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**Morphological, Pathological, and Biochemical
Comparisons Among Selected Downy Mildew
Pathogens of the Gramineae, Leguminosae and
Compositae**

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ABSTRACT

Conidiospore length, width, length/width ratios, volume, and nuclear number were determined for isolates of Peronosclerospora philippinensis, P. sacchari, and P. sorghi and integrated with previous and new information on host range, symptoms, isozymes, and aminopeptidase profiles to make conclusions on the reliability of these determinations in identifying and/or delineating species of this genus.

The length/width ratio of mature conidia of Peronosclerospora separated the P. philippinensis-P. sacchari "complex" from both "true" (typical) P. sorghi and the atypical P. sorghi from Thailand. Conidial volume was a good characteristic for separating atypical P. sorghi in Thailand from all other organisms. Conidiophore characteristics were too variable to be of assistance.

Isozyme analysis was very useful and in concert with morphological measurements showed P. sorghi from Thailand not to be closely related to any other isolate in this study. Isolates of P. sacchari and P. philippinensis shared alleles at 24 isozyme loci and were determined probably to represent a single species. Isozyme analysis readily separated isolates of Peronosclerospora from those of Bremia lactucae and Peronospora tabacina and demonstrated the potential of this technique in fungal taxonomy. Aminopeptidase profiles were essentially identical for all isolates of Peronosclerospora and therefore had no value for differentiation.

The thailand isolates of P. sorghi produced the greatest number of conidia per cm² infected-leaf area, P. sacchari and P. philippinensis intermediate, and "true" P. sorghi the least on Pioneer 3369A maize. Evidence was obtained indicating that isolates originating from hosts other than maize eventually adapt to become more aggressive on maize.

A technique was developed by which spore prints of peronosporaceous fungi, free of immature conidia, could be obtained for biometric studies. When spores were measured in lactophenol-cotton blue, no significant pregermination swelling could be detected in any of three fungal species tested and it is recommended that this be used as a standard mounting medium for both dried and fresh peronosporaceous conidia.

Peronospora radii de Bary 1863, a new record in Israel, was found in January 1985 as the cause of a downy mildew of cultivated marguerite daisies, var. "Scheine von Nizza" (a cultivar of Argyranthemum frutescens: Anthemideae = Compositae) in the Coastal Plain. The same fungal species was found in February 1986 on the very common, wild Chrysanthemum coronarium (Anthemideae) near the previous site.

Introduction

Peronosclerospora downy mildew pathogens of graminaceous crops are among the most destructive pathogens in the tropics and subtropics. Although to date they have had little importance in more temperate regions, the rapid expansion of P. sorghi (the sorghum downy mildew pathogen of sorghum and maize) in the United States from Texas to Indiana and Illinois suggests that these pathogens may have the potential to cause large losses in the United States (4).

Furthermore, recent research results at the USDA Foreign Disease-Weed Science Research Unit, Frederick, Maryland, demonstrate a high susceptibility of U.S. maize to foreign isolates of Peronosclerospora sorghi, P. sacchari, and P. philippinensis (Bonde, unpub.). Maize breeding lines highly resistant to downy mildew in the United States are highly susceptible to isolates of Peronosclerospora, including P. sorghi, from Southeast Asia. Some pathogen isolates are capable of readily infecting several common perennial grasses in the United States, which potentially could act as overwintering hosts in the absence of crop plants (1, 2).

Downy mildews of lettuce and safflower (both of the Compositae), caused by Bremia lactucae Regel, may or may not be the same on these two hosts as well as on certain wild weeds of the family. Likewise, there is much confusion on the identity of Peronospora spp. on plants of the Leguminosae, such as peas, common vetch, alfalfa, and clovers, as well as on certain plants of the Compositae. The diseases on these crops can be serious in both Israel and the United States.

The confusion over specific pathogen involvement is a major problem in evaluating downy mildew diseases from a global perspective and determining

if research results and control methods developed in one area of the world might be applicable to another. In several instances, taxonomy within the genera is confused because, at most, only slight differences in morphology of recognized species exist (7). Furthermore, apparently morphological plasticity exists within the species. Exconde et al. (3), when inoculating various hosts with P. philippinensis, obtained striking differences in size and shape of conidiophores and conidia. Leu (5) in Taiwan determined that temperature during sporulation and host cultivar both affected size and shape of P. sacchari conidia. Pupipat (6) was able to classify 35 isolates of P. sorghi on maize in Thailand into ten categories based on conidial shapes from globose to dumbbell to cylindrical.

The study presented here compared isolates of Peronosclerospora from throughout the world using several divergent techniques. Because, in many instances, isolates were compared simultaneously on the same hosts under essentially identical environment conditions, better comparisons were made than would otherwise have been possible. The techniques used include morphology, symptomatology, isozymes, and numbers and staining properties of nuclei. Several of the studies initiated are to be continued as other critical cultures are collected for comparison such as Peronosclerospora maydis from Australia and Indonesia, and P. philippinensis from India.

The main objective of this study was to determine the dependability of using morphological, certain biochemical, and karyological techniques for denoting speciation within representative downy mildew taxa. Correlations among these factors were to be made and methods to overcome problems developed, if possible. Techniques were to be developed in order to standardize methods for measuring the size, shape, and volume of conidia,

as well as length and width of conidiophores. We believe that as a result of this study, certain changes in the classification of specific downy mildew pathogens will eventually be made.

Relevant Bibliography

1. Bonde, M. R., and G. L. Peterson. 1981. Host range of a Taiwanese isolate of Peronosclerospora sacchari. Plant Dis. 65:739-740.
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6. Pupipat, U. 1976. Corn downy mildew research at Kasetsart University. Kasetsart J. 10:106-110.
7. Schmitt, C. G., J. M. Woods, C. G. Shaw, and E. Stansbury. 1979. Comparison of some morphological characters of several corn downy mildew incitants. Plant Dis. Reprtr. 63:621-625.

OBJECTIVES OF RESEARCH

- a. To compare morphologically, isolates of the genera Peronosclerospora (from maize and sorghum), Bremia (from lettuce, safflower, and related weeds), and Peronospora (from peas, common vetch, alfalfa, clovers, and chick peas) from several parts of the world. Detailed comparisons were made of available isolates of Peronosclerospora from maize, sorghum, and sugarcane from several geographic areas of the world. Morphological studies were conducted on a new Peronosclerospora discovered on prairie cupgrass (Eriochloa contracta) in Texas and the fungus was named Peronosclerospora globosa Kenneth and Kubicek, sp. nov. Where applicable, pathogens were compared on the same host cultivars under essentially identical environmental conditions.
- b. Determine effects of environment and host cultivar during growth of the pathogen, and of specimen preparation, on morphology of the isolates, and develop standard techniques.
- c. Determine feasibility of starch gel electrophoresis of proteins and deoxyribonucleic acid for differentiating species within genera of downy mildew pathogens.
- d. Compare numbers of nuclei and staining properties of nuclei among isolates to determine if these may be valid criteria to distinguish pathogen species.
- e. Where applicable, compare host ranges of pathogen isolates using the same host cultivars or accessions.

PUBLISHED DATA

DESCRIPTION OF COOPERATION

Past and Present Cooperation. The research proposed was prepared jointly by the participants while Dr. Robert Kenneth was visiting the United States during August, 1982. During the course of the project, the participants have been in contact by mail and telephone.

The project was begun at Frederick, Maryland in September, 1983. Because Dr. Kenneth was on sabbatical leave at Texas A&M University, College Station, Texas at that time, his portion of the project began one year later. While at Texas A&M, Dr. Kenneth and the Principal Investigator were in close contact by telephone.

Cultures of Peronosclerospora (including those from Israel) were collected at the USDA, Foreign Disease-Weed Science Research Unit, Frederick, Maryland where host plants were inoculated under standard conditions. Leaf samples supporting conidiophores and conidiospores, and mature conidiospores on glass slides were sent to Rehovot, Israel and Beltsville, Maryland. Microscopic measurements were made at Rehovot by Dr. Kenneth and Research Associate Dr. Israel Ben-Ze'ev and nuclear studies performed by Dr. Farr at Beltsville. Conidial volumes of spore samples were determined with a particle counter and computerized terminal at Frederick. Isozyme analysis of conidial spore suspensions on the same isolates used by scientists at Rehovot and Beltsville were conducted at Frederick by Dr. Jessie Micales, Research Associate. Statistical treatments of some of the data collected at Rehovot was performed by computer at Frederick.

Dr. Kenneth visited Frederick in August, 1984 while on sabbatical leave at Texas A&M University. Dr. Bonde visited Rehovot in September, 1985 and Dr. Ben-Ze'ev visited Frederick and Beltsville, Maryland in September, 1986 to

assist in preparing the final report for the project. Joint manuscripts are being prepared and these are included in this report.

Future Cooperation. Cultures of Peronosclerospora maydis are in the process of being obtained from scientists in Indonesia and Australia. These will be compared with the other species of Peronosclerospora.

A major paper (monograph) will be prepared discussing and correcting some of the taxonomy of the genus Peronosclerospora.

Further isozyme comparisons will be made using isolates of Bremia from the United States, Israel, and Europe.

Isolates of Peronospora radii from Israel will be further compared using isozyme analysis with other isolates of the genus.

BENEFIT TO AGRICULTURE

The benefits of the research findings obtained in this cooperative project are summarized as follows:

1. Isozyme analysis was shown to be an extremely powerful tool for sorting out taxonomic confusion in the genus Peronosclerospora. At the time this project was started, there had been almost no published work on isozyme analysis for taxonomic purposes where a genetic approach, as contrasted to "merely band counting", was used. The research presented here has received considerable interest. As a result, the paper "The use of isozyme analysis in fungal taxonomy and genetics" (Mycotaxon 27:405-449) was written; workshops teaching the technique are being considered. The experience obtained in this project will be applied to other plant pathogens.
2. Strong evidence was obtained corroborating the hypothesis that Peronosclerospora pathogens from hosts other than maize have the ability to adapt to maize. This process can be observed by an increase in rate at which corn tissue is colonized and an increase in the capacity for the pathogen to produce conidia on maize. The results suggest that "new" maize Peronosclerospora pathogens will be discovered and that a better means of identifying them is required.
3. A new species of Peronosclerospora was discovered on prairie cupgrass in Texas and may prove beneficial for sorting out taxonomic confusion in this fungal genus.
4. Peronospora radii was discovered for the first time in Israel and may become an important negative factor in the production and sale of marguerite daisies. Further research is required to study the epidemiology of this pathogen and effective control measures must be developed.
5. Better techniques were developed for standardizing the attainment of biometric data and information was obtained concerning morphological variation of specific downy mildew species.
6. Information was obtained indicating that Peronosclerospora sacchari and P. philippinensis are the same species. The atypical P. sorghi in Thailand should not be considered as this species. As additional isolates are obtained and examined by us, it is probable that there will be some changes made in the classification of some of these pathogens of maize. Their correct identification will aid in plant disease control measures.

LIST OF PUBLICATIONS

Current Publications:

1. Duck, N. B., Bonde, M. R., and Peterson, G. L. 1985. Comparison of sporulation of Peronosclerospora sorghi, P. sacchari, and P. philippinensis from several areas of the world. *Phytopathology* 75:625. (Abstr.)
2. Kenneth, R. G. and Ben-Ze'ev, I. S. 1985. Peronospora radii, the cause of an unusual downy mildew of marguerite daisy in Israel. *Phytoparasitica* 13:151-152. (Abstr.)
3. Micales, J. A., Bonde, M. R., and Peterson, G. L. 1986. The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxon* 27:405-449.
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5. Micales, J. A., Bonde, M. R., Peterson, G. L., and Fry, W. E. 1985. Differentiation of Peronosclerospora species by isozyme analysis. *Phytopathology* 75:1339. (Abstr.)
6. Micales, J. A., Huber, D. M., Bonde, M. R., Peterson, G. L., and Fry, W. E. 1985. Aminopectidase activity of Peronosclerospora sorghi, P. sacchari and P. philippinensis. *Phytopathology* 75:627. (Abstr.)

Submitted publications:

1. Ben-Ze'ev, I. S. and Kenneth, R. G. 1987. A biometric study in *Peronosporaceae*: Influence of water, lactophenol, and pregermination swelling on conidial dimensions. (Submitted to *Mycologia*)
2. Ben-Ze'ev, I. S., Kenneth, R. G., and Bonde, M. R. 1987. Peronospora radii de By., a causal agent of downy mildew of *Anthermideae*: complementary description and new hosts recorded in Israel. (Submitted to *Phytoparasitica*)
3. Kenneth, R. G. 1987. Will the real Java downy mildew please stand up? (Submitted to: *News1. International Working Group on Graminaceous downy Mildews*)
4. Kenneth, R. G. and Kubicek, Q. B. 1987. Peronospora globosa sp. nov., a downy mildew of prairie cupgrass, Eriochloa contracta, in Texas. (Submitted to *Mycologia*)
5. Micales, J. A., Bonde, M. R., and Peterson, G. L. 1987. Isozyme analysis and aminopectidase assays of the genus Peronosclerospora. (Submitted to *Phytopathology*)