Host Range and Phylogenetic Relationships of *Albugo candida* from Cruciferous Hosts in Western Australia, with Special Reference to *Brassica juncea*

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Abstract

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White rust, caused by *Albugo candida*, is a serious pathogen of *Brassica juncea* (Indian mustard) worldwide and poses a potential hazard to the presently developing canola-quality *B. juncea* industry in Australia. Nine isolates of *A. candida*, representing strains collected from *B. juncea*, *B. rapa*, *B. oleracea*, *B. tournefortii*, *Raphanus raphanistrum*, *R. sativa*, *Eruca vesicaria* subsp. *sativa*, *Capsella bursa-pastoris* and *Sisymbrium irio*, from different locations in Western Australia (W.A.), were tested on cruciferous host differentials to characterize their pathogenicity. In particular, these studies were aimed to determine the hazard to the newly emerging *B. juncea* industry in Australia from races or pathotypes of *A. candida* present. Pathogenicity tests with appropriate differentials demonstrated the presence in W.A. of a unique strain from *B. rapa* that did not show characteristics of either race 7A or 7V and clearly is a distinct new pathogenic strain within race 7. Different strains collected from W.A. differed in their host range, with the strains from *B. tournefortii* and *S. irio* being highly host specific, failing to be pathogenic on any other differentials. *B. tournefortii* was host to a strain attacking *B. juncea* and *E. vesicaria* subsp. *sativa*. The strain from *R. raphanistrum* showed a relatively wide host range among the differentials tested. *B. tournefortii*, *C. bursa-pastoris*, *R. raphanistrum*, and *S. irio* are common weeds within grain belt and horticultural regions in Australia. The *B. oleracea* isolate (race 9) was pathogenic to *B. juncea* 'Vulcan' whereas the isolate from *B. juncea* (race 2V) was

not pathogenic on *B. oleracea*. Similarly, the strain from *C. bursa-pastoris* (race 4) was pathogenic on *B. juncea* Vulcan but the *B. juncea* strain was not pathogenic on *C. bursa-pastoris*. In contrast, the strain from *R. sativus* (race 1) was pathogenic on *B. juncea* and the *B. juncea* strain was also pathogenic on *R. sativus*. Field isolates from *B. rapa*, *B. tournefortii*, *E. vesicaria* subsp. *sativa*, and *S. irio* were all nonpathogenic on *B. juncea*. Isolates from *B. juncea* and *R. raphanistrum* were pathogenic on *B. napus* (FAN 189). For the nine *A. candida* isolates from W.A., complete rDNA internal transcribed spacer region nucleotide sequence analysis showed a nucleotide identity range of 72.4 to 100% in comparison with previous Australian collections of *A. candida* and those previously reported in Europe and Asia. The *B. tournefortii* isolate of *A. candida* from W.A. formed a distinct clade on its own, with an identity range of 77.4 to 80.5% compared with the other isolates. Isolates from *R. raphanistrum* and *R. sativus* from W.A. were least similar to the other isolates, with a nucleotide identity similarity of only 72.4%. Characterization of the races of *A. candida* in Western Australia adds to the current knowledge regarding the diversity of this pathogen, allows choice of *Brassica* spp. or cultivars with resistance to races across different regions, and highlights the particular cruciferous weeds involved in pathogen inoculum carryover between successive cruciferous crops, particularly *B. juncea* crops.

The filamentous oomycete *Albugo candida* is considered to have descended from an aquatic nonfungal ancestor and belongs to the kingdom Straminipila (along with brown algae and diatoms; 21). Despite the separate phylogeny of oomycetes and true fungi (kingdom Eumycota), the biotroph *A. candida* causes plant disease in a manner similar to the biotrophic rust fungi, including penetration by germ tubes arising from asexual inoculum via stomata, mesophyll colonization of a compatible host tissue, and sporulation within spore-bearing pustules. Hence, the disease caused by *A. candida* has been referred to as white rust (29).

White rust is a serious disease of some types of rapeseed and mustard worldwide (51), forming white to cream-colored zoosporangial pustules on cotyledons, leaves, stems, and inflorescences, with staghead galls formed as the result of inflorescence infection (56). The combined infection of leaf and inflorescence causes yield losses up to 30 to 60% in severely affected fields in Canada

(9), 60% or more in India (31), and up to 20% in Australia (5,8). White rust is an important disease of cruciferous crops in Australia, including Western Australia (W.A.; 4–8,32–34,36). It is of particular significance to *Brassica juncea*, because canola-quality *B. juncea* is being developed in Australia to extend oilseed *Brassica* spp. production into the lower rainfall areas (11,40).

A. candida has a wide host range of some 63 genera and 241 species (10,48), including some weedy species (23). Many of the forms of *A. candida* found in the various crucifers exhibit a high level of host specificity (59). In Australia, it has been reported on 20 different plant genera, although it is generally restricted to introduced cultivated and weedy plants (41), including those that are widespread throughout the broad-acre and vegetable *Brassica* spp.-growing regions. It was first recorded in W.A. on *Raphanus raphanistrum* in 1929 (35), *B. napus* var. *napobrassica* and *B. rapa* in 1940 (13), *R. sativus* in 1944 (13), *B. oleracea* var. *botrytis* in 1965 (35), *B. napus* in 1970 (1,50), *B. tournefortii* in 1974 (2,50), and *B. rapa* var. *chinensis* in 1984 (3,50). White rust has also been observed on a few 'Norin'-type breeding lines of *B. napus* from Japan several decades ago (M. J. Barbetti, unpublished data) and, more recently, in a few *B. napus* breeding lines introduced from China (33). *B. juncea* cultivars previously reported as being resistant to *A. candida* race 2 (now designated as 2A) were subsequently found to be susceptible to a more virulent form of this race in Canada (designated as 2V; 42), including newly developed canola-quality *B. juncea* in Canada and Australia (26). *A. candida* race 2V was reported in W.A. in 2008 (30).

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Hiura (28) and Eberhardt (22) demonstrated the host specificity of *A. candida* by examining isolates of *A. candida* across a variety of different crop species. *A. candida* initially was classified into 10 races based on their specificity to different brassicaceous hosts (27,44). Recently, Minchinton et al. (37) reported 17 races of *A. candida* from various cruciferous species. Although there has been a preliminary attempt to define races in W.A. (30), the races currently prevalent on the cruciferous crop and weed species and that have potential to become a threat to the newly emerging *B. juncea* industry in the state have not been determined.

Many studies have already proven the sequence analysis of the internal transcribed spacer (ITS) region of rDNA to be a very powerful tool for the comparison of closely related species or isolates within the phylum Oomycota (14–19,41,43,54,57). However, detailed molecular systematic investigations within W.A. isolates of *A. candida* were lacking; hence, in the present study, sequence analysis of the ITS rDNA was carried out to better understand the interrelationships of this species complex from various host plants. In addition, molecular sequence and phylogeny data were evaluated in terms of their relationship to

the pathogenicity spectrum of the isolates of *A. candida* collected from a variety of different cruciferous hosts during field surveys in W.A. from 2007 to 2009.

Materials and Methods

Sample collection and inoculum preparation. Nine isolates of *A. candida* representing populations on cultivated hosts such as *B. juncea* (Indian mustard), *B. rapa* (turnip rape), *B. oleracea* var. *italica* (broccoli), *Eruca vesicaria* subsp. *sativa* (arugula, rocket-salad), and *R. sativa* (radish) and weeds such as *B. tournefortii* (wild turnip, Mediterranean turnip), *R. raphanistrum* (wild radish), *Capsella bursa-pastoris* (shepherd's purse), and *Sisymbrium irio* (London rocket) from different sites in W.A. (Table 1) were used in this study. The *A. candida*-infected leaves collected in the surveys were stored at -80°C . When inoculum was required, these leaves were removed from -80°C , zoospores were scraped from the leaves, and a zoosporangial suspension was applied on the cotyledons of growing plants using a drop inoculation technique as described in the section on pathogenicity tests. The leaves with pustules that developed on these plants were collected 10 days post

Table 1. Details of *Albugo candida* isolates obtained from surveys in Western Australia from 2007–2009 (in bold) and of the other isolates elsewhere to which molecular comparison was made^a

Number	Host species	GenBank accession number	Geographic origin	Collection year	Reference
1	<i>Brassica juncea</i>	HM587260	Wongan Hills, Western Australia	2007	This study
2	<i>B. juncea</i>	AY929826	Namyangju, Korea	1998	15
3	<i>B. juncea</i>	AY929827	Namyangju, Korea	2003	15
4	<i>B. juncea</i>	AY929828	Jeju, Korea	2004	15
5	<i>B. rapa</i>	HM587261	Perth, Western Australia	2007	This study
6	<i>B. rapa</i> var. <i>pekinensis</i>	GQ328843	Amiens, Queensland	2007	41
7	<i>B. rapa</i> var. <i>pekinensis</i>	GQ328841	Devon Meadows, Victoria	2006	41
8	<i>B. rapa</i> var. <i>pekinensis</i>	GQ328842	Ocean Grove, Victoria	2007	41
9	<i>B. oleracea</i> var. <i>italica</i>	HM587262	Wanneroo, Western Australia	2007	This study
10	<i>B. oleracea</i> var. <i>gemmifera</i>	GQ328823	Forth, Tasmania	2004	41
11	<i>B. oleracea</i> var. <i>italica</i>	GQ328848	Werribee South, Victoria	2005	41
12	<i>B. oleracea</i> var. <i>italica</i>	GQ328825	Dandenong South, Victoria	2003	41
13	<i>B. oleracea</i> var. <i>italica</i>	GQ328826	Dandenong South, Victoria	2003	41
14	<i>B. oleracea</i> var. <i>italica</i>	GQ328827	Cranbourne, Victoria	2004	41
15	<i>B. oleracea</i> var. <i>italica</i>	GQ328828	Werribee South, Victoria	2007	41
16	<i>B. oleracea</i> var. <i>italica</i>	GQ328829	Werribee South, Victoria	2006	41
17	<i>B. oleracea</i> var. <i>italica</i>	GQ328831	Munjimup, Western Australia	2007	41
18	<i>B. oleracea</i> var. <i>italica</i>	GQ328830	Forth, Tasmania	2007	41
19	<i>B. oleracea</i> var. <i>italica</i>	GQ328832	Harford, Tasmania	2007	41
20	<i>B. oleracea</i> var. <i>italica</i>	GQ328833	Lindenow, Victoria	2007	41
21	<i>B. oleracea</i> var. <i>botrytis</i>	GQ328838	Lindenow, Victoria	2007	41
22	<i>B. oleracea</i> var. <i>botrytis</i>	GQ328839	Werribee, Victoria	2007	41
23	<i>B. oleracea</i> var. <i>botrytis</i>	GQ328840	Gumeracha, South Australia	2007	41
24	<i>B. tournefortii</i>	HM587266	Bolgart, Western Australia	2008	This study
25	<i>Raphanus raphanistrum</i>	HM587267	Wongan Hills, Western Australia	2007	This study
26	<i>R. sativus</i>	HM587265	Perth, Western Australia	2008	This study
27	<i>R. sativus</i>	AY929841	Seoul, Korea	1990	15
28	<i>R. sativus</i>	AY929843	Busan, Korea	2002	15
29	<i>R. sativus</i>	GQ328844	Batemans Bay, New South Wales	2007	41
30	<i>R. sativus</i>	GQ328845	Richmond, Tasmania	2007	41
31	<i>Eruca vesicaria</i> subsp. <i>sativa</i>	HM587263	Perth, Western Australia	2008	This study
32	<i>Eruca sativa</i>	DQ418503	Daudkhel, Pakistan	1968	15
33	<i>Capsella bursa-pastoris</i>	HM587268	Wanneroo, Western Australia	2008	This study
34	<i>C. bursa-pastoris</i>	AY929829	Namyangju, Korea	1997	15
35	<i>C. bursa-pastoris</i>	AY929831	Yongin, Korea	2000	15
36	<i>C. bursa-pastoris</i>	AY929830	Seoul, Korea	1999	15
37	<i>C. bursa-pastoris</i>	AF271231	CAN1, Wales	1997	15
38	<i>C. bursa-pastoris</i>	GQ328834	Werribee South, Victoria	2006	41
39	<i>C. bursa-pastoris</i>	GQ328835	Cranbourne, Victoria	2004	41
40	<i>C. bursa-pastoris</i>	GQ328836	Werribee South, Victoria	2007	41
41	<i>C. bursa-pastoris</i>	GQ328837	Werribee, Victoria	2007	41
42	<i>Sisymbrium irio</i>	HM587264	Cunderdin, Western Australia	2008	This study
43	<i>Sisymbrium</i> sp.	GQ328822	Knoxfield, Victoria	2003	41
44	<i>S. luteum</i>	AY929844	Pyongchang, Korea	2002	15
45	<i>S. luteum</i>	AY929845	Pyongchang, Korea	2003	15
46	<i>S. loeselii</i>	AY929846	Bulgaria	...	15
47	<i>Amaranthus spinosus</i>	AY929824	Chunchon, Korea	2003	54

^a Common names of cruciferous hosts from which *A. candida* was isolated are *B. juncea* (Indian mustard), *B. rapa* (turnip rape), *B. oleracea* var. *italica* (broccoli), *B. tournefortii* (wild turnip, Mediterranean turnip), *R. raphanistrum* (wild radish), *R. sativus* (radish), *Eruca vesicaria* (arugula, rocket-salad), *C. bursa-pastoris* (Shepherd's purse), and *S. irio* (London rocket).

inoculation (dpi) in order to bulk up inoculum that was then stored at -80°C until needed.

Pathogenicity tests. In total, 16 cruciferous differentials were used to characterize the pathogenic behavior of *A. candida* (Table 1). The selection of the differentials was made on the basis of the history of the occurrence of white rust disease in W.A. (50). Seed of the 16 host differentials was sown into steam-treated potting mix (composted pine bark/cocoa peat/river sand, 2:1:1) in plastic trays with 8 cells, each cell 5 by 5 cm. Specifically, four plants were used for each genotype-isolate combination replicate and assessments were conducted separately on each cotyledon lobe (i.e., across the 16 cotyledon lobes/four plants/replicate of each treatment combination).

For this study, *A. candida*-infected leaves were removed from -80°C storage and the zoosporangia dispersed in deionized (DI) water and filtered through double cheesecloth to remove any plant debris. The concentration of the zoosporangial suspension was adjusted using a hemacytometer to 10^5 zoosporangia ml^{-1} before application to cotyledons (33). Ten days after sowing, fully expanded cotyledons were inoculated by a drop-inoculation technique where a single 10- μl drop of the zoosporangial suspension was spotted onto the adaxial surface of each of the two lobes of each cotyledon (33). Plants were then subjected to 4 days of enhanced humidity ($>98\%$ relative humidity) in a high-humidity chamber consisting of a sealed clear plastic container, where the inside of the lid was sprayed with DI water and 1 cm of free water maintained in the bottom of the container.

The experiment was carried out in a controlled environment room maintained at 18 and 13°C , day and night, respectively, with a 16-h photoperiod at a light intensity of $520 \mu\text{E m}^{-2} \text{s}^{-1}$, at the University of Western Australia, Perth, W.A. The experiment was repeated but only data from the first experiment are shown (Table 2). A complete randomized block design with four replications was used.

Disease assessments. At 12 dpi, disease severity was assessed using a 0-to-9 scoring system as used by Williams (58), where 0 = no symptoms on either leaf surface; 1 = small, pinpoint to large

brown necrotic flecks under the inoculation point on upper surface, occasional necrosis extending to lower epidermis but no sporulation; 3 = very sparse, one to a few minute, scattered pustules on upper surface, none to very few pustules on lower surface; 5 = few to many scattered pustules on upper surface but either none or a few scattered pustules on lower surface; 7 = many to a few pustules on upper surface with many scattered small to larger pustules on lower surface; and 9 = very few to no pustules on upper surface with many large coalescing pustules on lower surface.

DNA-extraction, polymerase chain reaction, and sequencing. Genomic DNA was extracted using the sporangiophores and sporangia formed on the upper or under surface of infected leaves. The presence of the sporangiophores and sporangia of white rust was confirmed under the dissecting microscope (Olympus SZ, $\times 100$ magnification), which were then scraped with a dissecting blade into 1.5-ml tubes. DNA was extracted using the method described by Cenis (12) where the material was washed first with 500 μl of TE buffer and pelleted, the supernatant was discarded, and the remaining material was ground directly in a 1.5-ml Eppendorf tube. After grinding, 300 μl of extraction buffer was added to the tube as described by Raeder and Broda (45) (viz., 200 mM Tris HCL [pH 8.5], 200 mM NaCl, 25 mM EDTA, and 0.5% sodium dodecyl sulfate). Subsequently, 150 μl of sodium acetate was added and Eppendorf tubes maintained at -20°C for 10 min. Samples were then centrifuged in a microfuge at $20,000 \times g$ for 5 min and the supernatant transferred to another tube. Then, an equal volume of isopropanol was added to the supernatant and, after 5 min at room temperature, the precipitated DNA was pelleted by centrifugation in a microfuge. After a wash with 70% ethanol, the pellet was vacuum dried for 10 min and then resuspended in 50 μl of TE.

For the selective amplification of the complete ITS region of rDNA, the oomycete-specific forward primer DC6 (20) (5-GAG-GGA-CTT-TTGGGT-AAT-CA-3) and LR-0 (reverse complementary to LR-0R) (38) (5-GCT-TAA-GTT-CAGCGG-GT-3) were used. Polymerase chain reactions (PCRs) were conducted in 40- μl reaction volumes, with each reaction tube containing 2.4 μl of tem-

Table 2. Reactions of different cruciferous host differentials to Western Australian isolates of *Albugo candida* obtained from nine different isolates collected from Western Australia in surveys in 2007 to 2009, presented with the mean disease severity (0-to-9 scale)^a

Host differential	Disease reaction for trial I, II (mean severity, 0-to-9 scale) ^a								
	<i>Brassica juncea</i>	<i>B. rapa</i>	<i>B. oleracea</i>	<i>B. tournefortii</i>	<i>Raphanus raphanistrum</i>	<i>R. sativus</i>	<i>Eruca vesicaria</i>	<i>Sisymbrium irio</i>	<i>Capsella bursa-pastoris</i>
<i>B. carinata</i> 94024.2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>B. juncea</i> 'Vulcan'	+(3.4), +(3.8)	0, 0	+(2.4), +(2.3)	0, 0	0, 0	+(1.5), +(2.6)	0, 0	0, 0	+(2.0), +(1.8)
<i>B. juncea</i> 'Commercial Brown'	+(5.7), +(5.3)	0, 0	0, 0	0, 0	+(3.0), 0	0, 0	0, 0	0, 0	0, 0
<i>B. napus</i> 'FAN 189'	+(2.0), +(1.0)	0, 0	0, 0	0, 0	+(1.4), +(1.0)	0, 0	0, 0	0, 0	0, 0
<i>B. napus</i> 'Surpass 501TT'	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>B. nigra</i> 90745	+(4.3), +(2.0)	0, 0	0, 0	0, 0	+(1.0), +(1.0)	0, 0	0, 0	0, 0	0, 0
<i>B. oleracea</i> var. <i>italica</i> 'Broccoli Sprouts'	0, 0	0, 0	+(3.9), +(2.7)	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>B. rapa</i> 'Torch'	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>B. rapa</i> 'Reward'	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>B. rapa</i> 'Shi Huang'	0, 0	+(4.3), +(3.9)	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
<i>R. raphanistrum</i> WARR25	0, 0	0, 0	0, 0	0, 0	+(1.4), +(1.3)	0, 0	0, 0	0, 0	0, 0
<i>R. sativus</i> 'White Icicle'	+(3.0), +(1.0)	0, 0	0, 0	0, 0	+(1.5), +(2.0)	+(4.1), +(3.9)	0, 0	0, 0	0, 0
<i>B. tournefortii</i> BTO2	+(1.5), +(1.5)	0, 0	0, 0	+(3.5), +(3.0)	0, 0	0, 0	+(2.0), +(1.8)	0, 0	0, 0
<i>E. vesicaria</i> subsp. <i>sativa</i> MJB1-06	0, 0	+(4.3), +(4.1)	0, 0	0, 0	0, 0	0, 0	+(3.7), +(3.9)	0, 0	0, 0
<i>S. irio</i>	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	+(2.3), +(2.0)	0, 0
<i>C. bursa-pastoris</i>	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	+(2.7), +(2.1)

^a Disease reaction = occurrence of sporulation. Mean disease severity was assessed using a 0-to-9 scoring system as used by Williams (58), where 0 = no symptoms on either leaf surface; 1 = small, pinpoint to large brown, necrotic flecks under the inoculation point on the upper surface, with occasional necrosis extending to the lower epidermis but no sporulation; 3 = very sparse, one to a few minute, scattered pustules on the upper surface, zero to very few pustules on the lower surface; 5 = few to many scattered pustules on the upper surface but either zero or a few scattered pustules on the lower surface; 7 = few to many pustules on the upper surface with many scattered, small to larger pustules on the lower surface; and 9 = very few to no pustules on the upper surface with many large, coalescing pustules on the lower surface.

plate DNA solution (approximately 100 ng), as prepared above, 20 µl of GoTaq Green Master Mix (Promega Corp.), 16 µl of MQ water, and 0.8 µl (each) of 100 µM primers. The thermal cycling parameters were denaturation for 1 min at 95°C, annealing for 1 min at 58°C, and extension for 2 min at 72°C. Thirty-five cycles were performed, with the first denaturation and last extension times extended to 5 and 10 min, respectively. The success of the amplification was monitored by using 5 µl of the PCR product by electrophoresis on 2% agarose gels and visualized by ethidium

bromide staining. The remaining 35 µl of the PCR products were then sent to Macrogen (South Korea) for purification and sequencing with the primers.

Sequence alignment and phylogenetic analysis. The evolutionary history was inferred using the minimum evolution (ME) method (47). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (24). The evolutionary distances were computed using the maximum composite likelihood method (53)

Fig. 1. Minimum evolution tree from internal transcribed spacer sequences showing relationship among *Albugo candida* collections of isolates from different hosts in Western Australia (in bold). Bootstrap numbers are only those given above major nodes. The sequence of *Albugo amaranthi* (AY929824) (54) from GenBank is included as outgroup.

and are in the units of the number of base substitutions per site. The ME tree was searched using the close-neighbor-interchange algorithm (39) at a search level of 1. The neighbor-joining algorithm (49) was used to generate the initial tree. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were, in total, 451 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (52), with alignment of the sequences performed using the CLUSTAL W (55) program. Sequences from this study were deposited in GenBank and the sequence data for the ITS rDNA region of 38 full-length sequences of *A. candida* isolates from other parts of the world used for comparison with the isolates in W.A. were retrieved from GenBank (Table 1; Fig. 1). The complete ITS rDNA region sequences from 9 W.A. isolates and 38 isolates from different parts of Australia and other continents were also analyzed in direct pairwise comparisons to establish percent identities between them (Supplementary Figure 1).

Results

***B. juncea* isolate.** *B. juncea* 'Vulcan', resistant to race 2A and susceptible to race 2V, and 'Commercial Brown', susceptible to race 2A (26), were tested to differentiate races 2A and 2V of *A. candida*. Both *B. juncea* differentials (Commercial Brown and Vulcan) showed significant sporulation from the *B. juncea* strain of *A. candida* (Table 2), confirming the presence of race 2V in W.A. This isolate from *B. juncea* also readily sporulated on the cotyledons of the *B. napus* test variety from China ('FAN 189') on *B. tournefortii*, *B. nigra*, and *R. sativus* (Table 2).

***B. rapa* isolate.** *B. rapa* 'Reward', resistant to race 7A and susceptible to race 7V, and 'Torch', susceptible to race 7A (42), were tested to differentiate races 7A and 7V of *A. candida* in addition to the Chinese *B. rapa* 'Shi Huang'. Both Reward and Torch showed no sporulation from the *B. rapa* strain of *A. candida*, whereas Shi Huang showed significant sporulation (Table 2). This isolate of *A. candida* from *B. rapa* also readily sporulated on the cotyledons of the *E. vesicaria* subsp. *sativa* MJB1-06 (Table 2).

***B. oleracea* isolate.** The *A. candida* isolate obtained from *B. oleracea* readily sporulated on *B. oleracea* var. *italica* 'Broccoli Sprouts' as well on *B. juncea* Vulcan (Table 2). Race 9 has been designated for strains attacking *B. oleracea* 'Diggers' (27,58).

***B. tournefortii* isolate.** This isolate was pathogenic only on *B. tournefortii* BTO2 and did not produce any sporulation on any of the other 15 differentials (including *B. juncea*) exposed to it (Table 2). There are no published race designations available for the pathogen on *B. tournefortii*.

***R. raphanistrum* and *R. sativus* isolates.** The *A. candida* isolate obtained from *R. raphanistrum* readily sporulated on *B. juncea* Commercial Brown, *B. napus* FAN 189, *R. raphanistrum*, *B. nigra*, and *R. sativus* (Table 2), whereas the *R. sativus* isolate sporulated not only on *R. sativus* 'White Icicle' but also on *B. juncea* Vulcan (Table 2). Race 1 has been designated for strains attacking *R. sativus* 'Early Scarlet Globe' (44) but no published race designation is available for strains attacking *R. raphanistrum*.

***E. vesicaria* subsp. *sativa* isolate.** The *A. candida* isolate obtained from *E. vesicaria* subsp. *sativa* was not pathogenic to *B. juncea* and sporulated only on *B. tournefortii* BTO2 apart from *E. vesicaria* subsp. *sativa* MJB1-06 (Table 2). There is no published race designation for strains attacking *E. vesicaria*.

***S. irio* and *C. bursa-pastoris* isolates.** The *A. candida* isolate obtained from the weed species *S. irio* only readily sporulated on the host from which it was isolated and did not attack any of the other 15 differentials (including *B. juncea*) inoculated with these isolates. In contrast, the *A. candida* isolate obtained from *C. bursa-pastoris* not only readily sporulated on the host from which it was isolated but also attacked *B. juncea* Vulcan (Table 2).

The results from the repeated trial confirmed the findings for all of the above isolates.

Phylogenetic analyses and nucleotide sequence identities. For the nine *A. candida* isolates from W.A., complete rDNA-ITS region nucleotide sequence analysis showed a range of 72.4 to 100%

(Supplementary Figure 1) nucleotide identity to the isolates used for comparison with previous Australian collections of *A. candida* and also those previously reported in Europe and Asia. The W.A. isolates from *B. juncea* showed close identity to *B. juncea* isolates of *A. candida* from Korea. Isolates from *B. juncea* from other states of Australia were not included in the phylogenetic studies of Petkowski et al. (41) in Victoria, Australia. The *B. tournefortii* isolate of *A. candida* from W.A. was the most distant from all others compared (Fig. 1), forming a distinct clade on its own, with an identity range of 77.4 to 80.5% compared with the rest of the isolates. *A. candida* isolates from *R. raphanistrum* and *R. sativus* from W.A. were least similar to others, with a nucleotide identity similarity of only 72.4%.

Discussion

Various reports in the literature suggest a range of biological races within *A. candida*. The current study explores the biological specialization occurring among the isolates collected from different host plants from various locations in W.A. Screening of differential hosts identified nine putative races among the isolates studied.

Pathogenicity tests indicated the presence of a different strain from *B. rapa* in W.A. compared with the Canadian isolates, one that was not pathogenic to *B. juncea*. This confirms that the W.A. isolate from *B. rapa* belongs to neither race 7A nor 7V and needs to be defined as a separate strain within race 7. Not only do the pathogenicity tests also confirm the presence of race 2V in W.A. (30), it is also likely that race 2V in Australia and Canada are similar. Although Rimmer et al. (46) used *B. juncea* 'Cutlass' as one of their differentials because it is susceptible to race 2V, the Vulcan that we used and Cutlass are related genotypes and, therefore, either can be used to designate the 2V race (R. Rimmer, *personal communication*). The results also showed that the strains from *B. tournefortii* and *S. irio* are highly host specific and did not sporulate on any of the differentials other than the host from which they were isolated. The strain from *B. juncea* had a relatively wide host range that included *B. tournefortii*. Therefore, as a weedy species, *B. tournefortii* has the potential to harbor *B. juncea*-attacking strains of *A. candida* in addition to the strain from *E. vesicaria* subsp. *sativa*. *B. tournefortii* is an endemic weed in the W.A. cropping belt (25) and white rust has been reported on this weed for a long period (2,50). The strain from *R. raphanistrum* also showed a relatively wide range of hosts among the differentials tested, as shown previously (30).

The strains attacking *R. sativus* and *E. vesicaria* subsp. *sativa* clearly warrant separate race designations. Future studies involving a more diverse set of host differentials should allow such designations, which would be most helpful to plant breeders in developing new cultivars with resistance to these new races and to agronomists in allowing strategic deployment of those new host resistances.

White rust is a major threat to *B. juncea* which is expected to expand its area of cropping in southern Australia, especially in regions experiencing relatively drier cropping seasons (11). Of the nine cruciferous taxa which yielded *A. candida* strains, only strains isolated from *B. juncea*, *C. bursa-pastoris*, *B. oleracea*, *R. raphanistrum*, and *R. sativus* were pathogenic on *B. juncea*. This may be a reflection of *B. juncea* being a relatively new crop compared with the more extensively cultivated *B. napus* oilseed rape. With time, it is likely that new strains will appear in *B. juncea* with a wider host range, especially to include the widespread weed *R. raphanistrum* and other cruciferous weeds currently prevalent in oilseed cropping areas. Such cruciferous weeds may play a role not only as carry-over hosts in rotations but also as a reservoir for the white rust pathogen to develop novel strains. The isolate from *S. irio* was unique because it was not pathogenic on any other host tested, nor was *S. irio* susceptible to any of the other strains tested. Race 5 has been designated for *S. officinale* but the isolate tested in the present study was from *S. irio*; therefore, further tests need to be conducted to confirm whether or not the strain from *S. irio* belongs to race 5. It should be noted that white rust does not affect European cultivars of *B. juncea*; rather, there, it has been confined to *B. rapa*

types. This might well influence the debate about breeding for drought resistance in *B. napus*, knowing it has resistance to *A. candida*, versus exploiting *B. juncea*'s drought resistance but having to manage its susceptibility to *A. candida*.

This study has established the prevalence of nine races in W.A. From comparing the complete ITS rDNA nucleotide sequences of the 9 *A. candida* isolates from W.A. with those of 38 previously collected Australian isolates of *A. candida* and also of those previously reported for isolates from Europe and Asia, it was possible to distinguish distinct groups within these isolates based on ITS rDNA nucleotide sequences, despite the small overall sequence divergence. Overall, the majority of these races have been reported previously from Australia (41) and were found to be similar phylogenetically to those analyzed in the current study. However, the isolate we obtained from *B. tournefortii* was phylogenetically the most distinct among those analyzed. The phylogenetic relationships identified in this study are expected to help overcome the technical difficulties usually encountered with host range studies, allowing a better understanding of host specialization.

The use in our study of a molecular systematic classification of different strains of *A. candida* in conjunction with determining the pathogenic behavior on a variety of cruciferous hosts of *A. candida* isolates from different hosts in W.A. provides, for the first time, reliability and repeatability in relation to defining races of *A. candida*. Defining the race status of *A. candida* in W.A. has provided a sound basis for the selection and deployment of new cultivars of both oilseed and horticultural *Brassica* spp. In particular, this new information is critical to the planned rapid expansion of the *B. juncea* industry in Australia with resistances to the races present in W.A. and in relation to increased interest to develop alternative *Brassica* spp. for industrial oil production.

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