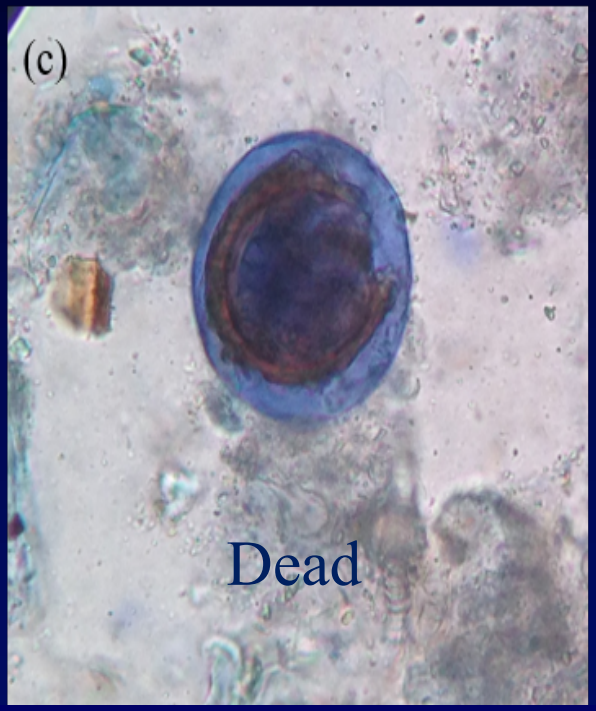
The following seed lots have no Oospores

- 1005 (cultivar-1),
- 1012 (cultivar-2), 1013 (cultivar-2)
- 1014 (cultivar-4)
- 1015 (cultivar-5)
- 1016 (cultivar-6)
- 1019 (cultivar-7), 1020 (cultivar-7), 1021 (cultivar-7)

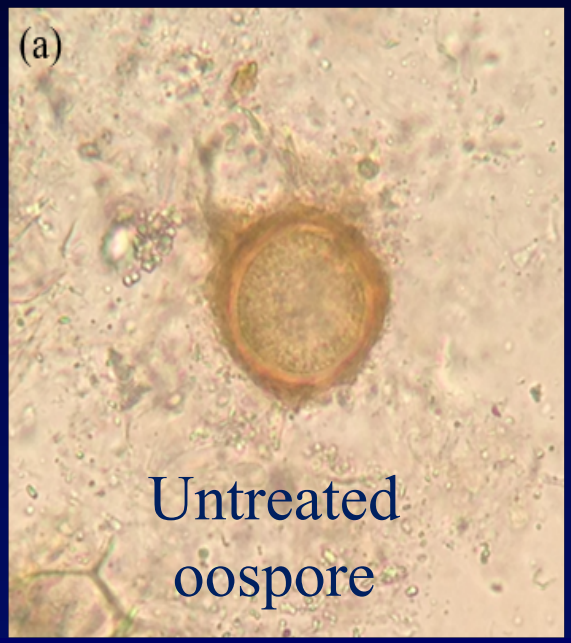
Types of Oospores were detected in seed lots



Oospore vitality using Trypan blue stain



Oospore vitality using plasmolysis



Oospore vitality test by Trypan blue stain

Lot#	Cultivars	Trypan Blue Stain		
		Living Oospore	Counted oospore	Vitality Rate (%)
1006	Cultivar-1	38	82	46.3
1007	Cultivar-1	30	97	30.9
1022	Cultivar-8	21	61	34.4
1023	Cultivar-8	9	31	29.0
1024	Cultivar-8	8	36	22.2
1026	Cultivar-8	27	54	50.0

Oospore vitality test by plasmolysis method

Lot#	Cultivars	Plasmolysis Method		
		Living Oospore	Counted oospore	Vitality Rate (%)
1006	Cultivar-1	39	74	52.7
1007	Cultivar-1	32	88	36.4
1022	Cultivar-8	26	66	39.4
1023	Cultivar-8	10	26	38.5
1024	Cultivar-8	11	28	39.3
1026	Cultivar-8	33	59	55.9

Conclusion and future work

- 6/16 (38%) seed lots contain oospores
- Four types of oospores were detected on seed surface
- Both Trypan blue stain and plasmolysis can test the vitality of oospore
- Oospore vitality rates are higher using plasmolysis method than Trypan blue stain
- Live oospores on seed surface might be responsible for spreading diseases
- Germination test of oospores are in progress

Thank you

Questions?