

# Pathogenic Races of *Gymnosporangium juniperi-virginianae* on Apple

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## ABSTRACT

Fourteen apple cultivars were inoculated under controlled conditions with standardized suspensions of basidiospores of *Gymnosporangium juniperi-virginianae*, collected in ten areas of the U.S. and in Ontario, Canada. Five pathogenic races were distinguished by cultivars on which aecia developed. Race 1 (Indiana and Michigan) was most restricted in host range, and incited aecia on only three test cultivars. Race 2 incited aecia on four cultivars, and Race 3

(Alabama and Arkansas) on six cultivars. Race 4 (New Jersey, New York, Maine, Iowa, and Kentucky) incited aecia on seven cultivars. Race 5 (Ontario) incited aecia on eight cultivars, the widest host range of any race. Within races, individual collections differed in production of pycnia and nonsporulating flecks on certain cultivars.

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Cedar apple rust, a major disease of apple in eastern North America (3), is incited by *Gymnosporangium juniperi-virginianae* Schw., a demicyclic rust with red cedar (*Juniperus virginiana* L.) and apple (*Malus sylvestris* L.) as alternate hosts. Basidiospores from telial galls on red cedar infect apple leaves and fruits. Pycnia develop on infected apple leaves and, probably after spermatization (10), aecia develop. Aeciospores infect red cedar, initiating the formation of telial galls, and thus completing the life cycle of the fungus.

Knowledge of the variation in pathogenicity of the fungus is a necessary preliminary to the development of rust-resistant apple cultivars. There are several reports of pathogenic races of *G. juniperi-virginianae* (4, 5, 9). However, the races were differentiated on symptoms resulting from inoculations with unknown

concentrations of viable basidiospores. Aldwinckle (2) demonstrated that inoculum concentration can account for qualitative as well as quantitative differences in lesions. Low concentrations of basidiospores resulted in a few pycnia, but no aecia, on a cultivar on which, with higher basidiospore concentrations, abundant pycnia and aecia were produced. Shay (12) concluded that there were 'no confirmed cases of physiologic race specialization on the apple host'.

In this paper, five pathogenic races of *G. juniperi-virginianae* are differentiated by the inoculation of 14 apple cultivars with standardized inocula under controlled conditions.

**MATERIALS AND METHODS.**—*Differential apple cultivars.*—Fourteen apple cultivars (Arkansas Black, Ben Davis, Cortland, Delicious, Empire, Golden

Delicious, Jonathan, McIntosh, Prima, Rome Beauty, Tolman Sweet, Turley, York Imperial, and Yellow Newtown) were selected for a range of reactions to *G. juniperi-virginianae* based on earlier experiments (2) or field observations (1). The cultivars on which Bliss (4) and McNew (9) based their differentiation of races were included.

Dormant apple scions were bench-grafted on seedling rootstocks and stored at 2 C until required. They were potted in a loam:sand:peat mix (1:1:1, v/v) in 13-cm plastic pots, grown in a greenhouse at 23 C, and trained to single shoots, which were inoculated when they were approximately 30 cm long.

*Collections of G. juniperi-virginianae.*—Fresh telial galls of *G. juniperi-virginianae* were obtained from Alabama (P. A. Backman, Auburn), Arkansas (R. C. Rom, Fayetteville), Indiana (E. B. Williams, Lafayette), Iowa (Lois Tiffany and J. H. Hill, Ames), Kentucky (W. D. Armstrong, Princeton), Maine (C. Granger and D. Stark, Augusta), Michigan (A. L. Jones, East Lansing), Missouri (D. F. Millikan, Columbia), New Jersey (R. White-Stevens, New Brunswick), New York (collections at Stone Ridge in eastern N. Y. and at Dresden in western N. Y.), and Ontario, Canada (J. Northover, Vineland Station). The galls were collected when the telial horns had been at least partially extruded. Galls were frozen on receipt.

*Production of inoculum.*—The galls were thawed at room temperature, soaked in tap water for 30 minutes,

and placed in a chamber at 18.5 C and 100% relative humidity (RH) for 15 hours to induce teliospore germination. Basidiospores were washed from the telia with a fine spray of water from a modified DeVilbiss atomizer and the concentration adjusted by dilution to 267,000 basidiospores per ml with a hemacytometer. This concentration was adequate to eliminate influence of low inoculum level on symptom type (2).

*Inoculation.*—Freshly prepared inoculum suspension was sprayed on the six youngest leaves on the terminal of each plant (2) to produce a uniform coating of droplets about 2 mm in diameter. Glass slides coated with 'Parlodion' (Mallinckrodt Chemicals, St. Louis, Missouri) were also sprayed with the inoculum. Plants and slides were placed in an illuminated chamber at 18.5 C and 100% RH for 48 hours. The plants were then returned to the greenhouse (23 C) and the percentage germination of the basidiospores on the glass slides was determined microscopically.

*Symptomatology.*—Symptoms began to appear on leaves approximately 10 days after inoculation. In a fully compatible host-parasite interaction, disease development progressed through three stages: (i) invasion and hyphal proliferation resulting in a nonsporulating lesion or 'fleck' (N), (ii) formation of pycnia (P) within the lesions, and (iii) formation of acacia (A). In less-compatible interactions, no macroscopic infection (0) or only the first or second of these stages appeared. The presence of pycnial lesions and flecks was recorded at 3

TABLE 1. Symptoms incited by 12 collections of *Gymnosporangium juniperi-virginianae* on leaves of 14 apple cultivars

Cultivar	Collection location (Canadian province or American states) <sup>a</sup>											
	Ontario (Canada)	NJ	NY (east)	NY (west)	ME	IA	KY	AL	AR	MO	IN	MI
Rome Beauty	A <sup>b</sup>	A	A	A	A	A	A	A	A	A	A	A
Prima	A	A	A	A	A	A	A	A	A	A	A	A
Jonathan	A	A	A	A	A	A	A	A	A	A	A	A
Ben Davis	A	A	A	A	A	A	—	A	A	A	P	P
Yellow Newtown	A	A	A	A	A	P	A	A	A	P	P	P
York Imperial	A	A	A	A	A	A	A	A	A	P	P	N
Tolman Sweet	A	A	A	A	A	A	A	P	P	P	P	N
Turley	A	P	P	P	P	P	0	P	P	N	N	N
Cortland	N	P	P	P	P	P	N	P	P	N	N	N
Golden Delicious	—	P	P	P	P	P	N	N	N	N	N	N
Empire	P	P	P	P	P	N	N	N	N	0	0	N
Arkansas Black	0	P	P	P	N	N	N	N	N	N	N	N
Delicious	N	P	P	N	N	N	—	0	N	0	N	N
McIntosh	0	P	N	N	N	N	N	N	N	N	N	N

<sup>a</sup>Abbreviations: NJ (New Jersey), NY (New York), ME (Maine), IA (Iowa), KY (Kentucky), AL (Alabama), AR (Arkansas), MO (Missouri), IN (Indiana), and MI (Michigan).

<sup>b</sup>A = acacia, P = pycnia, N = nonsporulating lesion ('fleck'), 0 = no macroscopic symptom, — = missing.

TABLE 2. Aecial production in differential apple cultivars incited by five races of the cedar apple rust fungus, *Gymnosporangium juniperi-virginianae*

Apple cultivars	Races				
	1	2	3	4	5
Rome Beauty, Prima, Jonathan	+ <sup>a</sup>	+	+	+	+
Ben Davis	-	+	+	+	+
Yellow Newtown, York Imperial	-	-	+	+	+
Tolman Sweet	-	-	-	+	+
Turley	-	-	-	-	+
Cortland, Golden Delicious, Empire, Arkansas Black, Delicious, McIntosh	-	-	-	-	-

<sup>a</sup>Symbols: + = aecia present; - = no aecia.

TABLE 3. *Gymnosporangium juniperi-virginianae* races identified in collections of telial galls from apple trees in different American states and one Canadian province

State	Race
Indiana	1
Michigan	1
Missouri	2
Alabama	3
Arkansas	3
New Jersey	4
New York <sup>a</sup>	4
Maine	4
Iowa <sup>b</sup>	4
Kentucky	4
Ontario, Canada	5

<sup>a</sup>Collections from eastern and western New York State both contained Race 4.

<sup>b</sup>Inocula from the Iowa collection did not incite aecia on cultivar Yellow Newtown, but were designated as Race 4 since they did incite aecia on cultivars Tolman Sweet and York Imperial.

weeks, and of aecial lesions at 10 weeks after inoculation. Interactions were scored as N, P, A, or 0 according to the most advanced stage observed. Leaves with advanced stages also showed the earlier symptoms.

RESULTS.—In three experiments, three trees of each of the 14 differential apple cultivars were inoculated with basidiospores from 12 collections of *G. juniperi-virginianae*. Basidiospores of all collections had high germination rates. The most advanced stage of infection

for each cultivar-collection combination was determined (Table 1). The rust collections were divided into five races on the basis of cultivars on which they incited aecia (Table 2). The collection with the widest host range (Race 5), from Ontario, Canada, incited aecia on eight cultivars: Rome Beauty, Prima, Jonathan, Ben Davis, Yellow Newtown, York Imperial, Tolman Sweet, and Turley. Collections with the most restricted host range (Race 1) were from Indiana and Michigan, and incited aecia only on Rome Beauty, Prima, and Jonathan. The other collections (Races 2, 3, and 4) were intermediate. The Iowa collection incited aecia on York Imperial and Tolman Sweet, but only pycnia and flecks on Yellow Newtown. Nevertheless, it was designated Race 4 with the other collections that incited aecia on all three of these cultivars. No collection incited aecia on Cortland, Golden Delicious, Empire, Arkansas Black, Delicious, or McIntosh.

Some collections designated as the same race were distinguishable by cultivars on which they incited pycnia, but not aecia (Table 1), but this criterion was not used to subdivide races.

DISCUSSION.—The initial evidence of the existence of pathogenic races of *G. juniperi-virginianae* was the observation of varying susceptibility of the same apple cultivars in different geographical regions. Miller (11) pointed out that much of this apparent diversity was probably due to misidentification of quince rust (caused by *G. clavipes*) or hawthorn rust (caused by *G. globosum*) as cedar apple rust. However, Bliss (4) and McNew (9) were able to demonstrate a few apparent differential reactions on some apple cultivars. Crowell (5) reported 'biological strains' of *G. juniperi-virginianae* from eight states. None of these reports (4, 5, 9) specified whether the concentration of viable basidiospores in the inoculum was standardized, and differential inoculum levels could have accounted for some of the differential reactions. Low inoculum levels cause fewer rust lesions on leaves and, if inoculum level is sufficiently low, a cultivar with a capacity for producing few aecia per lesion may fail to produce any aecia at all (2). If *G. juniperi-virginianae* is heterothallic (10) and segregates 1:1 for compatibility, at low inoculum levels perhaps only one sexual strain would be present rendering impossible the dikaryotization necessary for aecia formation. This effect could explain reports of pathogenic races based on the absence of certain types of lesions on particular cultivars (4, 5, 9).

McNew (9) identified collections from Harrison County, Iowa, and Morgantown, West Virginia, as 'possible parasitic races' on the basis of inciting aecia on Tolman Sweet and York Imperial. Seven of our collections incited aecia on these cultivars, including one from Iowa. A collection of galls from West Virginia (which is not listed in Table 1 since it only produced inoculum of 0.8 germinable basidiospores per  $\mu$ liter) incited aecia on Rome Beauty, Prima, Jonathan, and Yellow Newtown and pycnia on Ben Davis, York Imperial, Tolman Sweet, and Cortland. If this collection was Race 2, the low inoculum concentration could have prevented aecial formation on Ben Davis, York Imperial, and Tolman Sweet.

The concept of a race as employed here is somewhat different from that used for other rusts. The host of interest, the apple, can only be infected by basidiospores,

which segregate for genetical factors. Furthermore, since there is no uredial stage, isolates of constant genotype cannot be maintained on the primary host, the red cedar. Although there has been substantial progress in artificially culturing *G. juniperi-virginianae* (6, 7, 8), techniques are still too unreliable and time-consuming for practical use. Thus, races differing in pathogenicity to apple cultivars must be based on mixtures of segregating basidiospores. A race here comprises the sum of the pathogenicities of the segregates in the population. These may include segregates with wide, narrow, and complementary host ranges. That a collection from a particular state was characterized as a race with a wide host range (Table 3) does not exclude the possibility that segregates with narrower host range were also present. They would be masked by segregates with wider or complementary host ranges.

Almost all the better-known cultivars reacted to the races that had wider host range with symptoms at least as susceptible as have been reported in the field (1). The exception was Golden Delicious, which is regarded as very susceptible in northeast North America, but less susceptible in other regions (1). Aecia did not develop after inoculation with any race in this study. However, in a later test in which New York galls were used to evaluate the susceptibility of over 100 cultivars, aecia developed on five Golden Delicious plants (H. S. Aldwinckle, unpublished). Otherwise, the cultivar reaction fitted Race 4. These data suggest a sixth race.

The existence of races of *G. juniperi-virginianae* requires apple breeders wishing to incorporate useful resistance to include the more pathogenic races in their inoculum.

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