

# Isozyme Analysis as an Indicator of Synonymy of the Causal Agents of Gall Rust on Sand and Virginia Pine

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## ABSTRACT

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Isozyme patterns of homogenized aeciospores of *Cronartium quercuum* from sand pine and Virginia pine were identical and unlike patterns for aeciospores from loblolly, jack, or shortleaf pines, which also differ from each other. These results, together with those of previous inoculation tests, are evidence that strains of *C. quercuum* from sand and Virginia pine should be assigned the same special form classification, *C. quercuum* f. sp. *virginianae*.

Sand pine, *Pinus clausa* (Chapm. ex Engelm.) Vasey ex Sarg., is a locally important southern pine that occupies relatively sterile sites where other pines grow poorly. Sand pine occurs only on deep, infertile sands and is most common in central Florida. Two geographic races have been distinguished: the Ocala race, which occurs from northeast to central Florida, and the Choctawhatchee race, which is found in a small area in northwestern Florida and extreme southern Alabama. Sand pine is quite resistant to insects and diseases that are serious pests of other southern pines (2). The species is also tolerant of drought and temperature extremes. Sand pine is, however, attacked by *Cronartium quercuum* (Berk.) Miyabe ex Shirai, which can produce globose galls on stems and branches. Although the galls are conspicuous, they usually do not cause serious damage.

Taxonomically, sand pine is closely related to Virginia pine, *P. virginiana* Mill. Both are listed as members of the subsection *Contortae* of the genus *Pinus* (6). Virginia pine is susceptible to a gall rust caused by *C. q.* f. sp. *virginianae* Burdsall & G. Snow. The designation "formae speciales" was assigned by Burdsall and Snow (1). Because there are no apparent morphological differences among isolates of *C. quercuum* from Virginia, jack (*P. banksiana* Lamb.), shortleaf (*P. echinata* Mill.), slash (*P. elliottii* Engelm. var. *elliottii*), or loblolly (*P. taeda* L.) pines, it seemed appropriate

to apply this designation to the rust that occurs on each of these species. Recently, Powers et al (8) used isozyme analysis to distinguish among the special forms of *C. quercuum* as well as among various *Cronartium* species.

The galls produced by *C. q. virginianae* in Virginia pine are globose, usually not very damaging, and resemble the gall rust on sand pine. These two pine species have been inoculated with rust spores from several pine species, including Virginia pine, but have not been inoculated with the rust from sand pine (3,7). In these tests, aeciospores from the various pine species were used to inoculate northern red oak (*Quercus rubra* L.) to produce basidiospores used in pine inoculations. The inocula derived from Virginia pine caused infection (galls) on 54% of sand pine seedlings and 43% of Virginia pine seedlings. In recent preliminary tests, inocula from both sand and Virginia pines produced galls on both pine species (G. A. Snow, unpublished). Again, each treatment produced a higher percentage of infection in sand pine than in Virginia pine. Kuhlman and Kaneko (4) have also compared basidiospores derived from five formae speciales of *C. quercuum*, including *C. q. virginianae* and *C. q. banksianae* Burdsall & G. Snow, on the basis of color, size, and length/width ratios. They found that the latter two rusts can be grouped together and differentiated from the others on the basis of length/width ratios. Jack pine is in the subsection *Contortae* of the genus *Pinus*, as are Virginia pine and sand pine. Thus, the rust fungi (*C. quercuum*) that cause galls on pines in the subsection *Contortae* appear to be very closely related.

In the previous study (8), in which isozyme analysis was used to differentiate between species and formae speciales within the genus *Cronartium*, glucose

phosphate isomerase (GPI) and esterase gave the best results of the 16 enzyme systems tested. In the present study, isozyme analysis using these two enzymes was carried out to define the relationship between the gall rust fungi on Virginia and sand pines. This method can be used to detect differences that cannot be detected by inoculation tests.

## MATERIALS AND METHODS

For the present study, aeciospores of *C. quercuum* were collected from loblolly pine in Texas, Mississippi, Alabama, Georgia, North and South Carolina, and Maryland; jack pine in Minnesota; shortleaf pine in Mississippi; Virginia pine in South Carolina; and the Ocala race of sand pine in central Florida. Aeciospores from rust on the Choctawhatchee race of sand pine were not available because that race of pine is much more resistant to the disease than is the Ocala race. The *C. q. banksianae* aeciospores were a mass collection from 10 individual galls. The aeciospores of *C. q. virginianae* were mass collections from 10 galls in 1984 and from 10 galls in 1985. The aeciospores from sand pine were from a mass collection of seven individual galls collected along a 24-km transect in the Ocala National Forest in Florida. The *C. q. echinatae* Burdsall & G. Snow aeciospores were a mass collection from five individual galls. The nine samples of *C. q. fusiforme* (Hedgc. & N. Hunt) Burdsall & G. Snow included eight single-gall isolates and one mass collection from 10 galls from Georgia.

Aeciospores of the various species and formae speciales were collected from pine hosts and processed and stored according to the procedures outlined by Roncadori and Matthews (9). Briefly, aeciospores were collected from fruiting rust galls, sifted through 150- $\mu$ m mesh screens, and placed in a desiccator containing  $\text{CaCl}_2$ . The aeciospores were kept in the desiccator at approximately 4 C for 2-3 wk until they became powdery in texture. Then 300 mg of aeciospores was placed in a 5-ml freeze-drying ampoule and dried for 2 hr at approximately 15-20  $\mu$ m of Hg.

For isozyme analysis, 50 mg of aeciospores was ground for 30 s in 0.5 ml Tris-MCl buffer solution (pH 6.8) with a Brinkmann homogenizer. Insoluble

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particles were sedimented on a high-speed microcentrifuge for 15 min at 15,000 g. The supernatants were collected, and the protein content was measured by the Bio-Rad protein assay method. The concentration of each sample was adjusted to 60 µg of protein per 50 µl of buffer. Glycerol (20%, v/v) and a trace of bromophenol blue were added, and 50 µl of the sample was subjected to electrophoresis on 7.5% polyacrylamide slab gels at constant voltage of 200 V until the tracking dye had traveled to within 0.5 cm of the bottom of the gel. The discontinuous buffer system was prepared according to Laemmli (5). The gels were then stained for esterase and GPI as recommended by Shaw and Prasad (10) and Siciliano and Shaw (11). Three different runs of the aeciospores were made, each providing identical results.

## RESULTS AND DISCUSSION

With staining for GPI (Fig. 1), the banding produced from homogenized aeciospores from Virginia pine (lanes 2 and 3) was identical with that produced from spores from sand pine (lanes 4 and 5). The bands for all other forms of *C. quercuum* (lanes 1 and 6-15) are quite different from these. With staining for esterase, (Fig. 2), patterns for aeciospores from Virginia and sand pine were again identical (lanes 2-5). This banding also differed from that of all other special forms of *C. quercuum*. The banding in lanes 7-15 produced by aeciospores of various isolates of *C. q. fusiforme* indicates that there is some variability within this formae speciales (Fig. 2). These *fusiforme* isolates came from several geographic areas, and some of the isolates were derived from fusoid and some from globose galls. However, we have not been

able to consistently distinguish between morphological types using isozyme patterns.

The banding produced by aeciospores of *C. q. virginianae* in the three tests we conducted and the five tests reported in a previous paper (8) is remarkably consistent. Of all the formae speciales of *C. quercuum* tested, *C. q. virginianae* is the most uniform. GPI zymograms of aeciospores of *C. q. virginianae* and of the sand pine rust fungus were identical, as were esterase zymograms of aeciospores from Virginia and sand pine. Thus, zymogram analysis supports classifying the rust occurring on each pine as *C. q. virginianae*. The additional information provided by inoculation tests and morphological studies of the basidiospores of various formae speciales of *C. quercuum* also strongly suggest the synonymy of the gall rust fungi on sand and Virginia pine.

Although the natural ranges of Virginia pine and sand pine are separated by more than 300 km, the same rust is apparently found on each species. There are several possible explanations for this anomaly: 1) the rust evolved when the two pines occupied the same geographic range, 2) the rust evolved separately, but in parallel on the two pine species, or 3) the rust has recently been introduced from one host to the other. Currently available evidence suggests that the first of these explanations is the best one. Based on studies of fossil pollen, Watts (12) concluded that a pine species similar to sand and Virginia pine occupied much of Georgia and northern Florida during the last glacial period. It seems likely that *C. quercuum* developed on these trees and then migrated with the trees when they became separated into their present populations.

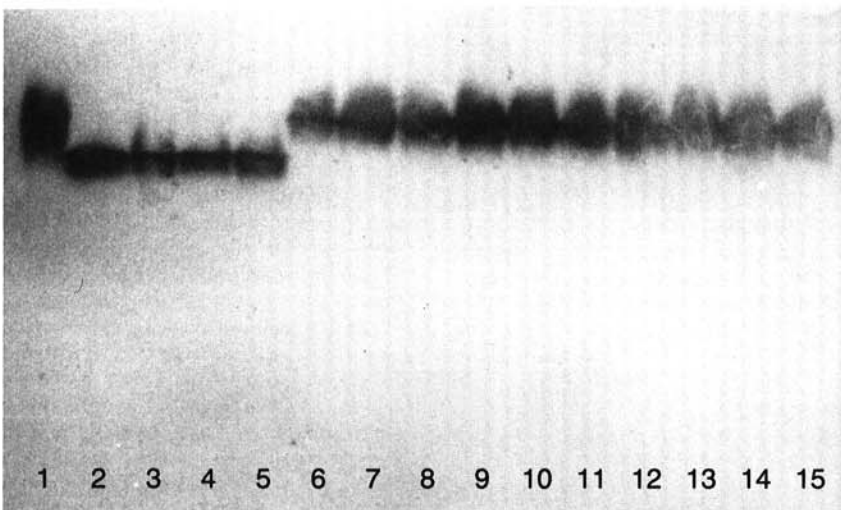


Fig. 1. Glucose phosphate isomerase patterns of aeciospores of *Cronartium quercuum* f. sp. *banksianae* (lane 1), *C. q. virginianae* (lanes 2 and 3), the sand pine rust (lanes 4 and 5), *C. q. echinatae* (lane 6), and nine isolates of *C. q. fusiforme* (lanes 7-15).

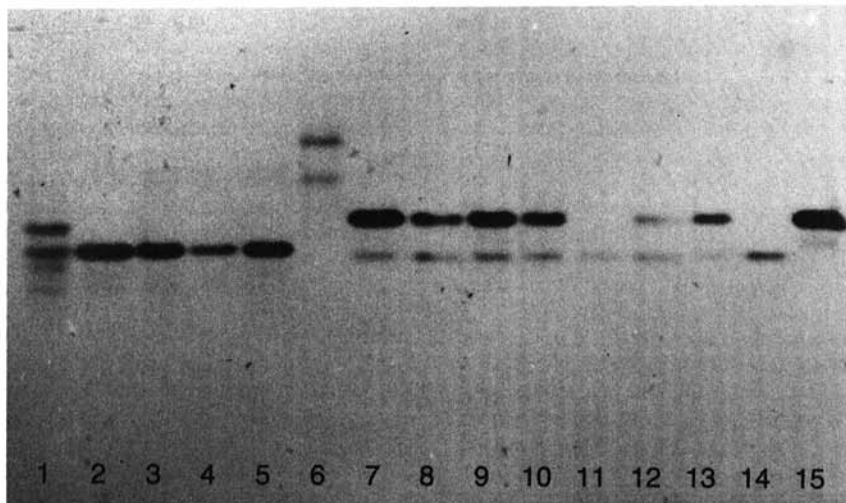


Fig. 2. Esterase patterns of aeciospores from *Cronartium quercuum* f. sp. *banksianae* (lane 1), *C. g. virginianae* (lanes 2 and 3), the sand pine rust (lanes 4 and 5), *C. q. echinatae* (lane 6), and nine isolates of *C. q. fusiforme* (lanes 7-15).

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