

Deriving uredinial cultures of *Puccinia graminis* from aecial infections on the alternate host



Melissa Carter¹, Melissa Lim², Douglas Luster^{*1}, Pablo Olivera³, Yue Jin⁴

¹US Department of Agriculture-Agricultural Research Service, Foreign Disease-Weed Science Research Unit, Ft. Detrick MD

²Institute for Agriculture and Trade Policy, St. Paul MN

³University of Minnesota, Department of Plant Pathology, St. Paul MN

⁴US Department of Agriculture-Agricultural Research Service, Cereal Disease Lab, St. Paul MN

*Correspondence: Dr. Doug Luster, doug.luster@usda.gov



UNIVERSITY OF MINNESOTA

INTRODUCTION

Barberry was determined to play a role in the life cycle of *Puccinia* rusts in the 1800s. Following the emergence of Ug-99, a renewed interest in the alternate host was born out of necessity to stay ahead of new virulence found in yearly rust surveillance around the world



Collaborators send dried leaf samples to the USDA lab in Frederick MD so researchers can begin the process of deriving isolates of *Puccinia* from aeciospores

LIFE CYCLE of PUCCINIA

The alternate host, common barberry, is used by *Puccinia* spp. to complete its sexual reproduction through the production of aeciospores

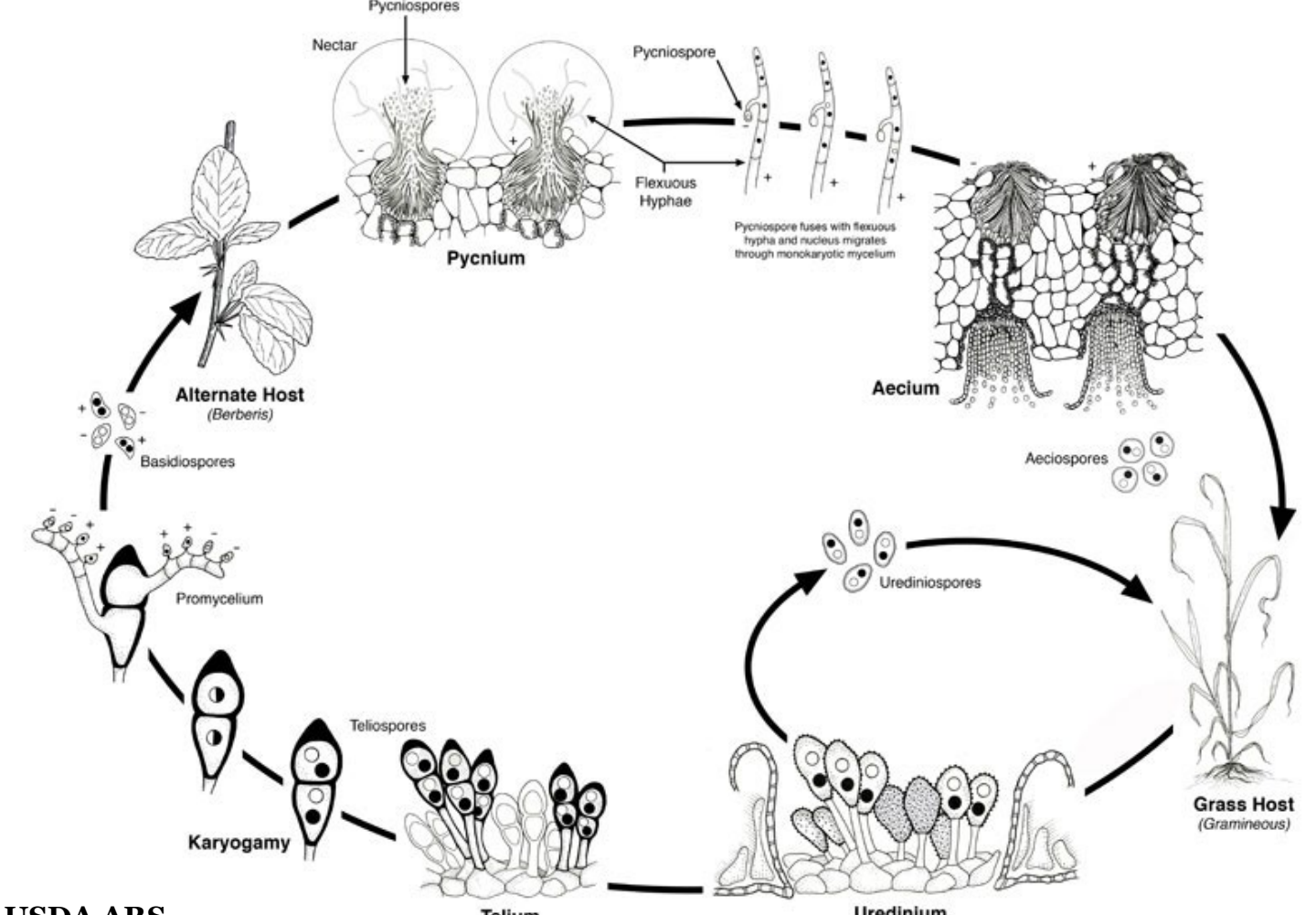


Photo credit: USDA ARS

BARBERRY COLLABORATION

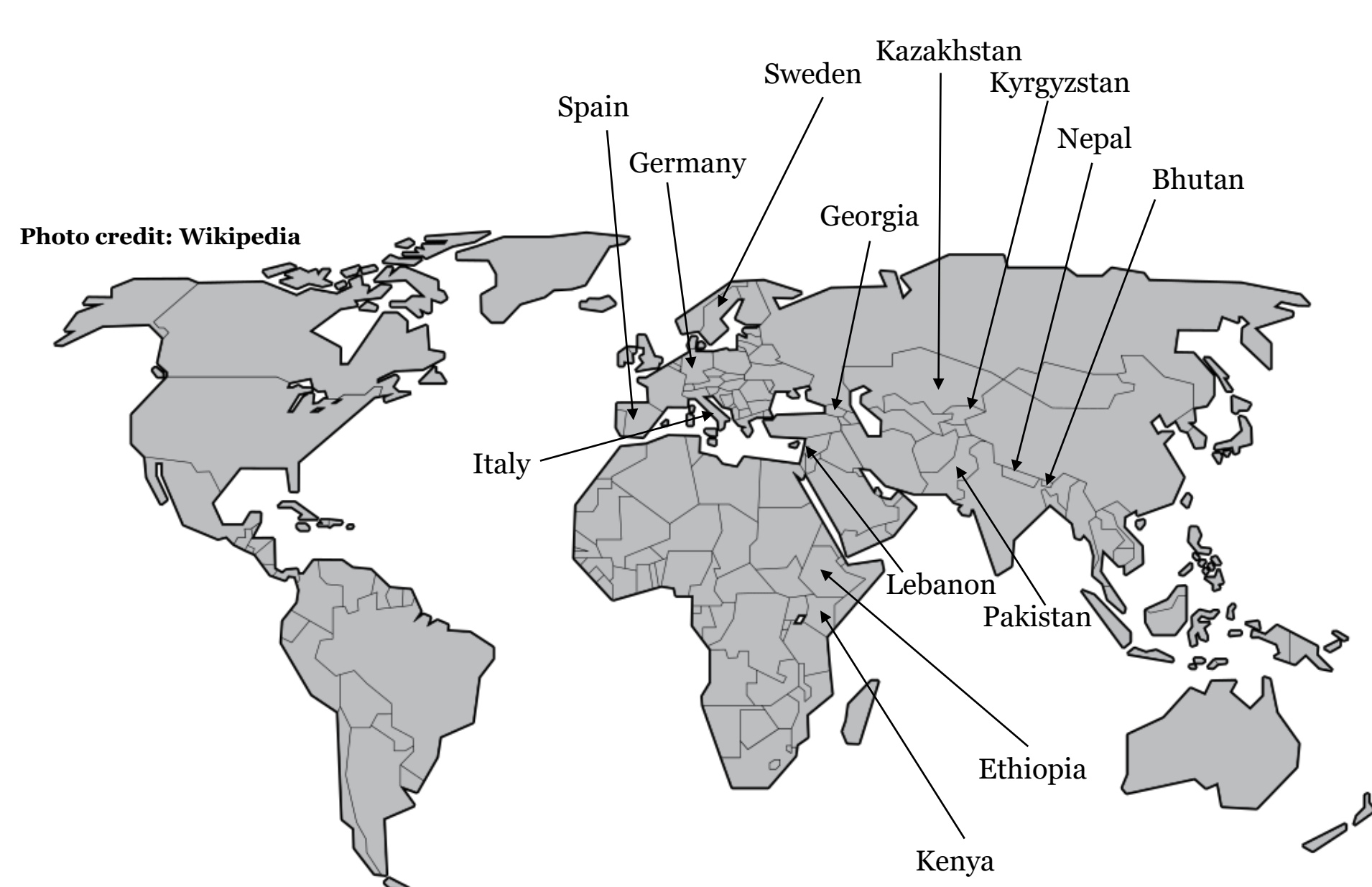


Photo credit: Wikipedia

Samples have been sent to us by our wonderful collaborators, who account for 13 unique countries around the world!

INOCULATION METHODS

METHOD 1 SUSPENSION

Suspend aecia-bearing leaves are above cereal seedlings

Place in 20C dew chamber for 48-hour incubation in the dark

Hydrated spores will fall onto plants and cause infection



METHOD 2 WET SPRAY

Collect and suspend aeciospores released during Method 1 into water and spray inoculum onto seedlings

Use water-resistant 3-D printed capsules for this protocol

Place in 20C chamber for 48-hour incubation in the dark



Photo credit: Nina Shishkoff

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METHOD 3 DRY SPRAY

Collect and suspend aeciospores from dried leaves into mineral oil and spray spore suspension onto seedlings

Use gelatin capsules in this protocol

Place in 20C dew chamber for 48-hour incubation in the dark



Photo credit: BGRI

Photo credit: CIMMYT

POST INOCULATION

Take plants from dew chamber and allow to dry

Place in growth chamber 20C day, 18C night

Fertilize with soluble 20-20-20 on day 1

Bag with cellophane on day 7 post-inoculation

Fertilize again on day 10

Check for infection on day 10 through 14



POST INFECTION



Harvest spores by vacuuming into capsules

Photograph infection on each cereal host

Save rust-infected tissue for genotyping

Dessicate spores 3-4 days prior to storage in cryotubes

Store in -80C short term; liquid nitrogen archival storage



ACKNOWLEDGMENTS



USDA is an equal opportunity provider and employer.

WHERE FROM HERE?

Capsules are sent to the ARS Cereal Disease Lab in December for single spore isolation, race phenotyping and SNP-based genotyping.