Reactions of Argentine and Australian Sunflower Rust Differentials to Four North American Cultures of *Puccinia helianthi* from North Dakota

SHAW-MING YANG, Research Plant Pathologist, USDA-ARS, Conservation and Production Research Laboratory, Bushland, TX 79012; E. F. ANTONELLI, INTA Castelar, Buenos Aires, Argentina; and A. LUCIANO and N. D. LUCIANI, Gualeguay, 446, 3100 Parana, Entre Rios, Argentina

ABSTRACT

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Four North American (NA) cultures (1, 2, 3, and 4) of *Puccinia helianthi* were tested on 12 Argentine (six from Antonelli [A] and six from Luciano and Luciani [LL] and three Australian (from Kochman and Goulter [KG]) rust differential cultivars and lines of sunflower (*Helianthus annuus*). Impira INTA NAL (LL) and Guayacan INTA NAL (LL) were susceptible to all four NA rust cultures; Morden 307-1 (A,LL) and 29-3-1-3-21 (KG) were susceptible to NA cultures 2 and 4; Guayacan INTA sel. Castelar (A) and F164A (LL) were susceptible to NA cultures 3 and 4; Pergamino 71/538 (A) was susceptible to culture 2 but resistant to 4; Impira INTA sel. 11 Castelar was susceptible to culture 3 but resistant to culture 4; F164A × Morden 307-1 (LL), 953-88-3-1-54 (KG), and 953-102-1-1-41 (KG) were susceptible to culture 4. INTA sel. 5 Castelar (A), Pergamino 78/287 (A), Saenz Peña 74-1-2 (A), and P386 (LL) were resistant to the four NA cultures. We hypothesize that the genes that condition rust resistance in Antonelli's Impira INTA sel. 5 Castelar, Impira INTA sel. 11 Castelar, Pergamino 71/538, Pergamino 78/287, Saenz Peña 74-1-2, and Luciano and Luciani's P94A and P386 are different from the genes R_1 and R_2 that were found in CM 90RR (or Guayacan INTA sel. Castelar or F164A) and CM 29-3 (or 29-3-1-3-21 or Morden 307-1), respectively. We propose using the term "cultures" instead of "races" in studies on the genetic relations of sunflower rust systems. A list of sunflower rust differentials is suggested for documenting the corresponding gene pairs in the sunflower rust systems and pathogenicity formulas are proposed for describing the cultures of *P. helianthi*.

Rust of sunflower (*Helianthus annuus* L.) caused by *Puccinia helianthi* Schw. is one of the most destructive diseases of sunflower in the world. *P. helianthi* is composed of races (cultures) of varying pathogenic characters (12,18). Four North American (NA) races (1, 2, 3, and 4) of *P. helianthi* have been identified on three standard Canadian sunflower rust differential lines (12). Kochman and Goulter (8) used six derivatives of the three standard Canadian sunflower rust differential lines to identify two Australian races (1 and 3). Luciano and Luciani (*unpublished*) have identified four races (1, 2, 3, and 4) of *P. helianthi* on seven sunflower rust differentials (Table 1) developed in Argentina (except Morden 307-1). Antonelli has identified 10 races of *P. helianthi* in Argentina on 10 sunflower rust differentials (1; E. F. Antonelli, *unpublished*) that were different from those of Luciano and Luciani. The relationship between Luciano and Luciani's races and Antonelli's races is unknown. The susceptibility of sunflower rust differential cultivars and lines of Antonelli, Kochman and Goulter, and Luciano and Luciani to the four North American (NA) cultures of *P. helianthi* had not been determined before this study.

The current system of naming races of *P. helianthi* is not helpful to sunflower breeders selecting appropriate parental material to breed for resistance or to sunflower pathologists looking for relationships between new and old races (13). Recently, three systems of naming races of *P. helianthi* have been proposed (9,13; E. F. Antonelli, *unpublished*) at the 11th International Sunflower Conference held at Mar del Plata, Buenos Aires, Argentina, in March 1985.

Sackston et al (13) proposed using avirulence/virulence formulas based on known effective/ineffective genes to designate races of *P. helianthi* on sunflower. This system cannot show that there is a "gene for virulence" and a "gene for susceptiblity" (L. E. Browder, *personal communication*). This system of naming races can be hypothetical until the resistance genes in the differentials are identified.

Kochman and Goulter (9) also

Table 1. Reactions of sunflower differentials to four North American cultures of *Puccinia helianthi*[a]

Differentials	Culture 1	Culture 2	Culture 3	Culture 4
Canadian (NDSF)[b]				
S 37-388	3,4	3,4	3,4	3,4
CM 29-3	0	3,4	0,1	3,4
CM 90RR	0	0,1	3,4	3,4
Argentine (Antonelli)				
Guayacan INTA sel. Castelar	0,1	0,1	3,4	3,4
Impira INTA sel. 5 Castelar	0	1,2	0,1	0,1
Impira INTA sel. 11 Castelar	0	1,2	3,4	1,0
Morden 307-1[c]	0	3,4	1,2	3,4
Pergamino 71/538	0	3,4	0,1	1,2
Pergamino 78/287	0	0	0	0
Saenz Peña 74-1-2	0	0	0	0,1
Argentine (Luciano and Luciani)				
Guayacan INTA NAL	3,4	3,4	3,4	3,4
Impira INTA sel. NAL	3,4	3,4	3,4	3,4
F164A	0	0,1	3,4	3,4
Morden 307-1	0	3,4	0,2	3,4
F164A × Morden 307-1	0	0,1	0,2	3,4
P94A	0	0	0,1,2	2,3
P386	0	0,1	0,1	0,1
Australian (Kochman and Goulter)				
29-3-1-3-21	0	3,4	1,2	3,4
953-88-3-1-54	0	1,2	1,2	3,4
953-102-1-1-41	0	1,2	1,2	3,4

[a] 0 = immune, no infection; 1 = very resistant, hypersensitive reaction, pustules less than 0.2 mm in the broadest part; 2 = resistant, pustules 0.2–0.4 mm; 3 = susceptible, pustules 0.4–0.6 mm; and 4 = very susceptible, pustules larger than 0.6 mm.
[b] North Dakota Seed Foundation, Fargo.
[c] Developed in Canada.

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proposed numbering the sunflower rust differentials according to single resistance genes (such as R_1, R_2, or R_1R_2) that the differential contains and to identify races (cultures) of *P. helianthi* numerically, depending on their virulence on each differential. They also proposed changing the four races, 1, 2, 3, and 4, which were identified on the Canadian sunflower rust differential lines, to races 0 (virulent on differentials with neither gene R_1 nor R_2), 2 (virulent on differentials with gene R_2), 1 (virulent on differentials with gene R_1), and 1,2 (virulent on differentials with genes R_1 and R_2 or R_1R_2), respectively.

Antonelli (*unpublished*) proposed using Habgood's (6) binary and decanary notations to name races of *P. helianthi*. He also proposed designating races by a world number followed by a local number with a letter prefix to indicate region or country. For example, Antonelli's race 827 (to which South American differentials 1, 3, and 4 are susceptible [1]) would be designated race 13-SA 827 (13 [designation of race] derived from binary values $2^0 + 2^2 + 2^3 = 1 + 4 + 8$, respective decanary values = 13, SA = South American origin of the race, and 827 = Antonelli's race 827). The advantage of this sytem is that the differentials can be used before the genetic basis of their resistance has been established (3), but the disadvantages are the difficulty in going from binary to decanary or back and the need to retain differentials in series even when they have become useless (3).

It is apparent that the aforementioned three systems of naming cultures of *P. helianthi* have shortcomings and are inadequate. The method of naming races in taxonomic approaches obscures the genetic relationship and is also indirect (2). Therefore, we use "cultures" instead of "races" in this manuscript, and we suggest using "cultures" in future studies of the genetic relationships between isolates of *P. helianthi* and sunflower.

To relate the cultures of *P. helianthi* to one another, the differentials used for inoculation, the method of inoculation, and environmental conditions at the time of inoculation and during incubation should be standardized. Standard methods of inoculation and environmental conditions for inoculation and incubation of the inoculated sunflower plants have been suggested recently (9), but standard differentials have not yet been proposed.

The objectives of this study were 1) to determine the reactions of Argentine and Australian sunflower rust differentials to the four NA cultures of *P. helianthi*, 2) to prepare a list of sunflower cultivars and lines for use as international standard differentials to identify and compare cultures of *P. helianthi*, and 3) to propose a method that would describe the pathogenic potential of cultures of *P. helianthi*. Results of preliminary study on the reactions of seven Argentine sunflower rust differentials to the four NA cultures have been published (16).

MATERIALS AND METHODS

Seeds of the sunflower rust differential cultivars and lines in Table 1 were supplied by E. F. Antonelli, J. Kochman (Department of Primary Industries, Toowoomba, 4350, Queensland, Australia), A. Luciano and N. Luciani, and H. L. Shands (Dekalb-Pfizer Genetics, Rt. 2, Box 8-AA, Glydon, MN). Seeds of the three Canadian differentials were provided by the North Dakota Seed Foundation, North Dakota State University, Fargo. Seeds of these cultivars and lines, except Antonelli's Guayacan INTA sel. Castelar and Luciano and Luciani's differentials, were increased by selfing in the greenhouse and also in the sunflower nurseries at Bushland, TX.

Urediospores from a single uredium of the four NA cultures (1, 2, 3, and 4) of *P. helianthi* from the USDA collection (T. Gulya, Fargo, ND) were increased on the Canadian differential lines (Universal suscept S 37-388 for culture 1, CM 29-3 for cultures 2 and 4, and CM 90RR for cultures 3 and 4) at Bushland, TX, in a growth chamber (21 ± 1 C day, 17 ± 1 C night, 12 hr light, and 65 ± 10% relative humidity). All four NA cultures showed a uniform infection type on Canadian sunflower rust differential lines. For example, NA culture 2 produced large, abundant pustules on CM 29-3 and S 37-388 but hypersensitive flecks or very few, small pustules on CM 90RR. NA culture 3 produced large pustules only on CM 90RR and S 37-388. NA culture 1 produced large pustules only on S 37-388, but NA culture 4 produced large pustules on all three lines. The urediospores produced on these lines were used to inoculate the Argentine and Australian differential sunflower cultivars and lines (Table 1).

Seedlings (four per 10-cm pot) of each differential cultivar and line at growth stages VE (first leaf of seedling beyond the cotyledons less than 4 cm long) to V2 (first pair of true leaves at least 4 cm long and second pair of true leaves less than 4 cm long) (14) were inoculated with each culture of *P. helianthi*. The cotton plug method, as described in previous papers (7,17), was used to inoculate the differential lines and also to increase the inoculum in the growth chamber. Before inoculation, seedlings of the differentials were atomized with sterile, demineralized water. Small plugs of sterile, wet, absorbent cotton were directly touched to the pustules on the leaves, and cotyledons of artificially inoculated seedlings were then pressed lightly onto the upper (adaxial) surfaces of true leaves (one cotton plug per leaf) of sunflower rust differentials. After inoculation, the seedlings in each pot were enclosed in a plastic bag and incubated in a growth chamber for 18–22 hr in darkness. The inoculated seedlings were then removed from the plastic bags and incubated in the same growth chamber for an additional 11–13 days. The three Canadian sunflower rust differential lines inoculated with the four NA cultures served as controls in each test. Inoculation tests were repeated three times.

Sackston's modified numerical rating system, as described in previous papers (12,17), was used to record the reaction types: 0 = immune, no infection, no pustules (uredia), no hypersensitive flecks nor lesions produced; 1 = very resistant, presence of hypersensitive flecks or lesions, or very small pustules, the broadest part smaller than 0.2 mm, with or without chlorotic halo; 2 = resistant, small pustules, the broadest part smaller than 0.4 mm but larger than 0.2 mm, with or without chlorotic halo; 3 = susceptible, pustules larger than 0.4 mm but smaller than 0.6 mm, with or without chlorotic halo, separate or coalesced; and 4 = very susceptible, pustules larger than 0.6 mm and with many urediospores, with or without chlorotic halo, some pustules coalesced to form larger pustules. A transparency chart showing three actual sizes of pustules, as shown previously (17), was used to characterize the pustules formed on the inoculated leaves of the differentials.

RESULTS

Virulence and purity of the four NA cultures of *P. helianthi* were confirmed on the three standard Canadian and seven Luciano and Luciani's sunflower rust differentials. Reactions of Argentine and Australian sunflower differentials to the four NA cultures of *P. helianthi* are shown in Table 1. The NA culture 4 produced pustules in various sizes (0.3–0.5 mm at the broadest part) on P94A. Antonelli's differentials, Impira INTA sel. 5 Castelar, Saenz Peña 74-1-2, and Pergamino 78/287, and Luciano and Luciani's P386 were resistant to all four NA cultures of *P. helianthi*.

DISCUSSION

In the following discussion, it is assumed that the responses of the selected differential cultivars and lines to sunflower rust cultures are the same wherever tested. As far as we know, the rust cultures from North America, Argentina, and Australia have never been compared on these differentials at the same location, under the same conditions, and at the same time.

The three NA cultures (1, 2, and 3) of *P. helianthi* were similar to Luciano and Luciani's cultures 1, 2, and 3, respectively, from Argentina; however, NA culture 4 was different from Luciano and Luciani's culture 4 because P94A was resistant to Luciano and Luciani's culture 4 (A. Luciano and N. D. Luciani, *unpublished*) and was susceptible to NA culture 4 (Table 1).

NA culture 4 was virulent only on Guayacan INTA sel. Castelar of Antonelli's differentials and Morden 307-1 (Table 1). Antonelli's culture 793 was also virulent only on these two differentials (1) but was different from NA 4 and resembled Luciano and Luciani's culture 4 in being avirulent on P94A (E. F. Antonelli, *unpublished*).

Both NA culture 2 and Antonelli's culture 340 (1) were virulent on Morden 307-1 and avirulent on Guayacan INTA sel. Castelar. Pergamino 71/538 was susceptible and Impira INTA sel. 11 Castelar was resistant to NA culture 2, but the former was resistant and the latter was susceptible to Antonelli's culture 340 (1). NA cultures 1 and 3 were similar to the behavior reported for Antonelli's cultures 115 and 805, respectively (1).

NA cultures 1 and 3 gave the same reaction as Kochman and Goulter's cultures 1 and 3 from Australia, although we did not use Kochman and Goulter's S 37-388 RR or G 9-17-8-11 (both had R_1 gene) for testing the pathogenicity of NA culture 3; lines 29-3-1-3-21 (which contained the R_2 gene) and 953-88-3-1-54 and 953-102-1-1-41 (which contained both R_1 and R_2 genes) were resistant to NA culture 3. Cultures 2 and 4 have not yet been found in Australia (8).

Flor's (4) gene-for-gene hypothesis is applicable to sunflower rust (11,18). Two genes, R_1 and R_2, condition rust resistance in sunflower (11,18). By using the diagonal check (10), it is apparent that the genes that condition rust resistance in Antonelli's Impira INTA sel. 5 Castelar, Impira INTA sel. 11 Castelar, Pergamino 71/538, Pergamino 78/287, Saenz Peña 74-1-2, Luciano and Luciani's P94A and P386 are different from genes R_1 and R_2 that were found in CM 90RR and CM 29-3, respectively (11). Whether there are similar genes among these cultivars and inbred lines needs further study. Genetic analysis of some of these sunflower cultivars and lines resistant to rust is under investigation at the Bushland laboratory.

The results of our study indicate that the Argentine and Australian sunflower rust differentials are useful for determining the relationship among cultures of *P. helianthi*. Therefore, we propose that the sunflower cultivars and lines listed in Table 2 be used as international standard sunflower rust differentials to identify and compare cultures of sunflower rust in the world. We selected only those differentials that are available to researchers for studying the cultures of *P. helianthi*. The list was prepared from our study and also from the works of Antonelli (1), Kochman and Goulter (8), Luciano and Luciani (*unpublished*), Putt and Sackston (11), Sackston (12), Sackston et al (13), and Senetiner et al (15). Because two lines, B66-B100 and 71-538, of Sackston et al (13) were not available for distribution (W. Sackston, *personal communication*), the two lines were deleted from the table.

The pathogenicity formula similar to that proposed by Green (5) and adapted for sunflower by Sackston et al (13) is used to describe cultures of *P. helianthi*. Each culture would be given a number in sequence before the pathogenicity formula is determined. Along with the culture number, we suggest using letters (AF for Africa, AS for Asia, AU for Australia, CA for Central America, E for Europe, NA for North America, and SA for South America) to indicate geographical origins of the cultures.

The sunflower rust differentials in Table 2 will be used for preparing pathogenicity formulas for cultures of *P. helianthi*. A small letter is given to each sunflower rust differential listed in Table 2, and the letter instead of the resistance gene (but resistance genes may be used in the future if more resistance genes in sunflower are identified) is used to prepare the pathogenicity formulas of the cultures of *P. helianthi* because only two genes (R_1 and R_2) that conditioned rust resistance in sunflower so far have been found. The differential that has the same gene for rust resistance receives the same letter. The letters of the differential cultivars or lines on which the cultures are avirulent are listed on the left and the letters of those on which cultures are virulent are listed on the right of the diagonal. For example, the culture number and pathogenicity formula of Antonelli's culture 115 of *P. helianthi* as shown in Table 3 are SA culture 1: b,c,e,f,g,h,i,j,k,l,m,n/a, because the

Table 2. Sunflower rust differential lines proposed for international use to compare pathogenicity of cultures of *Puccinia helianthi* on sunflower

Letters assigned to differentials[w]	Sunflower rust differential cultivars and lines
a	S 37-388 NDSF, Guayacan INTA NAL, Impira INTA NAL
b[x]	CM 29-3 NDSF, 29-3-1-3-21, Morden 307-1
c[y]	CM 90RR NDSF, F164A, Guayacan INTA sel. Castelar
d[z]	F164A × Morden 307-1, 953-88-3-1-54, 953-102-1-1-41
e	Impira INTA sel. 5 Castelar
f	Impira INTA sel. 11 Castelar
g	LC 74/75-20620
h	MP557
i	P94A
j	P386
k	Pergamino 71/538
l	Pergamino 78/287
m	Saenz Peña 74-1-2
n	SPS894

[w] A small letter is given to each sunflower rust differential, and the differential that has the same gene for rust resistance receives the same letter.
[x] Sunflower has R_2 gene.
[y] Sunflower has R_1 gene.
[z] Sunflower has R_1R_2 genes.

Table 3. Cultures with pathogenicity formulas of previously described races of *Puccinia helianthi*[w] according to their reactions on differentials[x]

Culture numbers	Pathogenicity formulas[y]	Previously described races	Reference
	North American (NA) cultures		
1	b,c,d,e,f,i,j,k,l,m/a	1	12
2	c,d,e,f,i,j,l,m/a,b,k	2	12
3	b,d,e,i,j,k,l,m/a,c,f	3	12
4	e,f,j,k,l,m/a,b,c,d,i	4	12
	South American (SA) cultures[z]		
1	b,c,e*,f,g,h,i*,j*,k,l*,m*,n/a*	115	1
2	c,e*,g,h,i*,j*,k,l*,m*,n/a*,b,f	340	1
3	b,e*,g,h,i*,j*,k,l*,m*,n/a*,c,f	805	1
4	e*,f,g,h,i*,j*,k,l*,m*,n/a*,b,c	793	1
5	b,c,e*,g,h,i*,j*,k,l*,m*,n/a*,f	465	1
6	b,c,e*,g,h,i*,j*,k,l*,m*/a*,f,n	745	1
7	b,c,e*,f,i*,j*,k,l*,m*,n/a*,g,h	886	1
8	b,e*,f,g,h,i*,j*,k,l*,m*,n/a*,c	767	1
9	b,e*,g,h,i*,j*,k,l*,m*/a*,c,f,n	827	1
10	e*,f,g,h,i*,j*,l*,m*,n/a*,b,c,k	823	1

[w] Described by Sackston (12) and Antonelli (1).
[x] Differentials listed in Table 2.
[y] The letters of the differential cultivars or lines in Table 2 on which the cultures are avirulent are listed on the left and the letters of those on which cultures are virulent are listed on the right of the diagonal.
[z] Asterisks indicate from E. F. Antonelli, *(unpublished)*.

culture was avirulent on the differentials b,c,e,f,g,h,i,j,k,l,m, and n and virulent only on the differential a (1; E. F. Antonelli, *unpublished*). The culture numbers and pathogenicity formulas of the four NA cultures and Antonelli's 10 cultures are shown in Table 3. Three new cultures from Sackston et al (13) were not included because the three cultures might belong to three of Antonelli's 10 rust cultures (17), and also, two of their differentials were not listed in Table 2.

The advantages of this method of describing cultures of *P. helianthi* are 1) the pathogenic potential of each culture can be easily identified and understood, 2) the cultures can be compared with each other directly, and 3) the pathogenicity formula can be prepared even though the genotype of the differential for a particular cultivar or line is unknown.

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