Uromyces ciceris-arietini, the Cause of Chickpea Rust: New Hosts in the Trifolieae, Fabaceae

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ABSTRACT

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Uromyces ciceris-arietini has been reported on *Cicer arietinum* (chickpea) and *Medicago polyceratia.* Plants of *Medicago polymorpha* in Riverside and San Diego, CA were collected with severe rust caused by *U. ciceris-arietini*. To confirm the identification and potential new host range, a monouredinial isolate of *U. ciceris-arietini* from *M. polymorpha* was inoculated on eight accessions each of *C. arietinum* and *M. polyceratia*. All plants showed symptoms of the disease. Consequently, a range of fabaceous hosts were evaluated for their reaction to *U. ciceris-arietini*. New hosts for *U. ciceris-arietini* included 29 species of *Medicago*, specifically *M. arabica*, *M. blancheana*, *M. ciliaris*, *M. constricta*, *M. coronata*, *M. doliata*, *M. granadensis*, *M. intertexta*, *M. italica*, *M. laciniata*, *M. lanigera*, *M. lesinsii*, *M. lupulina*, *M. minima*, *M. murex*, *M. muricoleptis*, *M. orbicularis*, *M. praecox*, *M. radiata*, *M. rigidula*, *M. rotata*, *M. rugosa*, *M. sativa*, *M. sauvagei*, *M. scutellata*, *M. soleirolii*, *M. tenoreana*, *M. truncatula*, and *M. varia*, and three species of *Melilotus*, specifically *M. italicus*, *M. speciosus*, and *M. spicatus*. This isolate of *U. ciceris-arietini* produced no symptoms on plants in the 33 accessions tested in the genera *Anthyllis, Astragalus*, *Lotus*, and *Lupinus.* DNA sequences are provided to aid in the identification of this pathogen.

Uromyces ciceris-arietini Jacz. causes a widespread and economically important rust on *Cicer arietinum* L. (chickpea, garbanzo, gram) (6,9,10,12,16,19,20,25,26). In India, *U. ciceris-arietini* oversummers on *Medicago polyceratia* (L.) Trautv. (≡*Trigonella polyceratia* L.) at higher, cooler elevations during periods too hot for survival in fields of *C. arietinum* at lower altitudes (20). *Medicago polyceratia* is the only other reported host of *U. ciceris-arietini* (20,24). Plants of *Medicago polymorpha* L. with severe rust were observed in a seed-increase nursery at Riverside, CA, in 1996 and at San Diego, CA, in 2005 and 2006. This rust was subsequently identified as *U. ciceris-arietini* based on morphology of teliospores and urediniospores (6). We evaluated the reaction of a range of plant species in the Fabaceae to a monouredinial isolate of *U. ciceris-arietini* from *M. polymorpha* and report new hosts in the tribe Trifolieae. Additionally, *U. ciceris-arietini* was described and illustrated. The internal transcribed spacer region (ITS) and 5′ region of the nuclear large subunit (nLSU) of the ribosomal DNA (rDNA) were sequenced as DNA barcodes to aid in future identifications of *U. ciceris-arietini* (22).

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MATERIALS AND METHODS

Plant sources. Seeds of *Melilotus* accessions were supplied by the USDA, ARS, NCRPIS, Iowa State University Regional Plant Introduction Station, Ames, IA. All other seeds were obtained from the USDA Western Regional Plant Introduction Station, Pullman, WA. Most *Medicago* accessions were from core collections of annuals (7) and alfalfa (3,4) selected to represent broad genetic diversity. Seeds were planted in 8 cm^2 pots of Jiffy-Mix Plus (Jiffy Products of America Inc., Batavia, IL) potting mix. The pots were placed in chambers at 20 to 25°C and a 16-h photoperiod. The resulting plants, with a minimum of two true leaves, were inoculated 14 to 21 days later.

Inoculum. A monouredinial isolate of *U. ciceris-arietini* (6) designated as RM1, was the inoculum source for all tests. RM1 was isolated from a plant of *Medicago polymorpha*, accession W6 5587, collected from a seed-increase nursery at Riverside, CA, in June 1996. Isolate RM1 was subcultured and maintained on potted plants of *M. polymorpha* in a growth chamber at 25°C and a 16-h photoperiod. Representative voucher specimens of diseased accessions are deposited at the U.S. National Fungus Collections as BPI 879192–879198. Additional specimens and viable urediniospores of RM1 stored in liquid N are currently maintained by the first author.

Test procedures. Urediniospores were collected with a cyclone collector (5) (G-R Electric Manufacturing Co., Manhattan, KS). Inoculum was prepared by adding freshly collected urediniospores at a rate of 100 mg/100 ml distilled water containing two drops of Tween 20 (Sigma Chemical Co., St. Louis, MO) to reduce surface tension. The inoculum was stirred until the spores were well dispersed. Freshly prepared inoculum was sprayed onto the potted plants to the point of runoff, and immediately the plants were enclosed in plastic boxes to maintain near 100% relative humidity. The boxes were placed in darkness at 20°C in a growth chamber. Approximately 24 h later, the plants were removed from the boxes and the growth chamber settings were changed to a 16-h photoperiod at 25°C. Beginning the fifth day after inoculation, plants were examined for rust symptoms daily for 16 days. Plants of *M. polymorpha* W6 5587 were included in each test as susceptible controls. Minimum numbers of plants evaluated in each accession were 10 in the Fabaceae tribes Galegeae, Genisteae, and Loteae, and 30 in the tribes Cicereae and Trifolieae. At least 75 plants were evaluated in each of the nine accessions of *Medicago sativa* and *M. varia*. Each accession was included in at least two tests. Infection types used to evaluate rust reactions (Table 1) were similar to those designated for alfalfa rust (14). Plants with infection types 2, 3, or 4 supported ured-

iniospore production and were considered hosts. Urediniospores from plants of each host accession were examined microscopically to determine numbers of germ pores.

DNA sequencing. The ITS and nLSU rDNA regions were sequenced to provide reference sequences for *U. ciceris-arietini* as no DNA sequences for this taxon are currently available in publicly accessible databases such as GenBank. Genomic DNA was extracted from rust sori isolated from BPI 879192, a representative diseased specimen with the RM1 isolate, using the MoBio UltraClean Plant DNA extraction kit (Carlsbad, CA) with the following modifications: tubes were processed twice in a FastPrep (Bio101, Vista, CA) for 30 s at 5.0 m/s with at least 10 min room-temperature incubations between runs; 60 µl of solution P1 was added and tubes were incubated at 55°C overnight. The ITS and nLSU gene regions were amplified using primer pairs ITS5u and ITS4u (18) and Rust2inv (1) and LR6 (27), respectively, and sequenced with an ABI 3130 (Applied Biosystems, Foster City, CA) using the polymerase chain reaction (PCR) primers and these additional nLSU primers: LR3 (27) and LROR (17). Raw sequences were assembled and edited using Sequencher 4.8 (Gene Codes, Madison, WI).

RESULTS

Morphology. Morphological features of RM1 (Fig. 1) were consistent with *U. ciceris-arietini* as described by Cummins (6) and Punithalingam (19). **Spermogonia** and **aecia** lacking. **Uredinia** foliicolous and amphigenous or caulicolous; scattered to crowded; abundant; erumpent becoming pulverulent; cinnamon brown. **Urediniospores** globose, subglobose to broadly ellipsoid or irregular; 23-31 × 20-23 µm; surface echinulate; walls variable in thickness from 2 to 4 µm with predominately 6 or 7 scattered pores covered by hyaline caps, yellow to yellowish-brown to cinnamon brown. **Telia** with similar growth habit as uredinia, but fewer in number; erumpent becoming pulverulent; dark brown. **Teliospores** globose, subglobose, ellipsoid to obovoid or slightly irregular; 19-23 × 17-22 µm; surface verrucose with warts spaced 1 to 2.5 µm apart; walls with uniform thickness from 2 to 3 µm with a single apical pore, 1 to 1.5 µm thick at pore, dark brown; pedicel hyaline and broken near hilum with a portion remaining attached to the teliospore.

Host range. *U. ciceris-arietini* RM1 caused rust symptoms on plants in the tribes Cicereae and Trifolieae (Table 1). Hosts in Trifolieae included 29 species of *Medicago* and three species of *Melilotus* (Table 1). All species of *Medicago* and *Melilotus* tested produced symptoms. RM1 produced no symptoms on any plants of the 33 accessions in 14 species and subspecies tested in the genera *Anthyllis*, *Astragalus*, *Lotus*, and *Lupinus* within the tribes Galegeae, Genisteae, and Loteae (Table 1).

Pathogenesis. Small yellowish-green spots approximately 1 mm diameter appeared on leaves of *C. arietinum* on the eighth day (day 8) after inoculation. The spots enlarged, became yellow, and yielded open uredinia on day 10. On the other hosts, small pale green spots appeared on leaves on day 6, and exposed uredinia were present on day 8. Uredinia occurred on both sides of the leaves of all hosts and later developed on the petioles and stems of some hosts. Sori on *Cicer* were noticeably fewer than on most other hosts. Urediniospores examined from plants of every host accession had the diagnostic feature for this rust species, namely predominately six or seven scattered germ pores.

A wide range of reactions was expressed among and within host accessions (Table 1). All plants were severely rusted in the eight accessions of *M. polyceratia* and six accessions of *M. polymorpha*. Plants in each of the five *M. lupulina* accessions were either symptomless or severely rusted (Table1). Plants in the 13 accessions of *M. truncatula* expressed a wide range of reactions including all resistant to all severely rusted (Table 1). Only 2 to 4% of plants in four of the nine alfalfa accessions exhibited open pustules, which were small, sparse, and contained few urediniospores. *Melilotus* accessions generally expressed more resistance than did those of *Medicago* (Table 1).

DNA sequencing. A total of 1,662 base pairs from the ITS region and the 28S ribosomal RNA gene were sequenced from two fragments. The sequences were deposited in GenBank as accessions GQ914999 and GQ914998, respectively. A BLAST search of the nLSU of *U. ciceris-arietini* resulted in no exact matches, with the

Table 1. Reactions of plant accessions in the Fabaceae to monouredinial isolate RM1 of *Uromyces ciceris-arietini*

Tribe Genus, species, accession(s)[a]	Reaction(s)[b]
Cicereae	
Cicer arietinum 'Hermosillo' 502991, 'Nabin' 462191, Nokhodsiah' 251514, 'Sataya' 468932, 'Sonora 80' 468949, 'UC-5' 462203, 'Union' 503003, 'White Spanish' 462205	3Y
Galegeae	
Astragalus hamosus 516492, 535459	0
Genisteae	
Lupinus albus 244572, 457927, 469095, 516624, 615409	0
L. angustifolius 167938, 615407, 615408	0
L. cosentinii W6 17967, W6 17970	0
L. luteus 224493, 240750, 505855	0
Loteae	
Anthyllis cornicina 302835	0
A. hamosa 535573	0
A. vulneraria 516488, 516489, W6 178041	0
A. vulernaria subsp. *carpatica* 542779	0
A. vulneraria subsp. *polyphylla* 503143	0
Lotus creticus 516613, 535592	0
L. edulis 244281, 304068, 368895	0
L. glinoides 246736	0
L. halophilus 238336, 300237	0
L. palustris 311427, 308806	0
L. pedunculatus 631960	0
Trifolieae	
Medicago arabica 495200, 495212	4
M. blancheana 505415	1
M. blancheana 495223	4
M. ciliaris 498784	1,2
M. constricta 534177	3,4
M. coronata 489805	4
M. disciformis 487317	1
M. doliata 534202	4
M. granadensis 498813	3,4
M. intertexta 535607	2,3
M. italica 459188	1,2,3,4
M. laciniata 498918	3,4

(*continued on next page*)

[a] Accessions are Plant Introduction numbers unless stated otherwise.

[b] Reactions observed on plants by 21 days after inoculation: 0 = no macroscopic symptoms; 1 = chlorotic and/or necrotic flecks but no open uredinia; 2 = small, sparse, open uredinia within necrotic or chlorotic areas; 3 = uredinia erumpent within distinct chlorotic lesions; 3Y is same as 3 except the chlorosis is distinctly yellow; 4 = uredinia large, erumpent without surrounding necrosis or more than slight chlorosis. Plants with reactions 2, 3, or 4 were considered hosts.

closest match being *Uromyces viciae-fabae* isolate AFTOL-ID 986 (GQ914998.1) at 98% maximum identity (1041/1062 identical positions). A BLAST search of the ITS also resulted in no exact matches, with the closest identified match being *Uromyces trifolii-repentis* isolate R217 (EU014070.1) with 91% maximum identity (553/602 identical positions).

DISCUSSION

The known host range of *U. ciceris-arietini* was increased greatly within *Medicago* and also added *Melilotus* spp. (Table 1). These hosts include widely distributed agronomic crops and weeds indigenous to important areas of chickpea production (7,11,15,16,19,20). As suggested by our data, the known host range is confined to some members of the Vicioid clade of Fabaceae, a clade that includes tribe Trifolieae (29). In the case of *Medicago polymorpha*, *U. ciceris-arietini* has been observed as a host in both field and laboratory conditions. For the remaining new hosts, since age of the plant and a high level of inoculum in greenhouse infection experiments may overestimate host status (2), some reservation about whether or not infection would be epidemiologically significant in the field is warranted.

Although *U. ciceris-arietini* has been recognized as a distinct species by many authors, some authors have considered *U. ciceris-arietini* to be a synonym of *Uromyces anthyllidis* J. Schröt., which has been reported on numerous species of *Medicago* including *M. polymorpha* (9–11,15) and a species of *Melilotus* (10). This has led to some confusion about host ranges and rusts on *Cicer*. *U. anthyllidis* has been regarded as a collective (28) or broad species complex including numerous synonyms of *Uromyces* species and forms on various host genera within the Fabaceae (10,12,13), but Guyot (12) restricted the host range of *U. anthyllidis* sensu stricto to *Anthyllis* species. Jorstad (13) included several names as synonyms of *U. anthyllidis* because their teliospores were similar and "of the *U. anthyllidis* type." However, he also noted that the numbers of urediniospore germ pores reported for *U. anthyllidis* sensu lato varied with host (13). Examples of this variation in pore number were 3 or 4 on *Ononis laxiflora*; 4 or 5 on *C. arietinum*, *Medicago littoralis*, *M. minima*, and *M. orbicularis*; and up to 7 on *Lotus sessilifolius* and *M. truncatula* (13). Jorstad (13) identified *U. ciceris-arietini* from the uredinial state only and included it in *U. anthyllidis* sensu lato because at that time *U. anthyllidis* caused the only known rust of *C. arietinum*. However, his description of thin-walled urediniospores more closely fits *Uromyces striatus* J. Schröt. (6), another species that causes rust on *C. arietinum* (11,23).

Other authors have stressed differences between *U. ciceris-arietini* and *U. anthyllidis*. Urediniospores of *U. ciceris-arietini* can be distinguished from *U. anthyllidis* by slightly thicker walls (2 to 4 μm) with 4 to 8 (predominately 6 or 7) scattered germ pores (6,8,11,12,16,19,21,25). Urediniospores of *U. anthyllidis* sensu stricto typically have thinner walls (1.5 to 3 μm) and predominately 3 to 5 germ pores (12,21). *Uromyces striatus*, another species found on *Cicer*, is distinguished from both *U. ciceris-arietini* and *U. anthyllidis* by its striate teliospores (6,11). Sydow and Sydow (25) reported that the uredinia of *U. anthyllidis* were amphigenous; whereas most uredinia of *U. ciceris-arietini* were hypophyllous. Others reported uredinia of *U. ciceris-arietini* as hypophyllous (19) and "as a rule hypophyllous" (16). Cummins (6) noted that uredinia of *U. ciceris-arietini* on *C. arietinum* were amphigenous, as occurred on all hosts in our tests. Punithalingam (19) retained *U. ciceris-arietini* as a separate species because of a lack of evidence that the rusts on *Anthyllis* and *Cicer* could be cross inoculated. Our work supports this conclusion based on the inability of *U. ciceris-arietini* isolate RM1 to cause rust on any of the species tested of *Anthyllis*, *Astragalus*, *Lotus,* or *Lupinus* (Table 1) that are the commonly reported hosts of *U. anthyllidis* (10). Further work on the morphology of infection structures may prove to be useful in distinguishing these rust taxa (8).

U. anthyllidis was reported twice on *M. polymorpha* in southern Queensland (11,15). Goulter (11) noted that chickpea lines were susceptible to urediniospores of *U. anthyllidis* sensu lato from *M. polymorpha* plants. A description of those urediniospores was not given. However, *U. ciceris-arietini,* identified by its thick-walled urediniospores with 4 to 8 pores, was reported to be widespread on chickpea in the area (11). In a disease survey of annual pasture legumes, also in southern Queensland, during 1992 and 1993 (15), *U. anthyllidis*, identified by its thick-walled

Table 1. (*continued from previous page*)

Tribe Genus, species, accession(s)[a]	Reaction(s)[b]
Trifolieae	
M. lanigera 498930	4
M. lesinsii 534233	2,3
M. lupulina 227452, 532942	0
M. lupulina 189128, 290723, 566869	4
M. minima 499072	3,4
M. murex 534231	3
M. muricoleptis 495401	1,2
M. orbicularis 566870	2,3,4
M. polyceratia 219711, 227395, 239911 239912, 244328, 369152, 369153, 517186	4
M. polymorpha W6 5587, 186329, 577394, 577395, 577396, 577397	4
M. praecox 495434	2,3
M. radiata 340800	4
M. rigidula 233250	2,3,4
M. rotata 495570	2,3,4
M. rugosa 368962	4
M. sativa and *M. varia* (alfalfa)	
African 536539	0,1
Chilean 536534	0,1
Flemish 536538	0,1,2
Indian 536536	0,1
Ladak 5365321	0,1,2
Peruvian 536535	0,1,2
Turkistan 536537	0,1,2
M. varia 536533	0,1,2
WISFAL 560533	0,1
M. sauvagei 499153	2,3
M. scutellata 487403	1,2
M. soleirolii 537242	1,2
M. tenoreana 499157	0
M. tenoreana 499159	4
M. truncatula 197360, 243884, 384633, 384665	0
M. truncatula 190089, 197341, 292434	0,1
M. truncatula 190084	0,1,2,3,4
M. truncatula 190086, 239875, 566891	1
M. truncatula 464815	3
M. truncatula 197361	4
M. turbinata 566893	1
Melilotus alba 440548	0,1
M. dentatus 223000	1
M. elegans 260271	1
M. indicus 308524	0,1
M. italicus 317638	2,3
M. officinalis 174276, 440560	1
M. speciosus 317650, 317652	2,3
M. spicatus 317644	3

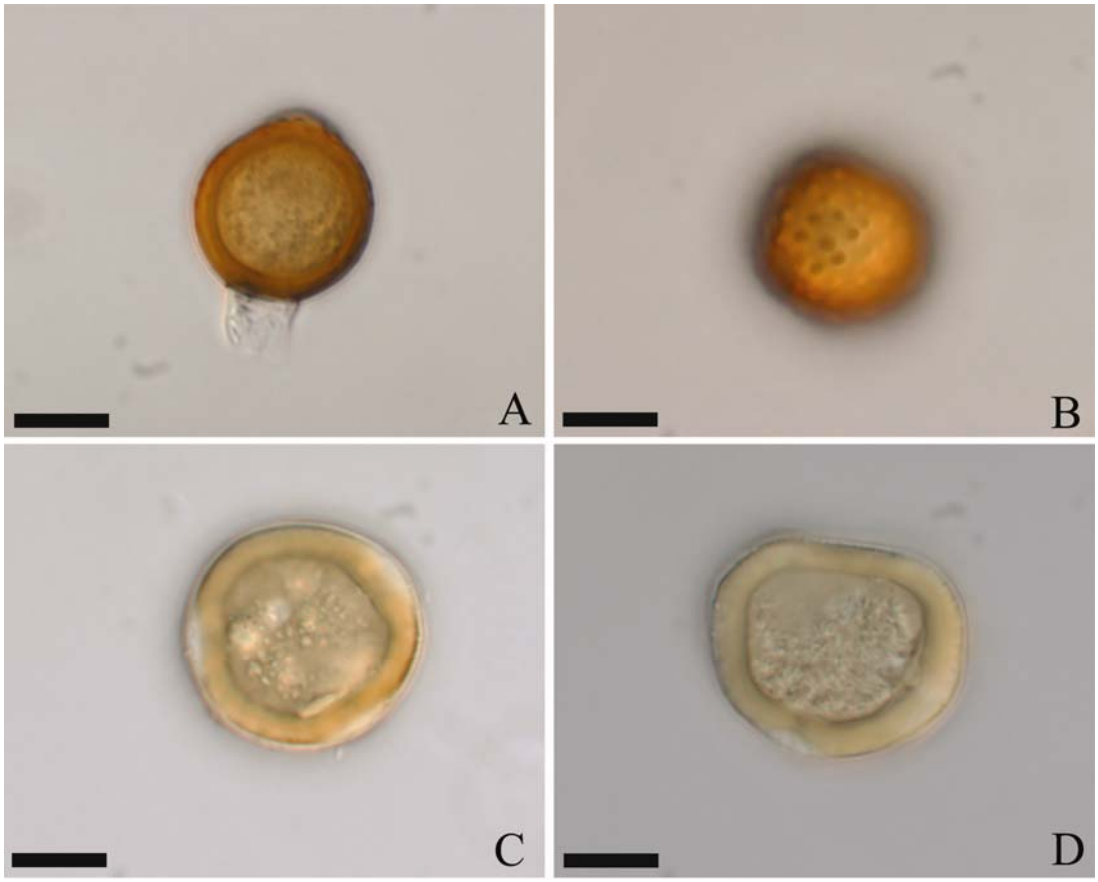

Fig. 1. *Uromyces ciceris-arietini*, monouredinial isolate RM1. **A,** Optical section of teliospore. **B,** Surface view of teliospore. **C and D,** Optical sections of urediniospores. Scale bars are 10 µm for all.

urediniospores with 5 to 8 pores, was reported as widespread on *M. polymorpha* (15). Based on this description, it would appear that the pathogen was actually *U. ciceris-arietini*. *U. anthyllidis* was also reported on *M. polymorpha* in Cameron County, TX, in June 1985 and April 1986 (9,10). However, *U. ciceris-arietini* was commonly noted on chickpea in this area in 1986 (10). Morphological examination of the specimens associated with these reports in the U.S. National Fungus Collections (Beltsville, MD) confirmed that all of the collections including those deposited as *U. anthyllidis* on *M. polymorpha* from Texas represent *U. ciceris-arietini*. No other reports of *U. anthyllidis* on Trifolieae in North America were found.

In terms of pathogenesis, it was noted in our tests that rust was less severe and the incubation period (time from inoculation until open uredinia appeared) was longer on *C. arietinum* than on all other hosts. This incubation period, however, is similar to that reported by others for *U. ciceris-arietini* on *C. arietinum* (16,20). Cummins (6) noted that *C. arietinum* plants from Mexico were so heavily infected by *U. ciceris-arietini* as to suggest an autoecious species with the primary infection occurring in the seedling stage. *U. striatus* also developed more slowly on *C. arietinum* than on other hosts (11).

In conclusion, *U. ciceris-arietini* is retained as distinct from *U. anthyllidis* sensu stricto. Based on the evidence presented here, the host range of *U. ciceris-arietini* is greater than that previously recorded and includes species of *Cicer*, *Medicago*, and *Melilotus*. Although laboratory infection experiments may overestimate host status for reasons previously discussed, our data suggest that numerous other hosts are susceptible to rust caused by this fungus. Further experiments on these other hosts will be required to determine if *U. ciceris-arietini* is a significant pathogen in the field and if infection on these hosts is potentially problematic for *Cicer* production.

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