Poster #656

GREER

POLLEN VIABILITY UNDER A STRESS CONDITION

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Abstract

Rationale

Experiments have suggested that pollen viability could be an indicator of air pollution. This testing could potentially replace the pesticides and heavy metals measurement currently performed to evaluate that parameter. The purpose of this exploratory study was to ascertain the efficacy of a stain to evaluate the viability of different pollen species under an artificial stress condition.

Methods

Timothy grass, short ragweed, and white oak pollen species were stored at 35oC for one month. Pollen viability was measured at time 0 and after 1, 5, 10, 20, and 30 days of storage using p-phenylenediamine to detect the presence of myeloperoxidase. A minimum of 150 pollen grains were examined by optical microscopy. Pollen grains were considered viable if they turned totally black. All samples were extracted at the end of the study. Bradford protein content was measured and qualitative protein profile was analyzed by SDS-PAGE.

Results

The discrimination between viable and non-viable pollen was efficient for oak and controversial for ragweed. The percents of viable Timothy grass, oak, and short ragweed pollen at time 0 were 93.1%, 58.8%, and 100%, respectively. The respective percents at the end of the study were 0.0%, 16.2%, and 18.3%. No substantial changes in total protein content and protein profile were observed at any testing time compared with the respective baseline levels.

Conclusion

Myeloperoxidase staining could be a sensitive marker of pollen integrity for some species. This test could have potential applications in different areas. Additional experiments in the laboratory should be performed before initiating field experiments.

Introduction

The need for assessing pollen viability is essential when pollen is used in artificial pollinosis and breeding experiments. It is also an imperative approximation to understand sterility problems, evolutionary ecology, and in hybridization programs.

Many air pollutants cause plant deterioration. The effects of air pollution on plants are mostly restricted to the observation of plant vegetative structures, particularly leaves. Mature pollen is hygroscopic, and thus it can absorb moisture from the atmosphere and also any associated air pollutants. A selective effect of atmospheric contaminants on pollen viability has been described, depending on the species.

Various stains that indicate pollen viability have been used to determine pollen viability to replace pollen germination and microscopic observations of pollen tube growth because it is a rapid process. These stains include 2,3,5-triphenyl tetrazolium chloride (TTC) and iodine plus potassium iodide (IKI). However, these stains also stain non-viable pollen.

Objective

The purpose of this exploratory study was to ascertain the efficacy of a stain to detect the presence of myeloperoxidase in various pollen species under an artificial stress condition.

Materials and Methods

Pollen Species

Five, 0.5 gram vials of the following grass, weed, and tree pollen species:

- Timothy grass (*Phleum pretense*)
- Short ragweed (*Ambrosia artemisiifolia*)
- White oak (*Quercus alba*)

Pollen Stress

One vial, stored at <0ºC at the beginning of the study, was used as a control (time 0).

- The remaining vials were stored at 35ºC for up to one month.
- One vial was removed after one, 5, 10, 20, and 30 days of storage at 35ºC and were kept at <0ºC for testing at the end of the storage period

Pollen Viability Staining (after each stress time)

Staining with p-phenylenediamine to detect the presence of myeloperoxidase was performed as follows:

- *Reagent preparation*: One vial of peroxidase indicator reagent (Sigma catalog #390-1VL) was mixed with 200 µL of 3% hydrogen peroxide and 50 mL of Trizmal buffer concentrate (Sigma catalog #903C) diluted 1:9 with distilled water and warmed to 35ºC.
- *Reagent storage*: In the dark at 2-8ºC for 15-20 days.
- *Pollen staining*: A pollen sample was mixed with the staining on a microscope glass slide using an applicator. Pollen staining was evaluated after 15-30 minutes. A minimum of 150 pollen grains were counted under a magnification of 400X using an optical microscope.
- *Interpretation*: Pollen grains were considered viable if they turned totally black. black.

Pollen Allergen Extracts Preparation (at the end of the study)

- *Extraction*: 1:20 (w/v) in 0.1 M ammonium bicarbonate for 48 hours under rotation at 2-8ºC.
- *Clarification*: By centrifugation at 2,500 rpm at 20-25ºC and filtration through 0.4 µm filters.

Total Protein Content

- According to the Bradford method in microplates using bovine serum albumin as the standard.

SDS-PAGE

- *Assay conditions*: 12% polyacrylamide gels, according to the discontinuous buffer system of Laemmli under reducing conditions.
- *MW standards*: Broad range.
- *Electrophoresis conditions*: Mini Protean II Dual Slab Cell (BIO-RAD Laboratories) at 200 V.
- *Staining*: Coommasie Blue.

Results

Pollen Viability

- The three pollen species indicated a decrease in the percent of viable grains at the study period (Table I).

TABLE I: Percent of Viable Pollen Detected

Extract	Days Stored at 35ºC	% Viable Pollen
White oak (*Quercus alba*)	0	93.1
	1	93.5
	5	84.8
	10	90.2
	20	90.4
	30	0.0
Timothy grass (*Phelum pratense*)	0	58.8
	1	54.3
	5	35.1
	10	54.7
	20	25.6
	30	16.2
Short ragweed (*Ambrosia artemissiifolia*)	0	100
	1	96.2
	5	98.1
	10	78.1
	20	92.2
	30	18.3

- The myoperoxidase staining was particularly efficient to detect the viability of oak pollen and controversial to test ragweed pollen (Figures 1, 2, and 3).

FIGURE 1: Microscopic Photographs of White Oak Pollen Obtained at Time 0 (A) and after Storage at 35ºC for 30 Days (B)

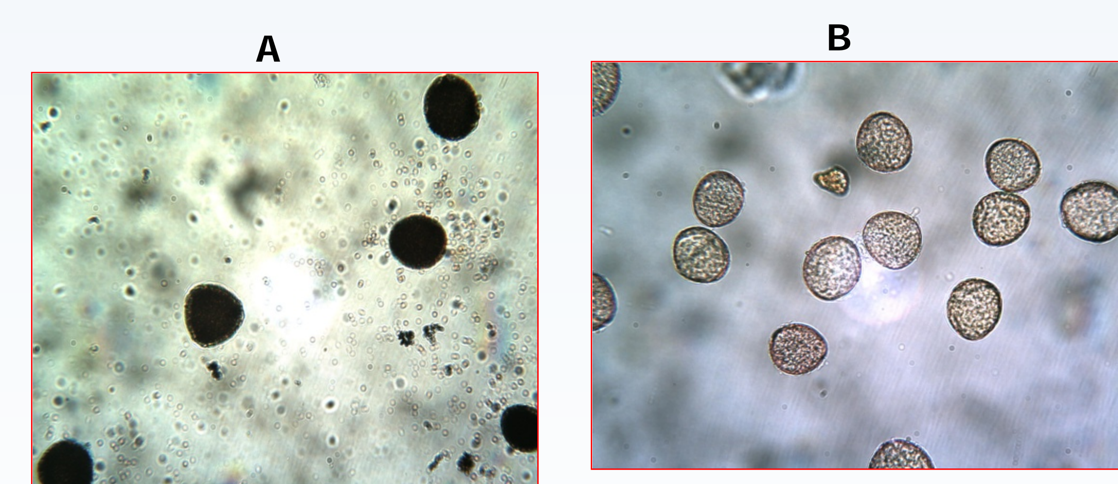


FIGURE 2: Microscopic Photographs of Timothy Grass Pollen Obtained at Time 0 (A) and after Storage at 35ºC for 30 Days (B)

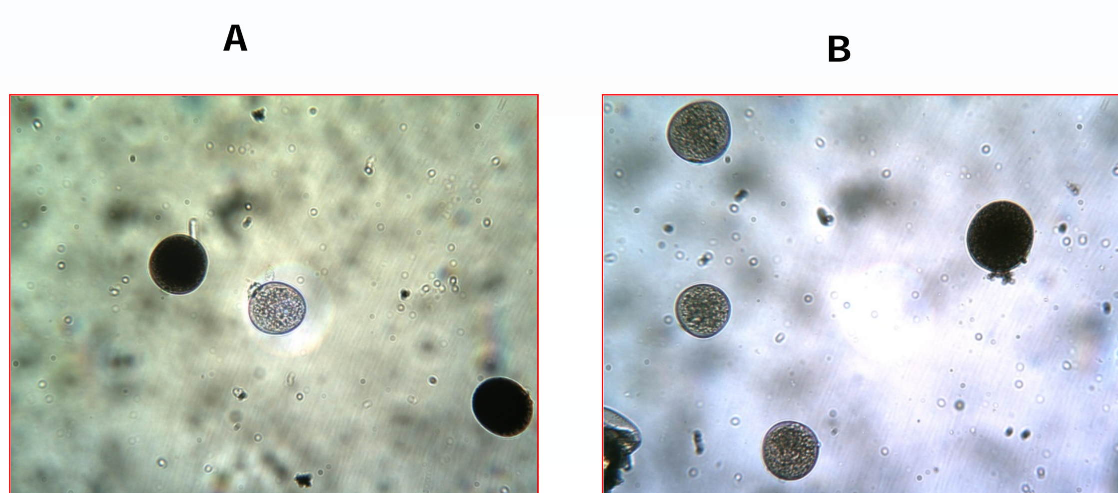
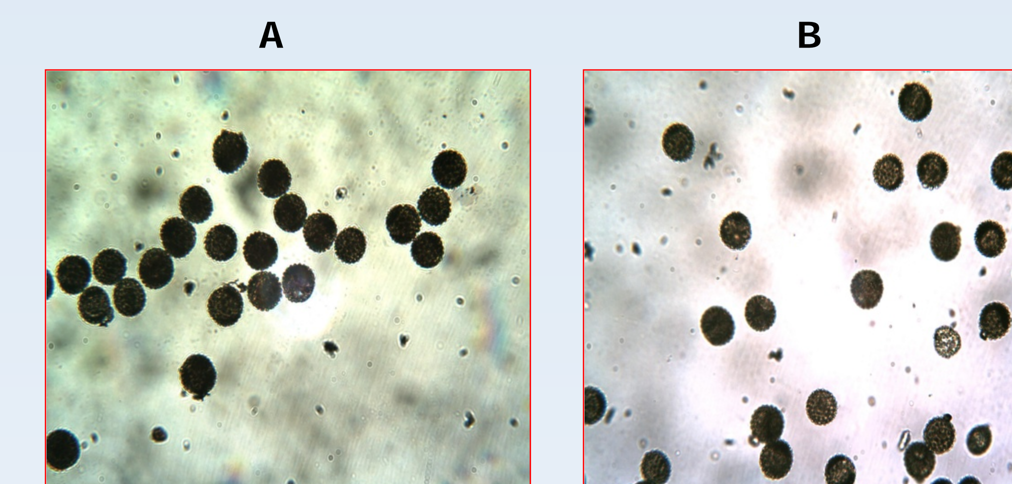


FIGURE 3: Microscopic photographs of Short Ragweed Pollen Obtained at Time 0 (A) and after Storage at 35ºC for 30 Days (B)



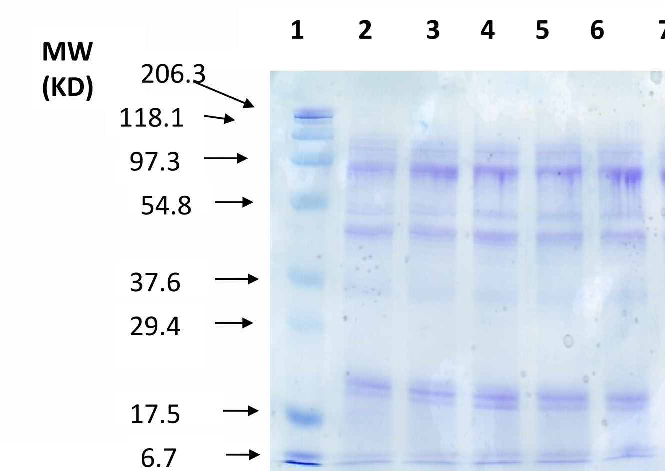
- The Bradford assay did not indicate any obvious changes in total protein content at any of the testing times compared with the values detected at time 0 (Table II).

TABLE II: Bradford Protein Content of Pollen

Extract	Days stored at 35ºC	Protein content (µg/g)
White oak (*Quercus alba*)	0	4,699
	1	4,877
	5	5,609
	10	5,005
	20	5,313
	30	6,708
Timothy grass (*Phleum pretense*)	0	22,921
	1	21,392
	5	22,524
	10	23,431
	20	20,061
	30	24,702
Short ragweed (*Ambrosia artemisiifolia*)	0	22,059
	1	15,867
	5	23,583
	10	18,445
	20	13,583
	30	14,295

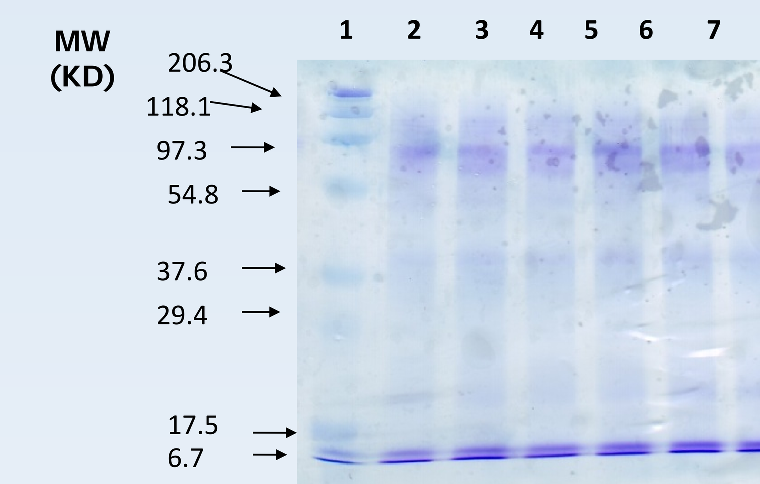
- The SDS-PAGE analysis failed to identify any changes in qualitative protein profile (Figures 4-6)

FIGURE 4: SDS-PAGE of White Oak Pollen



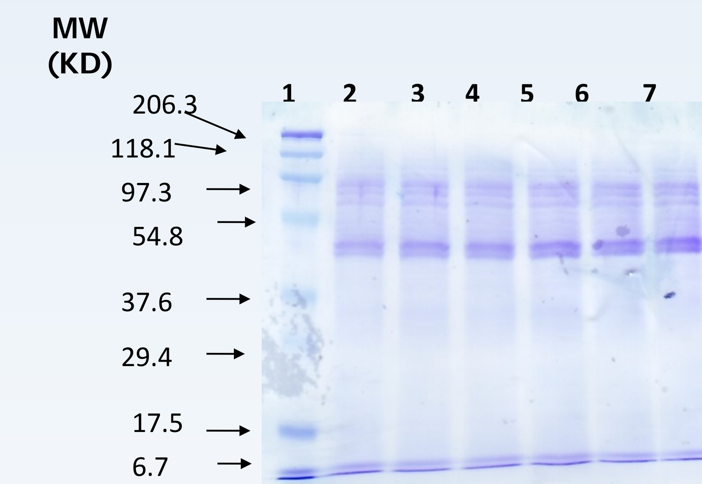
Lane 1: MW standards. Lanes 2, 3, 4, 5, 6, and 7: Pollen stored at 35ºC for 0, 1, 5, 10, 20, and 30 days, respectively

FIGURE 5:SDS-PAGE of Timothy Grass Pollen



Lane 1: MW standards. Lanes 2, 3, 4, 5, 6, and 7: Pollen stored at 35ºC for 0, 1, 5, 10, 20, and 30 days, respectively

FIGURE 6:SDS-PAGE of Short Ragweed Pollen



Lane 1: MW standards. Lanes 2, 3, 4, 5, 6, and 7: Pollen stored at 35ºC for 0, 1, 5, 10, 20, and 30 days, respectively

Conclusions and Relevance

- Pollen viability could potentially be a qualitative marker of integrity for some pollen species if the technique/s are improved.
- Additional experiments should be performed with a number of pollen species stored under various accelerated stress conditions to determine what species should be further studied.
- Real-time experiments should also be performed under natural conditions.
- While pollen viability is a test that has been restricted to agricultural research, its usefulness should be expanded to other areas, including the area of air pollution.

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