Germination of a spinach seedling infected with *Peronospora effusa;*
evidence of contaminated seed as a source of spinach downy mildew epidemics

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Introduction

Several occurrences of downy mildew in commercial plantings of spinach in winter tunnels in the Northeast since 2014 (Fig. 1) were the impetus for research undertaken to determine if contaminated seed could be the source of such outbreaks. In January 2023, seed was obtained of four cultivars that had been associated with outbreaks as well as seeds of other organically produced cultivars to look for evidence of living seedborne downy mildew oospores.

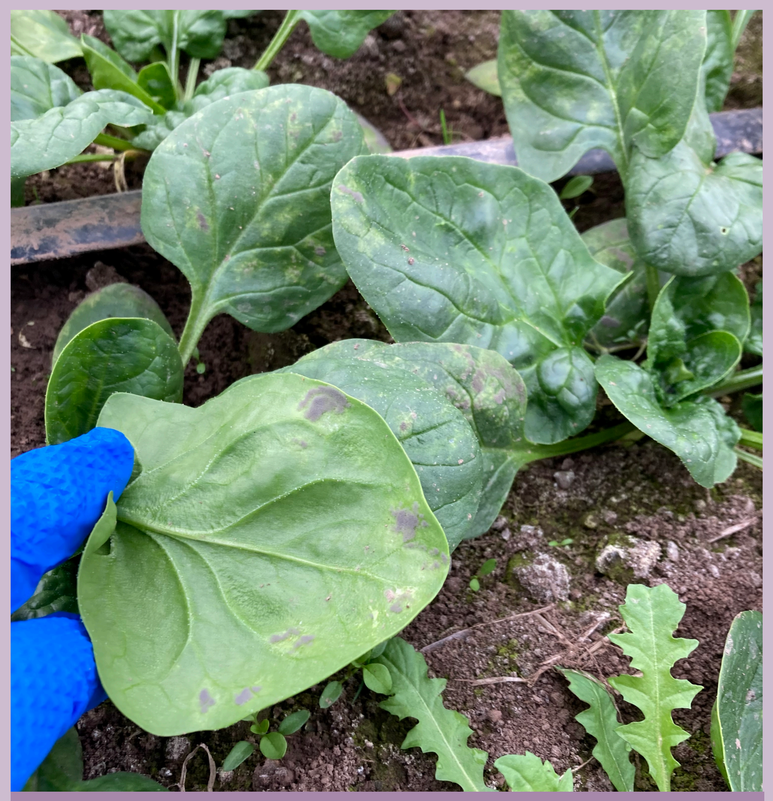

Fig. 1- Downy mildew on organic spinach of Cultivar A lot y in a winter tunnel in NY.
(photo by Teresa Rusinek, Cornell Cooperative Extension)

Materials and Methods

Organic seed (250-5000 seed packages) of four spinach cultivars were purchased from two retail seed sources in early 2023 and examined for infested seed. Four subsamples (approx. 100 seeds each) were taken from 1000- to 5000-seed packets and two from each 250 seed packet. Seeds treated with a soluble coating were washed. Each subsample was placed under a dissecting microscope (an Olympus SZH10 with a 10-80x zoom) and each seed checked for the presence of oospores. Oospores were visible as small red-brown spheres embedded in the pericarp tissue surrounding the seed. They could be present scattered in tissue or in aggregate clumps (Fig. 2). Seeds with visible infestation were placed in Petri plates on moistened filter paper and a sliver of oospore-infested tissue removed with a razor blade and placed on a microscope slide where it was covered with a drop of 4M NaCl solution. Slides were examined at 40X using a Nikon Eclipse 80i to count the total number of oospores present in the tissue and the number of oospores demonstrating plasmolysis from exposure to the salt solution (Fig. 3). The moist chambers with infested seed were placed on a greenhouse bench at 18-20 C. Seeds were examined for germination and emerging seedlings for signs of downy mildew infection. Infected tissue was placed in 70% ethanol to clear the tissue, then examined microscopically at a 40x magnification. DNA extractions were performed using samples from infested seed coat and cotyledon tissue (FastDNA kit, MPBio according to manufacturer's instructions). DNA was amplified using cox2 primers and a nested ITS reaction, and amplicons cleaned using ExoSapIT (Thermo Fisher). Cleaned amplicons were then Sanger sequenced (Eurofins genomics) and the resulting sequences trimmed, aligned, and compared to the NCBI blastN database using Geneious software.

Table 1- Percent infestation of spinach seed with oospores of *Peronospora effusa* and their percent viability.

			Percent infested seed per subsample[e]				
Cultivar, lot, packet size	**Germ. date[c]**	**Sample date[d]**	**1**	**2**	**3**	**4**	**% oospore plasmolysis[f]**
Cultivar A[a], lot y (5000 seed)	11-22	1-23	1.8	1.7	2.6*	0.8	32.0
Cultivar A[a], lot y (1000 seed)	11-22	1-23	0.9	1.0	0.0	3.8	28.7
Cultivar A[b], lot z (2x250 seed)	11-22	2-23	0.9	3.8	2.8	0.0	5.3
Cultivar A[b], lot z (2x250 seed)	11-22	2-23	5.9	1.0	3.0	0.01	4.0
Cultivar B[a], lot x (1000 seed)	11-22	1-23	1.9	2.9	1.7	2.7	ND
Cultivar B[a], lot x (1000 seed)	11-22	1-23	0.9	1.9	1.9	2.9	43.0
Cultivar B[b], lot w (2x250 seed)	11-22	3-23	0.0	1.0	1.9	0.9	ND
Cultivar B[b], lot w (2x250 seed)	11-22	2-23	0.0	1.0	0.0	0.0	21.0
Cultivar C[a], lot n (1000 seed)	11-22	3-23	0.0	0.0	0.0	0.0	0.0
Cultivar C[a], lot n (1000 seed)	11-22	3-23	0.0	0.0	0.0	0.0	0.0
Cultivar C[b], lot m (2x250 seed)	1-23	3-23	0.0	0.0	0.0	0.0	0.0
Cultivar C[b], lot m (2x250 seed)	1-23	3-23	0.0	0.0	0.0	0.0	0.0
Cultivar D[b], lot t (2x250 seed)	2-23	3-23	0.0	0.0	0.0	0.0	0.0
Cultivar D[b], lot t (2x250 seed)	2-23	3-23	0.0	0.0	0.0	0.0	0.0

a=Brand A; b= Brand B; c= date on package indicating the time of germination testing; d= date seeds were examined for oospores; e= packets were subsampled in four lots of approx. 100 seeds except for packets of 250 seeds, where two packets were used to get four subsamples; f= when possible, some oospores were scraped off infested seeds and immersed in a 4M NaCl solution to determine viability from % plasmolysis, which was averaged over subsamples where oospores were present.

* seed from this subsample germinated to produce an infected seedling

Results (Table one)

Infestation rates from seed subsamples were 0-6%, but in Cultivar A, an abundance of oospores were observed on each infested seed. Cultivar B had similar infestation rates, but fewer oospores per seed. Two additional cultivars, with coated seeds, had no visible oospores after the coat was dissolved. When oospores were p[aced in 4M NaCl, some plasmolyzed, indicating viability (up to 43%). One of the 62 infested seeds germinated to produce a systemically infected seedling (Fig. 4). Mycelium with haustoria was observed inside tissue from the root tip to the leaf tips. DNA sequencing confirmed the presence of *Peronospora effusa*. These results yield additional confirmation that the spinach downy mildew pathogen is seed-borne.

Discussion

These results suggest that seed-borne inoculum represents one way outbreaks can originate. It is important to know how epidemics begin especially when a suspected source can be controlled and especially for organic growers because organic fungicides have not proven adequately effective for controlling downy mildew epidemics.

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Fig, 2- Spinach seed of Cultivar A lot y infested with oospores of *Peronospora effusa*.

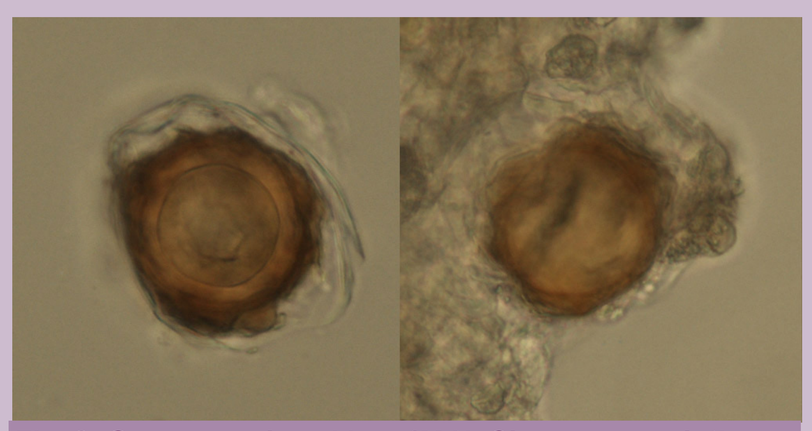

Fig. 3- Oospores placed in a 4M NaCl aqueous solution; nonplasmolyzed (left) and plasmolyzed (right).

Fig. 4- Infected spinach seedling germinating from an infested seed of Cultivar A lot y.