THE PATHOGENICITY OF FUNGI ISOLATED FROM SUGAR MAPLE SEEDS

Carol A. Janerette
Plant Physiologist, Forest Service,
U.S. Department of Agriculture,
Beltsville, Maryland

The most prevalent fungi isolated from sugar maple seeds were species of **Alternaria, Aureobasidium, Epicoccum, Penicillium,** and **Rhizopus.** The seedlings grown in the presence of these fungi exhibited pathogenic symptoms including chlorotic and necrotic lesions, malformed leaves, and stunted growth.

Seed transmission of plant pathogens has been recognized as an important factor in disease dissemination for many years. Although the post harvest deterioration of herbaceous seeds has been studied extensively *(12, 3, 4, 10)*, little attention has been given to tree seeds and the relationships between seed borne pathogens and tree diseases.

Stratification (storage under cold, moist conditions) is required for the germination of sugar maple (*Acer saccharum* Marsh.) seeds in the laboratory. During stratification, various micro-organisms develop on the seeds. Mycelial growth is so dense in some cases that the seeds are completely covered; however, seeds exhibit high germinability despite the presence of these organisms. Any effects on seedling development have not been determined.

This paper reports results of experiments designed to isolate and identify the micro-organisms present on representative sugar maple seeds and determine if they influence sugar maple seedling development.

Materials and Methods

Seeds with germination potential ranging from 71 to 100 percent were collected from three trees in northern Vermont (table 1). Filled fruits were

separated from empty ones by floatation in pentane *(2)*, and stored in sealed plastic bags at 10 percent moisture content at -10° C. Prior to use, seeds were removed from fruits.

Isolation procedures.—Fifty seeds from each lot were washed in 2.5 percent sodium hypochlorite (Na OC1) for 3 minutes and rinsed three times with sterile distilled water. Five replicates of 10 seeds each were placed on potato dextrose agar (PDA) and Czapek agar with 6 percent sodium chloride in petri plates and incubated at 25° C. Microorganisms growing on the plates around the seeds were repeatedly subcultured until each was obtained in pure culture and identified.

Effects on seedling development.—Two-hundred sugar maple seeds were soaked in distilled water for 14 days, to hasten germination *(8)*, and stratified at 3° to 4° C for 15 days in aluminum foil packets *(7)*. Sixty of the seeds that had germinated during stratification were individually planted in 15 ounce styrofoam cups containing moist peat moss.

Each fungus that had shown a frequency of greater than 10 percent in any seed lot was grown on PDA in a petri plate until the plate was completely covered with mycelia. One plate of each fungus was separately homogenized with distilled water making a final volume of 200 milliliter.

Ten replicates containing a single germinated seed planted in peat moss was moistened with 20 milliliters of the homogenized fungus solution; this procedure was repeated for each fungus that was tested.

Inoculated seeds were grown in a greenhouse for 49 days under

Table 1.—*Germination potential of sugar maple seeds from which micro-organisms were isolated*

Seed source	Year collected	Percent germination[1]
A	1974	99.6
A	1971	99.0
A	1970	99.6
B	1974	99.6
B	1972	98.7
B	1970	100
C	1974	81.7
C	1972	71.2
C	1969	96.9

[1]Germination tests conducted after collection and prior to use showed no differences.

long day conditions along with controls: seeds inoculated with a homoginate made from PDA and water.

At the close of the experiment, each plant was carefully examined. Tissue samples taken from plants with pathological symptoms were incubated on PDA, and the isolated fungi were identified.

Results and Discussion

Similar genera of microorganisms were isolated from seeds taken from each of the trees used as sources (table 2); however, the abundance and diversity varied from tree to tree and from year to year. Examination of freshly harvested sugar maple pericarps commonly reveals the presence of fungal mycelia indicating that infection occurs while the seed is on the tree.

Organisms similar to those isolated from sugar maple seeds have also been isolated from seeds of other forest trees *(13, 14, 15, 9, 5)* and from the outer bark of sugar maple trees *(11).*

Fungi isolated from sugar maple seeds (table 2) are generally classified as saprophytes and have not been considered as sugar maple parasites.

There was no apparent correlation between the prevalence of micro-organisms and seed deterioration in storage; however, there was a relationship between the frequency of infection and seed deterioration (table 2). Seed viability decreased as the prevalence of fungi increased (table 2). A similar observation was made by Shea *(13)* with Douglas-fir seeds and Kilpatrick *(10)* with Crambe seeds.

Five genera of fungi were isolated from more than 10 percent of the sugar maple seeds (table 2).

Sugar maple seedlings grown in the presence of those fungi isolated with the greatest frequency displayed pathological symptoms such as chlorotic and necrotic lesions on the leaves, stunted growth, and malformed leaves (table 3). Fungi used as inoculum were reisolated from sterilized tissue samples taken from diseased plants. Fungi similar to those isolated from sugar maple seeds have also been isolated from diseased Douglas-fir seedlings grown in the field *(1).* Leukel

Table 2.—*Genera of micro-organisms isolated from sugar maple seeds*

Seed lot	Germination potential of seed lot	Frequency of seed infestation	
		10 percent or more	Less than 10 percent
	percent		
B-1970	100	None	*Candida*
A-1974	99.6	None	*Alternaria, Bacillus Mucor, Rhizopus*
B-1974	99.6	None	*Aureobasidium, Candida, Penicillium, Rhizopus*
A-1970	99.6	*Aureobasidium*	*Alternaria, Bacillus Candida, Mucor, Phomopsis, Rhizopus*
A-1971	99.0	*Penicillium, Rhizopus*	*Alternaria, Aureobasidium, Candida, Ulocladium*
B-1972	98.7	*Aureobasidium, Rhizopus*	*Candida, Penicillium*
C-1969	96.9	*Aureobasidium, Penicillium*	*Alternaria, Bacillus, Candida, Cladosporium, Rhizopus*
C-1974	81.7	*Alternaria, Aureobasidium, Epicoccum, Rhizopus*	*Candida, Bacillus, Cladosporium, Mucor, Penicillium, Phomopsis*
C-1972	71.2	*Alternaria, Aureobasidium, Epicoccum, Penicillium, Rhizopus*	*Bacillus, Candida, Phomopsis, Ulocladium*

and Martin *(12)* conducted a similar study with sorghum. They found that when species of *Alternaria, Fusarium, Penicillium*, and *Rhizopus were* isolated from sorghum seeds and subsequently used as inoculum, there was reduced emergence and seedling blight.

Sugar maple regeneration has been mainly left to chance *(16)*, and field plantings usually have low survival *(6)*. It is probable that fungi borne on sugar maple seeds become active pathogens when conditions are favorable and exert detrimental effects on germinating seeds and young seedlings in the field.

Table 3.—*Influence of fungi isolated from sugar maple seeds on the development of sugar maple seedlings*

Fungus	Seedling height	Leaf symptoms[1] [2]		
		Chlorotic lesions	Necrotic lesions	Malformed leaves
	(CM)			
None (Control)	12.7 ± 1.8	0	0	0
Aureobasidium	8.4 ± 1.3	XX	0	0
Alternaria	10.0 ± 1.6	XXX	X	X
Epicoccum	*7.0 ± 2.6*	XXX	XX	0
Penicillium	11.1 ± 1.3	XXX	0	X
Rhizopus	8.0 ± 2.9	XX	XX	X

[1] Symptom absent = 0

[2] Symptom present = X *(1-3* plants); XX (4-6 plants); XXX (7-10 plants).

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