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REVIEW PAPER

Agronomic challenges from novel pathotypes of *Albugo candida* to the emerging *Brassica juncea* industry in Western Australia

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Summary. It can be difficult to predict the outcome when a newly introduced crop is challenged by a specialized obligate phytopathogen. This case study explores the potential of *Albugo candida*, which causes white blister (WB) of Brassicas, to evolve into a major challenge to the broad acre crop *Brassica juncea* in the Western Australian (W.A.) agricultural landscape. *Brassica juncea* is currently emerging as a viable replacement oilseed crop to *B. napus*, especially in parts of W.A. with diminishing rainfall. WB is known to be a greater threat to *B. juncea* than to *B. napus*. Studies to date indicate significant genetic diversity of *A. candida* populations in this region, with many pathotypes evolving on exotic and native weed flora, and these may result in the development of strains that pose increased threats to *B. juncea* crops than those already encountered. Information gathered on pathogenic behaviour and defense mechanisms will assist understanding the nature of the appearance of novel pathotypes, and enable development of strategies to enhance host resistance to *A. candida* in *B. juncea.* Strategies to manipulate agronomic practices, such as weed control, may also help to reduce the hazards posed by newly evolving pathotypes in this region.

Key words: white blister, oilseed crops, *Brassica*, evolution.

Introduction

All grain crops grown worldwide have spread from their geographical points of origin. The movement of seed and plant material to distant regions is likely to result in the transport of propagules of pathogens resident on or within the propagation material. It is also possible that pathogens native to local flora may adapt to the newly introduced plant species. An example is the native rust pathogen *Uredo ranfelli* in Central and South America attacking introduced *Eucalyptus* spp. (Ferreira, 1983).

In this review, the evolution or emergence of novel pathotypes of the white blister rust (WBR) pathogen *Albugo candida* (Pers.) (O.) Kunze is followed in the Western Australian (W.A.) agricultural landscape. This region is characterized by a Mediterranean type of environment (Sivasithamparam, 1993). This paper is a case study, exploring the potential emergence of novel pathotypes of *A. candida*, an obligate oomycete pathogen attacking *Brassica juncea*. This crop species is currently being adopted as a replacement for *Brassica napus* in the drier parts of the W.A. grain belt.

This review provides an evaluation of the potential hazards facing the emerging *B. juncea* industry in W.A., and is a case study of the evolution of novel pathotypes of an obligate plant pathogen in a geographically isolated region. This is likely to be relevant to other obligate pathogens with similar lifestyles, which must evolve in a given environment.

Emerging *Brassica juncea* industry in Australia

Brassica juncea (Indian mustard) is an allotetraploid species in the Brassicaceae that has arisen by

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hybridisation between *Brassica nigra* (2n = 16) as the female parent and *Brassica rapa* (2n = 20) as the male parent, followed by chromosome doubling of the F1 (Oram *et al.*, 2005). Cultivars of this species are generally grown for oilseed production, as green vegetables or as forage, and sometimes for their medicinal properties. *Brassica* spp. are the sources, globally, of the third-most important vegetable oil, after soybean and oil palm (Schippers and Mnzava, 2004).

During the past 10 years, canola (*B. napus*) has become an integral component of the cropping systems of the W.A. grainbelt. Adoption of canola in the low rainfall regions is hindered, however, by its low and variable yields, high input costs and the relatively high risk of crop failure due to drought and the fungal, after disease blackleg (Macroft, 1997). In Australia, a small proportion of the oilseed brassica area (about 3,000 ha) is sown to *B. juncea*, mainly for use as condiments (Oram *et al.*, 2005). In the early 1990's, canola-quality *B. juncea*, producing oil with low erucic acid and glucosinolate contents, was first developed in Canada, and has since been deployed into commercial production by the Saskatchewan Wheat Pool in collaboration with Agriculture and AgriFood Canada (Love *et al.*, 1991). Subsequently, in Australia, canola-quality *B. juncea* is being developed to extend Brassica oilseed production to the low rainfall areas particularly for the warm and dry part of the southern Australian wheatbelt, where it is better adapted than canola. Most of the varieties of *B. juncea* are highly susceptible to white blister (Burton *et al.*, 1999), including the newly released cv. Dune which is susceptible to this disease (Anonymous, 2007).

The pathogen

The filamentous oomycete *Albugo candida* (Pers.) (O.) Kunze is considered to have descended from an aquatic non-fungal ancestor, and belongs in the phylum Heterokontophyta (Sogin and Silberman, 1998). Despite the separate phylogeny of oomycetes and true fungi (Kingdom Eumycota), the biotrophic pathogen *A. candida* causes plant disease in a manner similar to the biotrophic rust fungi, including host penetration via host stomata by germtubes arising from asexual inoculum, mesophyll colonization of compatible host tissue, and sporulation within spore-bearing pustules. Hence, the disease caused by *A. candida* has been referred to as white blister rust (Holub *et al.*, 1995).

Obligate biotrophic lineages of *A. candida* are considered to be ancient in origin compared to the downy mildew pathogens. This may explain the sophisticated mechanisms of biotrophy and the high degree of pathogen diversity of the WBR pathogens (Thines *et al.*, 2009). All cruciferous crops in Australian agriculture and horticulture were introduced following European colonization of the continent. It is most likely that most, if not all, strains of *A. candida* were introduced with the crops. In general, phytopathogens introduced to new environments may be characterized by low genetic diversity. This has been suggested for *Phytophthora infestans* (Goodwin *et al.*, 1994), and is also evident in the stepwise evolution of novel pathotypes of *Puccinia striiformis* f. sp. *tritici* in Australia and New Zealand (Steele *et al.*, 2001).

Taxonomy

As an obligate parasite, the basis for species delineation of *Albugo* has, until recently, been host preferences of the pathogen. This has been limited by the paucity of material studied. Specimens used to establish taxonomic relations have often been only from single or few collections (Choi *et al.*, 2011b). Morphological assumptions, although valid and valuable, may not always be useful in taxonomic separations and have led to broad species concepts and the use of *A. candida* as a generalist species. Because of these complications, several species of white blister pathogens remain undescribed. The separation of the *A. candida* populations as pathotypes has been of great value in agronomy, especially for plant breeders. Delineation of "host specific forms" is certainly useful in the determination of suitable crops that can be grown in rotations and, in certain cases, to manipulate weed species. Host range of *Albugo* has been investigated to some depth in some recent studies. Choi *et al.* (2011a) proposed that expansion of WB pathogens from Brassicales to Fabales may have involved "host jumping". Recent evaluations have resulted in the allocation of WB pathogens of Asteridae to *Pustula*, and those on Caryophyllidae to *Wilsoniana* (Thines *et al.*, 2009). A recent phylogenetical and morphological investigation of *A. candida* specimens collected from about 30 host genera (Choi *et al.*, 2011b) found that *A. candida sensu stricto* has a broad host range, encompassing a large number of host plants belonging to Brassicales. Choi *et al.*, (2011a) pointed out the presence of previously overlooked species of *Albugo* with hosts in

this order. They revealed that *Albugo* specimens from *Alyssum montanum*, *Barbarea vulgaris*, and various *Rorippa* species, could be placed in three phylogenetically distinct clades that are closer to *A. candida sensu stricto* than any previously reported species. Their study also revealed that a large number of *Albugo* species remain still undiscovered, and that species close to *A. candida* exist. This information could help to elucidate the basis of the broad host range of *A. candida* as opposed to the narrow specialisation that is seemingly present in other species of *Albugo* on the Brassicaceae.

Based on ITS and cox2 sequence data of 72 *Albugo* specimens, predominantly from herbarium archives, and focusing on the widespread genus *Cardamine*, a high degree of phylogenetic diversity was revealed in *Albugo* (Sebastian *et al.*, 2010). In particular, the hypothesis that one host genus can be colonised by more than one white blister rust species is confirmed. In addition, it was revealed that there are hitherto overlooked lineages with close relationships to the generalist species *Albugo candida*. Evidence for at least three different species of *Albugo* infecting *Cardamine* were presented in the study. Based on molecular phylogenetic and morphological data, three new white blister rust species were described, *Albugo hohenheimia*, *Albugo hesleri*, and *Albugo leimonios* infecting *Cardamine hirsuta*, *Cardamine diphylla* and *Cardamine pratensis*, respectively. The fact that these species each have different ecological niches, prompted them to suggest that environmental factors may have played a role in the speciation process in *Albugo*. Their findings also suggested that other larger genera of the Brassicaceae may harbour unrecognized white blister rust species and that only a small fraction of the true biodiversity of white blister rusts is known at present.

The evolution of host preferences within WB pathogens is also complex. *Albugo candida* and *A. laibrachii* both infect *Arabidopsis* but they have been shown to be phylogenetically different, with *A. laibrachii* recorded only on *Arabidopsis* (Choi *et al.*, 2011a)

Geographic isolation may have enabled independent adaptations of pathogen strains to the same host. If two related but distinct species are parasitic on the same host in the same geographic region, this could suggest sympatric speciation (Thines *et al.*, 2009). The role of weed species in the evolution of pathotypes of WB pathogens has received relatively little attention. This issue is important in situations where new crop species, varieties or lines are introduced in relatively recent agricultural environments. Australia, therefore, could well be an ideal 'region' to study such adaptations.

In the studies of Petkowski *et al.*, (2010) involving DNA from *A. candida* from 11 hosts, a majority of the pathogen strains were found to be a common form (99% DNA sequence symmetry) of *A. candida*. In addition to a collection of a WB pathogen from *Cardamine hirsuta* made previously, they made three Australian collections from *C. hirsuta* of what they considered to be new species of the pathogen. They also reported that ten weed species are infected by WB pathogens, with only one species reported from the island of Tasmania. No material from weeds prevalent in W.A. was included in the study of Petkowski *et al.*, (2010).

Life cycle

Albugo candida is an obligate parasite that can develop only in living host tissues. It reproduces by asexual sporangia or zoospores and thick-walled sexual oospores. The presence of oospores in crop residues and/or perennial mycelium in living host (including weed) tissues enables the pathogen to survive between host growing seasons. A few infected plants may serve as primary sources of inoculum to establish a disease epidemic. In systemically infected plants, such as horseradish, the pathogen resumes growth early in the spring, invades host shoot primordia, develops along with the growing shoots, and produces pustules on all aerial parts (Anonymous, 1990). However, in Australia, oospores probably provide the sole source of carryover of *A. candida*, especially in regions with Mediterranean-type climates, where dry hot summers prevail (Figure 1).

The first external symptoms of white blister usually appear 5 to 20 d after infection, depending on the temperature and other environmental conditions, and within 3 to 14 d a new crop of sporangia are released, starting secondary disease cycles. Many secondary cycles may occur during a growing season. Under ideal conditions (cool and moist), a complete cycle may be completed every 8 to 10 d (Anonymous, 1990). Oospores develop in large numbers in distorted swellings and galls (including stagheads) in infected stems and pods of host plants. These thick-walled, over-wintering spores can resist drying as well as

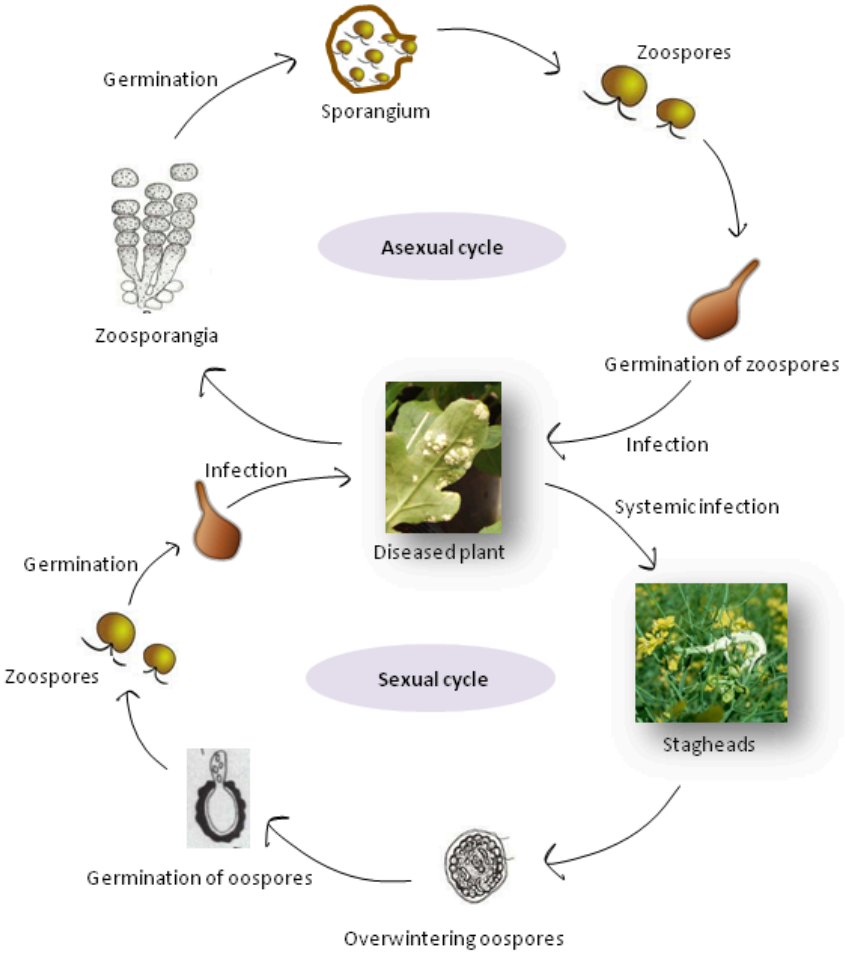

Figure 1. Life cycle of *Albugo candida* (adapted from Saharan and Verma, 1992). Reproduced with the permission of the publisher, IRDT).

temperature extremes. The oospores are responsible for long-term survival in plant debris and are liberated when the host tissues decay. The oospores germinate in the spring by producing zoospores like those formed by the sporangia (Anonymous, 1990).

Oospores in mature host stagheads serve as the main survival and dispersal propagules (Petrie, 1975). In addition, *A. candida* is spread through sowing seeds contaminated with oospores and by wind- and rain-borne zoosporangia. Occasionally, perennial mycelium in infected live plants may also initiate disease epidemics (Shukla *et al.*, 2003). These characteristics allow the pathogen to multiply and spread rapidly in the field under conducive environmental conditions.

Infection process

In all species of WB pathogens, oospores are the primary source of inoculum (Verma *et al.*, 1975; Saharan and Verma, 1992). Secondary spread is by means of sporangia, which are readily carried short distances by splashed water droplets or, to a certain degree, by air currents. Moisture on host surfaces is essential for germination of sporangia and infection by zoospores. The most likely primary infection sites are the emerging cotyledons of host plants (Verma *et al.*, 1975). Germ tubes enter the stomata of resistant hosts as readily as those of susceptible hosts (Verma *et al.*, 1975). In the former, mycelial growth of the pathogen ceases in the substomatal chambers, and a marked encapsulation forms around each hausto-

rium (Verma *et al.*, 1975). In congenial or susceptible hosts, the mycelium advances intercellularly with the production of haustoria (Verma *et al.*, 1975; Liu *et al.*, 1989; Liu and Rimmer, 1990).

Until formation of first haustoria, there appeared to be no difference in the infection process in susceptible (*B. juncea, B. campestris*), moderately susceptible (*B. hirta*), or resistant (*B. napus*) hosts. Growth in *B. napus* stopped 2–3 days after inoculation and a marked encapsulation was formed around each haustorium. In contrast, mycelial development increased rapidly after the formation of first haustoria in other hosts. Up to 14 haustoria were observed in each mesophyll cell and encapsulation was rarely observed around haustoria in susceptible host tissue. The massive amount of thallus produced by the pathogen was evident by susceptible hosts, which is evidence of the highly specialized type of parasitism evolved by *A. candida* (Verma *et al.*, 1975).

Samples of whole host cotyledons were examined by Liu *et al.*, (1989) by differential interference contrast microscopy after inoculating cotyledons of one resistant and three susceptible rapeseed lines/cultivars with zoospores of *A. candida* race 7. The time course of the infection process showed that germination of zoospore cysts occurred 2–3 h after inoculation and the infection was initiated with germ-tubes penetrating through stomata. Their studies also showed that the earliest event that distinguishes a compatible from an incompatible interaction occurred only after the formation of the first haustoria, and that resistance was not manifested until each host mesophyll cell had come into contact with the first haustorium. Most primary hyphae produced single haustoria in the resistant cultivar, and necrosis of the invaded host cells was first observed 12 h after inoculation followed by cessation of mycelial growth. The death of host cells was largely restricted to the penetration sites, whereas the adjacent non-penetrated cells remained apparently unaffected. In the susceptible hosts, necrosis of infected cells occurred only rarely, and hyphal growth continued unabated, resulting in mycelial ramification into the mesophyll and abundant haustoria were produced (Liu *et al.*, 1989). It is clear from these observations that the behaviour of the pathogen during infection processes can be significantly different on resistant as compared to susceptible hosts.

Under Indian conditions, first appearance of white rust disease on leaves and pods (staghead formation) of mustard occurred between 36 and 131 days after sowing (d.a.s.) and 60 and 123 d.a.s., respectively. Severity of WBR disease on leaves was favoured by 40% afternoon (minimum) relative humidity (RH), 97% morning (maximum) RH and 16–24°C maximum daily temperature. Staghead formation was significantly and positively influenced by 20–29°C maximum daily temperature and further aided by 12°C minimum daily temperature and 97% morning (maximum) RH (Chattopadhyay *et al*., 2011).

Physiological specialization

Biological specialization has long been noted in *A. candida*. Different strains exhibit host specificity, and races have been classified based on their ability to infect different species of Brassicaceae (Pound and Williams, 1963). Seventeen races of *A. candida* have been reported from various *Brassica* species, with strains from different *Brassica* spp. normally most pathogenic on the host genotype or species from whence they originated (Minchinton *et al.*, 2005). These are nonetheless able to grow, although normally not as well, on other *Brassica* spp. (Liu *et al.*, 1996).

Under experimental conditions, however, spores from some hosts have produced white blister lesions on hosts other than those listed in literature, particularly on seedling cotyledon leaves. This suggests that some pathotype specificities for the pathogen may not be clearly differentiated (Rachel, 2006). Individual pathotypes do not exhibit absolute adaptation to one particular host species and can often also infect heterologus hosts, especially those sharing a common genome (Tanhuanpaa and Vilkki, 1999). The genetic control of resistance in *B. rapa* towards *A. candida* is governed both by major and minor genes (Delwiche and Williams, 1974; Edwards and Williams, 1987; Kole *et al.*, 1996). To date, 409 RAPD primers have been tested for polymorphism but none have yielded suitable markers. Hence, an alternative strategy, based on the existence of conserved structures among different resistance genes, has been tested using amplified resistance gene analogs from resistant parents using different PCR primer pairs. These PCR products of *c*. 500 bp in size have been cloned and sequenced and putative white blister resistance genes located (Tanhuanpaa and Vilkki, 1999).

In Australia, *A. candida* has been reported on 20 different plant genera, although the pathogen is generally restricted to introduced cultivated and weed plants (Petkowski *et al.*, 2010). The classification of

species within the genus *Albugo*, which infects each host family, has often been impossible based on the morphological characteristics of the pathogen. Eberhardt, (1904) and Hiura (1930) demonstrated the host specificity of *A. candida* by predominately examining crop species, and classified *A. candida* into ten races based on their specificity to different brassicaceous hosts (Pound and Williams, 1963; Hill *et al.*, 1988). More recently, Minchinton *et al.*, (2005) reported 17 races of *A. candida* from various cruciferous species worldwide. The degree of host specificity within *A. candida*, however, has not been clearly defined. Khunti *et al.*, (2000) showed that *A. candida* isolates from *Brassica* can infect *Amaranthus viridis* (Amaranthaceae) and *Cleome viscosa* (Capperaceae, now included in Brassicaceae; APG, 2009), as well as *Brassica campestris* var. *rapa* (Choi *et al.*, 2006).

Assuming the current race designations, and allowing for interpretations of plant taxonomy, the recorded pathotypes of *A. candida* in Australia are likely to belong to races 1, 2, 3, 4, 5, 6, 7, 9 and 10. Host records indicate that races 1, 2V, 3, 4, 5, 6, 7, and 9 occur in New Zealand (Minchinton *et al.*, 2005). Recent field observations in New Zealand suggest that leafy turnip (*B. rapa*), grown for animal forage, is particularly susceptible to white blister (probably Race 7 of *A. candida*). Race 9 infects *B. oleracea*, and this race is likely to have caused the recent outbreaks of white blister that have occurred on broccoli in Australia and New Zealand. Variations in severity of white blister epidemics could be due to changes in the virulence of the pathogen population (Minchinton *et al.*, 2005). The primary host of race 7 is *B. rapa* (Verma *et al.*, 1975) and race 2 is primarily compatible with *B. juncea* (Pound and Williams, 1963), but it has also been found to be pathogenic on many genotypes of *B. rapa* (Petrie, 1988).

Resistance to *A. candida* race 2 in *B. rapa* and other oilseed *Brassica* spp., including *B. juncea*, *B. napus* and *B. carinata*, has been shown to be conferred by dominant alleles at single loci (Delwiche and Williams, 1974, 1981; Ebrahimi *et al.*, 1976; Tiwari *et al.*, 1988; Kole *et al.*, 1996), although there is some evidence of minor genes also being involved in the control and/or expression of resistance (Edwards and Williams, 1987; Kole *et al.*, 1996). *Brassica juncea* cultivars which were previously resistant to race 2 (now designated as race 2A) were subsequently susceptible to a more virulent form of this race of the pathogen (now designated as race 2V). A newly developed canola quality *B. juncea*, which is widely sown across the Canadian prairies, is susceptible to race 2V.

Qualitative hypersensitive response type resistance to *A. candida* race 2V has been developed by interspecific introgression of a resistance gene from *B. napus,* (Franke *et al.*, 1999). *B. napus,* being highly resistant to race 2A and race 2V of *A. candida*, has been successively backcrossed to *B. juncea*, and resistance to *A. candida* race 2V has been successfully introgressed into the canola quality *B. juncea*, and into oriental mustard and brown mustard, but only in Canada (Rimmer *et al.*, 2000). This material is now being used to develop improved canola quality *B. juncea*, oriental mustard and brown mustard varieties in the Saskatoon Research Centre breeding program (Gugel *et al.*, 1999). Resistance to both race 2A and 2V is an important requirement of all the future cultivars of *B. juncea* (Rimmer *et al.*, 2000) for Canada and Australia. The information available to date on the physiological specialisation of *A. candida* indicates the extent to which the pathogen can adapt on hosts within a single plant species.

Host range

Albugo causes considerable damage, both in many economically important agricultural crops and in common weeds (Farr *et al.*, 1989), and its hosts have been reported to include 63 genera and 241 species of cruciferous plants (Biga, 1955; Saharan and Verma, 1992). Among these, *Brassica* and *Raphanus* are the most important cultivated host genera. White blister, caused by one or more species of *Albugo*, occurs globally on beet (garden and sugar-beet types), Brussels sprouts, cabbage, cauliflower, Chinese cabbage, collards, garden cress, horseradish, kale, lettuce, mustards, parsnip, radish, rape, salsify (black and white), spinach, sweet potato, turnip, watercress, and possibly water-spinach. Additionally, various common weeds and herbaceous ornamentals are attacked by the pathogen (Anonymous, 1990).

In Australia, *A. candida* has been reported on 20 different plant genera, although it is generally restricted to introduced cultivated and weedy plants (Petkowski *et al.*, 2010). White blister was first reported in Australia on shepherd's purse (*Capsella bursa-pastoris*) in 1894, on Chinese cabbage (*Brassica rapa*) in 1895, and on radish (*Raphanus sativus*) in 1903. The first report of the disease on the *B. oleracea* group (cauliflower) was in 1980, so *A. candida* on this im-

portant group of vegetables is probably a relatively recent introduction. White blister also occurs on other crops such as horseradish (*Armoracia rusticana*), broccoli, Brussels sprouts and kale (*B. oleracea*), turnip, bokchoy, Shanghai and choysum (*B. rapa*), rocket (*Eruca sativa*), Indian mustard (*B. juncea*), mustard weed (*Sysymbrium* spp.), and water cress (*Rorippa* spp.). To date, *A. candida* has not been recorded on *B. oleracea* in Queensland, nor on cabbage (*B. oleracea*) in Australia (Minchinton *et al.*, 2005).

White blister is an important disease of brassicas in W.A. (Barbetti and Carter 1986; Li *et al.*, 2007a 2007b, 2008), and cruciferous weed species are widespread throughout the oilseed rape growing regions of W.A. The disease was first recorded in W.A. on *R. raphanistrum* in 1929 (MacNish, 1963), *B. napus* var. *napobrassica* and *B. rapa* var. *rapa* in 1940 (Chambers, 1959), *R. sativus* in 1944 (Chambers, 1959), *B. oleracea* var. *botrytis* in 1965 (MacNish, 1967), *B. napus* in 1970 (Anonymous, 1972; Shivas 1989), *B. tournefortii* in 1974 (Anonymous, 1974; Shivas, 1989), and on *B. rapa* var. *chinensis* in 1984 (Shivas, 1989). Symptoms have been recorded on cauliflower and broccoli and the disease has been found in all major vegetable *Brassica* growing regions in Australia (Rachel, 2006). White blister was also been observed on a few experimental genotypes of *B. napus* (particularly 'Norin' lines) from Japan several decades ago (M.J. Barbetti, unpublished), and more recently in a few experimental *B. napus* genotypes introduced from China (Li *et al.*, 2007a). *Brassica juncea* cultivars, previously reported as being resistant to a *A. candida* strain recorded as race 2 and currently recognised as pathotype 2A, were subsequently found to be susceptible to a more virulent form of this race in Canada (designated as pathotype 2V) (Petrie, 1994), including newly developed canola-quality *B. juncea* which is widely sown across the Canadian prairies (Gurung *et al.*, 2007). Pathotype 2V has also been reported in W.A. (Kaur *et al.*, 2008).

Severity of white blister can be affected by several factors, including the time of inoculation and the parts of host plants initially infected. *Albugo candida* causes both localized and systemic infections on plants of various cruciferous species. In localized infections, the pathogen mainly infects plant parts that contain chlorophyll. With systemic infections, extensive distortion, hypertrophy, hyperplasia and sterility of inflorescences ("stagheads") can occur (Verma and Petrie, 1980). Liu and Rimmer (1993) succeeded in obtaining deformed inflorescences resulting in stagheads in 40% of inoculated plants. However, Lakra and Saharan (1989) and Bains (1991) were unable to produce deformed inflorescences following similar inoculations of flower buds of susceptible *B. juncea* plants. To address such inconsistencies, Goyal *et al.*, (1996) reported that maximum staghead formation in 26-day-old [growth stage (GS) 3.1; Harper and Berkenkamp, 1974] *B. juncea* plants occurs in response to inoculating differentiating flower buds. In contrast, they found that inoculation of 35- and 45-d-old plants (GSs 4.1 and 5.0, respectively) produced fewer hypertrophies mainly in isolated flowers, while inoculation of the 7- and 13-d-old plants (GSs 1.0 and 2.1, respectively) did not produce any hypertrophied flowers. However, Goyal *et al.*, (1996) did not undertake inoculations at GS 3.2 nor at GS 3.3. Kaur *et al.*, (2011a) undertook investigations that defined the relative importance of the full spectrum of growth stages, from cotyledon stage to young pods present (viz. GS 1.0, 2.1, 3.1, 3.2, 3.3, 4.1, 4.2 and 4.3) in terms of inoculation and subsequent development of white blister. Secondly, they also defined symptom expression following inoculations of leaf laminae vs the growing points at seedling (GS 1.0) and first true leaf (GS 2.1) growth stages.

Host-pathogen interactions in the *B. juncea-A. candida* pathosystem are currently poorly understood. There are many reports indicating that there is poor correlation between the extent of white blister on foliage and pod deformation/stagheads. Li *et al.*, (2007a) reported that where some plants of certain cultivars of *B. juncea* showing almost immune response in leaves, subsequently developed severe pod hypertrophies as the crop matured. Kaur *et al.*, (2011a) indicated for the first time that infection of specific parts of plants at different stages of plant development can dictate if pod hypertrophies would subsequently develop in mature plants. The most severe and destructive white blister results in the deformations of the reproductive plant parts leading to the deformation of pods and loss of seed production. Thus, the development of such symptoms in plants during reproductive phases poses the greatest threat to crop yield. Earliest infection resulting in hypertrophies arose from infections of the growing points, not leaf laminae, even at the first true leaf stage. This suggests that genes determining resistance to foliar damage are different to those controlling pod hypertrophies. This phenomenon, and the development of

hypertrophies following inoculation of buds as they change colour or just after they open, resulting in pod hypertrophies, may have significant agronomic implications, especially in relation to the production and survival of carry-over inoculum of the pathogen in the field. Goyal *et al.,* (1996) proposed that infection has to occur while meristematic tissues are present, on which the biotrophic pathogen can establish as a systemic coloniser. These findings are particularly significant in relation to targeting of fungicide applications to maximise reduction of the impact of white blister on crop yield. Fungicide applications need to be targeted either at the emerging growing points at the initial true leaf stage of crop growth, and/or subsequently at later stages, specifically during early flowering. This could result in reduced and strategic application of fungicides for the effective management of the disease.

Susceptibility of a host to *A. candida* can be affected by the presence of other pathogens at inoculation (Kaur *et al.,* 2011b). *Albugo candida* is frequently associated with the downy mildew pathogen, *Hyaloperonospora parasitica*. Numerous instances are known of considerable damage from combined infections (Vanterpool, 1958; Sansome and Sansome, 1974; Saharan and Verma, 1992). Both of these pathogens infect all the aerial parts of host plants, and are very often observed intimately colonising host tissues. Combined infection by these two pathogens has been recorded as the cause of 17–37% yield losses in rapeseed-mustard crops in India (Kolte, 1985). According to Saharan (2010), the compatibility genes for association of *Albugo* and *Hyaloperonospora* pathogens of crucifers may be the same or situated on the same loci or tightly linked. These pathogens usually exist as specialised pathotypes on different cruciferous species and on cultivars within species, but generally asexual reproduction is the greatest on the host of origin (Pidskalny and Rimmer, 1985; Sherriff and Lucas, 1987; Petrie, 1988; Saharan and Verma, 1992; Mathur *et al.,* 1995; Nashaat and Awasthi, 1995; Silue *et al.*, 1996). *Hyaloperonospora parasitica* is commonly found parasitizing tissues colonized by *A. candida* (Chaurasia *et al.*, 1982; Bains and Jhooty, 1985).

Albugo candida predisposes the host tissues to susceptibility to *H. parasitica* (Bains and Jhooty, 1985). White blister resistance loci on three *Arabidopsis* chromosomes were also found to be closely linked to downy mildew resistance loci (Borhan *et al.*, 2004). Severe *A. candida* infection culminates in systemic 'staghead' symptoms in host inflorescences usually in association with *H. parasitica* (Awasthi *et al.*, 1997).

Kaur *et al.,* (2011b) demonstrated that although the co-occurrence of downy mildew caused by *H. parasitica* and white blister have been reported previously, resulting in severe yield reductions, none of the previous studies actually identified the role of *H. parasitica* in the severity of white blister on a host known to be resistant to downy mildew. The interaction of downy mildew and white blister displayed that even the levels of susceptibility showed in cultivars susceptible to white blister can be enhanced by the presence of the other biotrophic pathogen. The co-occurrence of the white blister and downy mildew pathogens on *B. juncea* in the study of Kaur *et al.,* (2011b) was the first to show the role of infection by the downy mildew pathogen on a host known to be resistant to it where that pathogen was still able to enhance the severity of white blister rust in the plant. This is a significant discovery in relation to the epidemiology of downy mildew. Stagheads of plants of *B. juncea* resistant to downy mildew can carry that pathogen asymptomatically. This is significant for the survival of the oospores of the downy mildew pathogen which can easily survive in the woody staghead tissues of WBR affected plants. Information on host range of WBR is critical for the understanding of the potential of other rotation crops as well as weeds, not only as alternate hosts, but also as sources of novel pathotypes developing at a site.

Host resistance

Mechanisms of host resistance to white blister in weed species are most likely to be similar to those investigated in crop species, varieties or lines tolerant or resistant to *A. candida* within the respective plant families.

Oomycetes colonize host plants by modulating host cell defenses through a variety of disease effector proteins (Kamoun, 2006). Pathogen Avr proteins can be 'effectors' that promote *A. candida* virulence (Dangl and McDowell, 2006). To be successful, effector proteins that suppress or otherwise manipulate key components of resistance have to be delivered at the appropriate time and site (Hein *et al.,* 2009).

Studies on the host-parasite interactions in white blister diseases have focused on the level of specificity among races of pathogens and genotypes of related

host species (Saharan and Verma, 1992). Even within the confines of race-cultivar specificity, the studies have been narrow in that no genetic information has been generated for *Albugo*, the causal organism. Oilseed *Brassica* spp. (traditional and doubled haploid genotypes), that are resistant, partially resistant, moderately susceptible, or susceptible to *A. candida* have been compared for phenotypic development and histology of host-pathogen interactions (Bansal *et al.*, 2005). A partially resistant genotype developed pinhead-sized pustules, which were mainly on the upper surfaces of cotyledonary leaves. Comparatively more mycelium was observed in the susceptible genotype compared with the partially resistant genotype. There was neither pustule development nor any mycelial growth in the resistant *B. napus* genotypes. In contrast, the pustules on leaves were similar to those in the partially resistant genotype, were of pinhead size, and occasionally coalesced in the moderately susceptible genotype. However, mycelial growth in the mesophyll tissues of the moderately susceptible genotype was comparable to that in the susceptible *B. rapa* cv. Torch, which showed large coalescing pustules. In the non-host *B. juncea* cv. Commercial Brown, while some mycelial growth was observed beneath the epidermal cell layers and in the mesophyll cell layers of cotyledonary leaf tissues, no pustules were formed (Bansal *et al.*, 2005).

Genes for white blister resistance were introgressed into *B. juncea* cv. RL 1359 from *B. napus*, *B. carinata* and *B. tournefortii* by Banga *et al.*, (2004) following interspecific hybridization. They found that seven genotypes had greater resistance than a susceptible check genotype. Backcrossing with these newly identified resistant stocks for five generations led to the synthesis of near isogenic lines of *B. juncea* cv. RL 1359, which differed only for gene(s) for white blister resistance. Biochemical analysis of the isogenic lines and the susceptible recurrent parent RL 1359 revealed association of white blister resistance with peroxidase and polyphenol oxidase activities, particularly under diseased compared with disease-free conditions. Amounts of total phenols, sugars, flavonoids and waxes were greater in leaves of the resistant genotypes. In contrast, cellulase activity was greater in the susceptible check, particularly under diseased conditions (Banga *et al.*, 2004). Inheritance of avirulence in *A. candida* is considered to be governed by a single dominant avirulence gene (Saharan, 2010). Genes for limited virulence (AC 23, AC 24 and AC 17) and wide virulence (AC 29, AC 27, AC 30, AC 18 and AC 21) have also been identified in the pathogen by Saharan (2010).

Prior to the penetration of plant epidermal cells and eventual tissue colonization, the life cycle of *A. candida* involves zoospore emergence from sporangia and the formation of appressoria on germ tubes. The biotrophic interactions between *A. candida* and certain crucifer species were studied by Gadagi and Pedras, comes after (2006) to determine the role of various crucifer phytoalexins on zoospore emergence and the formation of appressoria on germ tubes. Cellulose membranes impregnated with the solutions of test compounds were incubated with sporangia of *A. candida* in a bioassay to determine inhibitory effects of the compounds. Fifteen different phytoalexins were tested, and brassilexin, cyclobrassinin, wasalexin and caulilexin A completely inhibited zoospore emergence and also formation of appressoria of *A. candida* race 2V (Gadagi and Pedras, 2006).

Proteomic studies (Kaur *et al.*, 2011c) gave clear indication of the host proteins involved in the expression of resistance to white blister in the resistant cultivar (CBJ-001). The expressions of most cascades of these particular proteins were detected only in the resistant cultivar. These expressions were noted in a time frame which could be related to zoospore germination, formation of haustoria and subsequently in relation to the reproductive phase of the invading pathogen (Figure 3).

The earliest expressions, by 2 h post-inoculation (hpi), were of the pathogen-responsive protein 5 (PR-5) and the two isoforms of superoxide dismutase (SODs) (especially the Fe/Mn-binding isoform). These were also expressed at 4 hpi, by which time zoospore germination is known to occur (Liu *et al.*, 1989). This indicates that the resistant host is able to respond to the presence of the pathogen at, or even prior to, the entry into the host tissue. Similar early activation of host genes has also been reported for *Phytophthora clandestina* on a resistant cultivar of *Trifolium subterraneum* (Ma *et al.*, 2010). Kaur *et al.* (2011c) also found that there is no evidence of the expression of glutathione S-transferase (GST) or *O*-acetyl-L-serine(thiol)lyase (OAS-TL) which are related to stress management by the host during this period, although the transcription evaluations for these proteins were evident both at 2 and 4 hpi for these proteins. Proteins expressed only in the resistant cultivar at 8 hpi, when haustorium formation

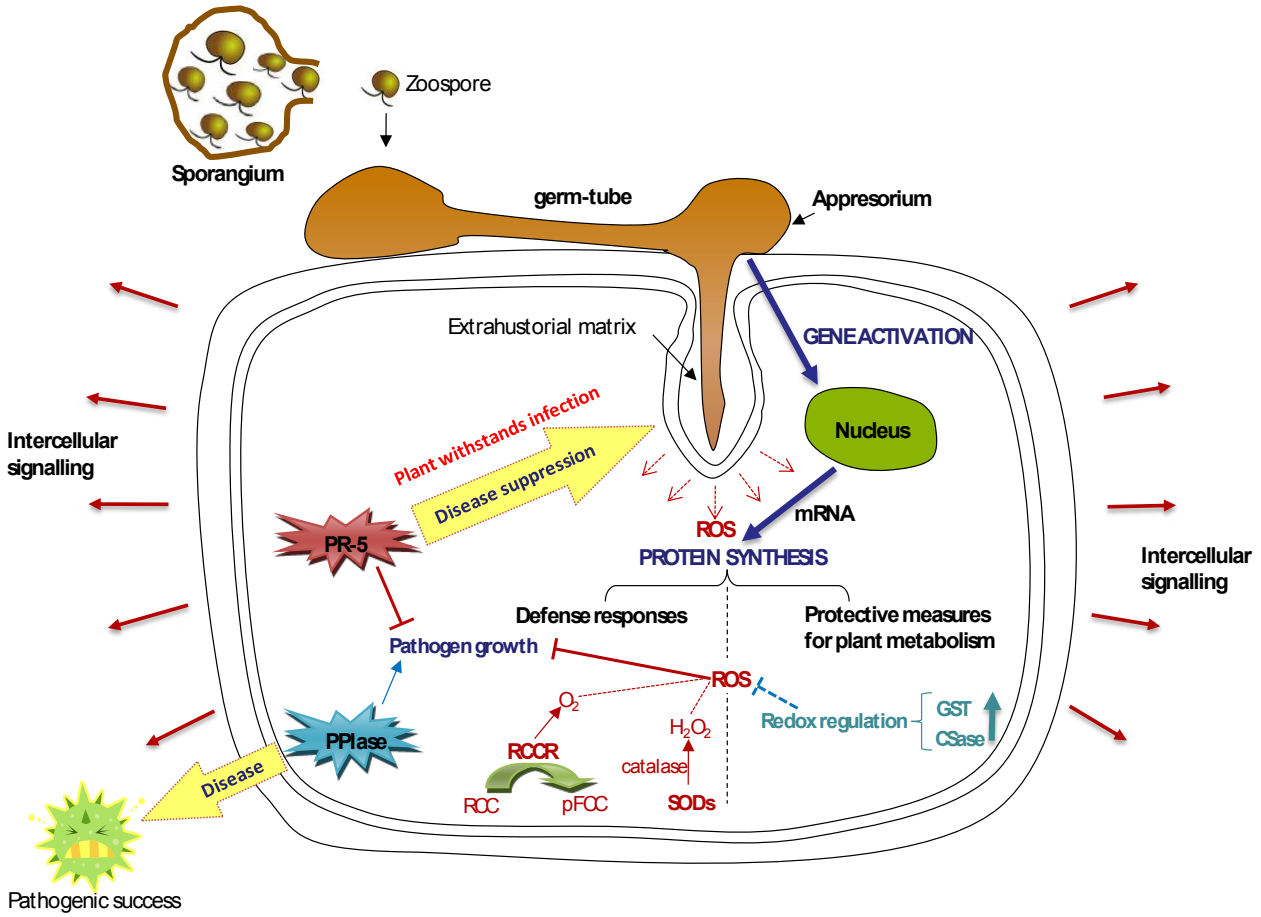

Figure 3. Major elicitor-induced changes proposed to occur during the interaction of *Brassica juncea* and *Albugo candida* to indicate the sites of activities and the outcomes resulting in the progression or suppression of the infection processes (Kaur, 2010). RCCR = Red Chlorophyll Catabolite Reductase, pFCC = Primary Fluorescent Catabolite, SODs = Superoxide Dismutases, ROS = Reactive Oxygen Species, GST = Glutathione S-transferase, CSase = Cysteine Synthas

has been noted for this pathogen (Liu *et al.*, 1989), included PR-5, GST, OAS-TL and SODs (especially the Fe/Mn-binding isoform). These proteins may play significant roles in the abortion of the parasitic phase in the resistant host. Recent studies with downy mildew (Li *et al.*, 2010) showed that both resistant and non-host effects against *H. parasitica* were clearly and initially evident at a critical phase where the number and size of the haustoria were evident. This was also the phase when there was significant decrease in the activity of red chlorophyll catabolite reductase (RCCR) and an increase in the activity of peptidyl-prolyl cis/trans isomerase (PPIase), both favouring susceptibility. Thus, it is highly likely that the activities of these proteins had significant roles in the progression or suppression of the infection process of the white blister pathogen. The assessment of the expression of proteins at 24 hpi is considered to coincide with necrosis of the invaded host cells by *A. candida* (Liu *et al.*, 1989) which was restricted in the resistant host. The resistant host continued to express proteins associated with suppression of infection through the expression of PR-5 peaking at this time. Expression of SODs (especially the Fe/Mn-binding isoform) also increased significantly at this time and together could have helped to reduce the extent of cell death associated with the expression of the disease in the resistant host.

The assessment of protein expression at 72 hpi coincides with the timing reported for the initiation

of the reproductive phase of the pathogen (Anon., 1990). The expression of the GST (helping the host to deal with the pathogenic stress) and the SODs (especially the Fe/Mn-binding isoform) were greatest at this time for these proteins, during the period monitored. These, with activities of other proteins favouring resistance and reducing susceptibility, could together be responsible for the suppression of sporulation. Pustule formation associated with sporulation is related to the reduction of the active photosynthetic area of the host, hence reduction in the sporulation could not only be very significant in maintaining host productivity and/or yield, but could also be important for curtailing the rate of secondary spread of white blister within a crop.

The increased expression of these defence-related proteins and the concurrent reduction of RCCR associated with susceptibility could explain the host-parasite interactions in this resistant pathosystem. In contrast, the increased activity of PPIase when haustorium formation occurs in susceptible cultivars could further support the view that haustorial formation is critical for pathogenesis. While focus here has been mainly on the proteins to give understanding of the host-parasite interactions in this *B. juncea*-*A. candida* pathosystem, other factors may also be involved in the suppression of infection processes that may also enhance the expression of resistance. Knowledge on host resistance (including that of the proteins involved in resistance) could provide useful indicators of the existence/absence of specific barriers that challenge the spread of the pathogenic strains on to taxonomically related hosts within or outside the host species.

Weeds as sources of new pathotypes

In Australia, white blister is seen as a greater threat to *B. juncea* production than to any other cruciferous crops, either for oilseed or vegetables (Burton *et al.*, 1999; Anonymous, 2007). In W.A., it is possible to study the potential for the introduction and/or evolution of new or exotic races that can challenge large scale cropping of *B. juncea*. Of *A. candida* races/pathotypes, only those from weeds common in the W.A. grainbelt pose the greatest and immediate threat as sources of novel pathogen strains. To date, *B. juncea* has been grown in W.A. as a condiment crop with only limited areas under cultivation (Oram *et al.*, 2005). Currently, however, there is a trend for *B. juncea* to replace *B. napus* as oilseed crops in areas where low or reduced rainfall prevents profitable *B. napus* cropping. In such situations the cruciferous weeds currently prevalent in *B. napus* fields may play roles both as carryover hosts in rotations and as reservoirs for the development of novel *A.candida* strains. *Raphanus raphanistrum* is currently the commonest cruciferous weed in and nearby to canola crops. As a weed introduced into W.A. over 150 years ago, this plant is an alternative host especially for *Leptosphaeria maculans* (which causes black leg of crucifers), which was recorded in W.A. about 85 years ago (Sivasithamparam *et al.*, 2005). For blackleg , this weed has probably also been a host for evolution and perpetuation of novel pathogen strains even in the absence of *B. napus* crops, as occurred during the 1970s and 1980s, following the initial onslaught of blackleg epidemics in south-west W.A. in the early 1970s. The phylogenetic studies of Kaur *et al.*, (2011d) demonstrated that the *A. candida* strain on *R. raphanistrum* is not only the first record on this host, but that it is phylogenetically close to strains from *R. sativus*. It is likely that strains of *A. candid*a may develop on *R. raphanistrum* that will severely attack commercial cultivars of *B. juncea* with more extensive cropping of this host in the future. This is expected as a pathotype from *R. raphanistrum* was virulent on *B. juncea* cv. Commercial Brown which was also attacked by the *A. candida* strain from *B. juncea* currently prevalent in W.A. Although the pathotype from *B. oleracea* can also attack *B. juncea*, the hazard is minimal to *B. juncea* as *B. oleracea* is a vegetable crop rarely cultivated in the rainfed grainbelt of W.A. White blister has been recorded in W.A. on shepherd's purse, wild mustard, wild radish and wild turnip, but the role of these weeds in spreading the disease has not been determined (Bokor, 1972). Until recently, *Capsella bursa-pastoris* (shepherd's purse) was the only weed prevalent in W.A. that was carrying an *A. candida* strain that can attack *B juncea* varieties utilized in Australia. Kaur *et al.*, (2011d; Figure 2) demonstrated that *B. tournefortii*, although susceptible to the strain from *B. juncea* and *E. vesicaria* ssp. *sativa*, carries a strain that can only infect itself. This strain shows strong phylogenetic variation to other strains of *A. candida*. The strain from *B. tournefortii*, which was incapable of attacking any other host tested, is clearly isolated from other strains in phylogenetic comparisons.

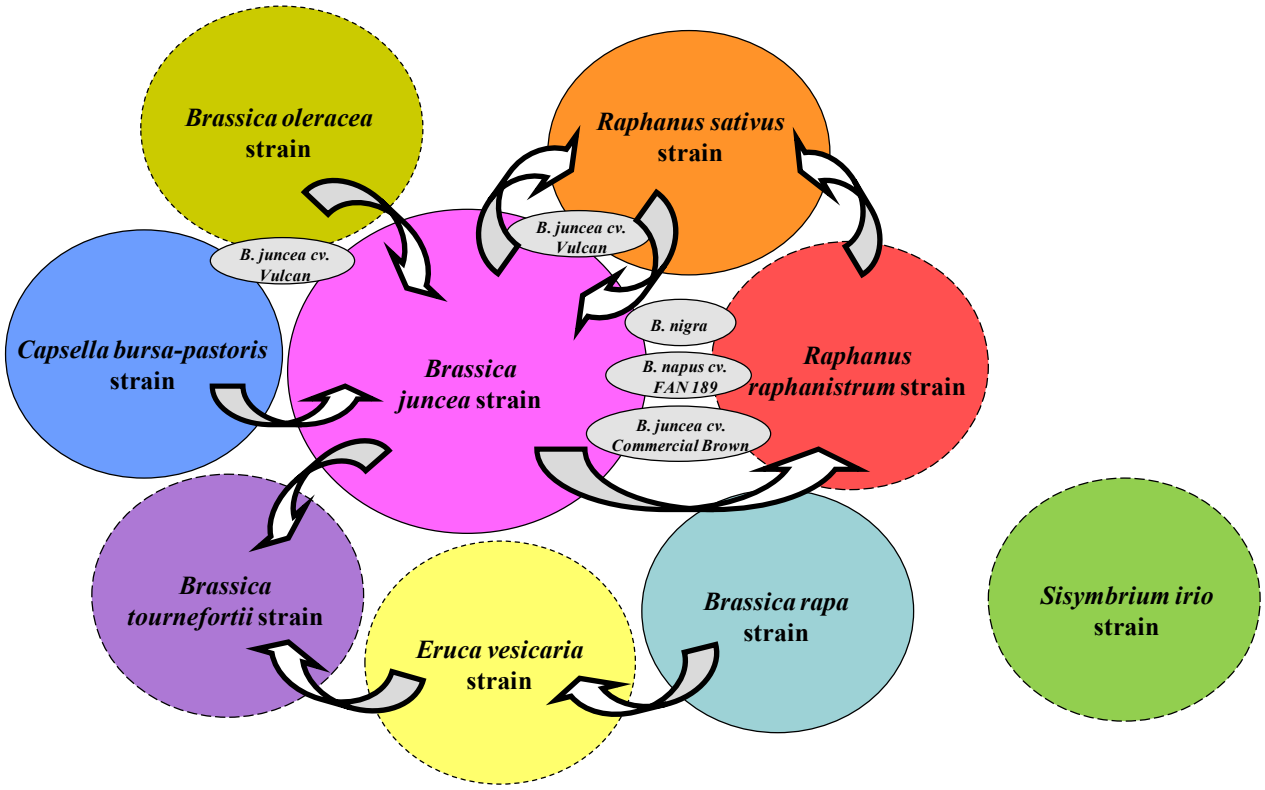

Figure 2. Host specificity and variability in virulence of *Albugo candida* strains collected from nine different cruciferous host species in Western Australia (Kaur, 2010). Circles indicate host of origin of strain and the arrows indicate the susceptibility of the host to the isolate. Dotted circles indicate the isolates reported in W.A. for the first time. *Sisymbrium irio* strain failed to cause disease on all hosts tested and vice versa.

However, it is possible under *B. juncea* cultivation, that *B. tournefortii* and *Sysimbrium irio* could develop other pathotypes that pose threats to *B. juncea* crops. *Sysimbrium irio* is also a widespread weed in W.A., and was not attacked by any of the strains tested, nor did the strain from this host attack any of the other hosts screened by Kaur *et al.*, (2011d). Although *B. rapa* is cropped in the W.A. grainbelt, the *A. candida* strain from this species (a novel pathotype from race 7) was incapable of attacking *B. juncea*. Surveys involving strains from a larger variety of cruciferous crops and weeds may provide a better picture of the frequency of occurrence of the pathotypes of *A. candida* in the agricultural and horticultural landscapes of W.A.

Overall, there are two major potential sources of new pathotypes for *B. juncea* in W.A. First, is the potential for imported seeds, especially of *B. juncea*, that may carry oospores of *A. candida* (Barbetti, 1981) resulting in the introduction of new strains into W.A. Imported seeds can be contaminated by *A. candida* strains from crops and/or weedy species, and thus introduce new races/pathotypes which currently do not occur in Australia. Secondly, there is potential for the development of new strains originating from weeds in the W.A. grainbelt. The changes in cropping practices, especially in relation to the introduction of herbicide tolerant oilseeds, the spectrum and occurrence of flora of cruciferous weeds may change drastically, affecting both the survival and evolution of *A. candida* pathotypes capable of developing on cruciferous weeds functioning as alternative hosts. Furthermore, volunteer plants from crops from previous rotations can also serve as weeds, as was pointed by Rieger *et al.*, (1999). For example, volunteer plants of *B. rapa* or *B. campestris* may serve as weeds in *B. juncea* crops providing carry-over hosts for *A. candida*.

Conclusion

The replacement of *B. napus* with *B. juncea*, especially in regions in W.A. with diminishing rainfall, is expected to result in the widespread occurrence of WBR on *B. juncea* in these regions.

Development of strategies to reduce the hazards from newly evolving pathotypes of *A. candida* is necessary to maintain the viability of this crop. As current work indicates that weed flora can be alternative hosts and can be sources of novel pathotypes, approaches to manage these weeds may also become critical. New knowledge of the pathogenic behaviour and host resistance mechanisms will assist in fortifying host resistance in *B. juncea*. The potential of a splash-borne obligate pathogen to evolve in a geographically isolated region to meet with challenges brought about by the introduction of new hosts and untested cultural practices has been highlighted in this review.

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