



**Special Lecture during 1<sup>st</sup> National Brassica Conference**

**White rust of crucifers : An overview of research progress**

PR Verma

1212 Lorne Avenue, Saskatoon, Saskatchewan, Canada, S7H 1X6

I owe my personal gratitude to the Government of Canada and to a well-known scientist and a dear colleague Dr. R.K. Downey for providing me a very conducive environment for research at the Agriculture Canada Research Station, Saskatoon where I spent 24 years doing what I enjoyed doing most.

I have been retired for fifteen years. Although, Dr. G.S. Saharan, Ex-Professor, CCSHAU, Hisar and myself have published very comprehensive monographs on both *Alternaria* and downy mildew of crucifers. My talk today, include only highlights of my research, and some suggestions for future research on three diseases namely white rust (*Albugo candida*), damping off and brown girdling root rot (*Rhizoctonia solani*), and Sclerotinia rot (*Sclerotinia sclerotiorum*). In addition to these diseases, there is another very serious disease in Canada called Blackleg caused by *Phomalingum* or *Leptosphaeria maculans*. Blackleg is known to have been introduced in Canada through seeds imported from Australia. Therefore, be aware be thoroughly checked for presence of *Phomalingum*.

The name rapeseed in Canada means both *Brassica napus* and *B. Campestris* or *B. rapa*. *B. napus* is completely resistant to white rust, whereas all *B. rapa* varieties are very susceptible. Upto late 1970's, white rust used to cause about 15 per cent yield losses in Canada. After my Ph.D. from the University of Saskatchewan, Saskatoon in Canada in July 1973, my job as a Research Associate at the Agricultural Canada Research Station in Saskatoon was to start a very comprehensive research programme on white rust. The Agricultural Canada Research Station in Saskatoon at that time probably had the world's largest collection of *Brassica* germplasm.

*Albugo candida* (Pers. Ex. Lev.) Kuntze. (*A. cruciferarum* S.F. Gray), the causal fungus of

the white rust disease of many cruciferous crops, causes both local and general infection. Local infections produce white to cream coloured pustules on the undersides of leaves and on stems or pods, while general or flower bud infections (Verma and Petri, 1980) cause extensive distortion, hypertrophy, hyperplasia and sterility of inflorescences (staghead). The staghead phase accounts for most of the yield loss attributed to this disease. The disease occurs in various parts of the world (Saharan and Verma, 1992) and causes extensive yield losses in Polish or turnip rape (*Brassica rapa* L.) (Harper and Pittman, 1974; Petrie, 1973), Indian mustard (*B. juncea* L.) Czern and Coss) (Bains and Jhooty, 1979; Perwaiz *et al.*, 1969), and radish (*Raphanus sativus* L.) (Kadow and Anderson, 1940; Williams and Pound, 1963). Although Canadian and European *B. napus* cultivars are not attacked, many cultivars of this species grown in China are susceptible (Fan *et al.*, 1983). Initially, the study was initiated with the principal objective to develop a reproducible growth chamber inoculation technique for screening germplasm and identification of biological races of *A. candida* infecting *B. rapa*. These and subsequent studies including epidemiology, chemical control, disease resistance and *in vitro* growth are discussed under this paper.

**Growth chamber inoculation technique**

Seeds of susceptible *B. rapa* cv. Torch were planted 2 cm deep in a soil-free growth medium (Stringham, 1971) in 10 cm square plastic pots. Seedlings were thinned to ten plants per pot. Plants were grown in a growth chamber with an 18-h photoperiod ( $312\mu\text{EM}^{-2}\text{S}^{-1}$ ) and at day-night temperature of 21°C and 16°C, respectively. Pots were placed in metal trays and watered by flooding the trays.

To prepare inoculum suspensions, zoosporeangia from pustules on fresh or frozen leaves infected with *A. candida* were suspended in deionized, distilled

water, filtered through cheese cloth, germinated for 2-3 at 5°C, and adjusted to 75000-100,000 zoospores per ml. the inoculum was sprayed on to plants with an atomizer until leaf run-off. Control plants were sprayed with distilled water. The plants were placed in a mist chamber (100% relative humidity) in the growth chamber for 72 h at 16°C to promote infection. Disease incidence and severity observations were recorded 10-days after inoculation.

### Identification of biological races of *A. candida* on *B. rapa*

Although physiological (biological) specialization has long been noted in *A. candida* (Eberhardt, 1904; Melhus, 1911; Hura, 1930; Napper, 1933; Pape and Rabbas, 1920; Savulescu, 1946; Togashi and Shibasaki, 1934; Biga, 1955), each of the above authors was hesitate to describe specialized races. While several methods of classification have been proposed, the method described by Pound and Williams (1963), based on host specificity, appears to be generally accepted.

Until 1975, six biological races of *A. candida* had been identified and classified based on compatibility on different crucifer hosts. (Pound and Williams, 1963): race 1 on *R. sativus*, race 2 on *B. juncea*, race 3 on *Armoracia rusticana* (Gaertn, Mey. and Sherb), race 4 on *Capsella bursa-pastoris* (L.) Medik., race 5 on *Sisymbrium officinale* (L.) Scop., and race 6 on *Rorippa islandica* (L.) Bess. Using these six differential hosts and accessions of *B. rapa* race 7 was proposed for *B. rapa* (Verma *et. al.*, 1975). Race 8 on *B. nigra* was later reported by Delwiche and Williams (1977).

### Oospore germination

The most conspicuous symptom of white rust and probably the major source of yield loss are distortion and hypertrophy of infected inflorescence called "staghead". When ripe, stagheads are almost entirely composed of numerous brown, thick-walled oospores, the form in which the pathogen survives during the off-season, and which are also the source of primary infection are also known as resting spores. Despite their importance in the epidemiology, conditions under which the oospores germinate have

largely been a mystery until our reports in 1974 (Petrie and Verma, 1974; Verma and Petrie, 1975). Prior to our work reports only De Bary (1866) and Vanterpool (1959) have described oospore germination in *A. candida*. Vanterpool (1959) reported germination as "always irregular and uncertain", never exceeding 4 per cent of the spores. In our studies (Petrie and Verma 1974; Verma and Petrie, 1975), we devised three reproducible methods all included very high percentage of germination.

In the first method, a small amount of finely ground staghead powder consisting largely of oospores was scattered over moist filter placed on wet cotton in a petri dish; the lid of the dish was also lined with moist cotton. The plates were incubated at 10-15°C for the period of upto 3 weeks.

In the second method, sterile deionized water or strile or non-strile tap water was allowed to drip slowly onto sintered glass filters of ultrafine porosity where small amounts of oospore powder were scattered. This was done in an attempt to mimic the leaching action that might occur during spring from melting sown or rain. Most of these experiments were run at 10-15°C.

In the third method, the one most routinely used in our laboratory now, a small amount of oospore powder was placed in 50 ml sterile water in a 125 ml flask and incubated at 200 rpm on a rotary shaker at 18-20°C for a period of 3-4 days. The spore suspension was then poured into a petri dish and kept stationary at 13°C for 24 or more. Counts of germinated oospore were made on materials mounted in lactophenol-aniline blue.

All three treatments induced germination of oospores in large numbers. Washing of oospores on a rotary shaker for 3-4 days followed by a day in still culture was the most rapid method and this gave the highest percentage germination for most of the samples 10 years old or more. Oospores required 2 weeks of washing on a sintered glass filter before maximum germination was obtained. On moist filter paper, maximum germination occurred after an incubation period of about 21 days.

Substantial numbers of oospores retained their viability for at least 20 years in dry storage in the laboratory. The highest percentage germination was recorded in the sample which had been collected only 2 weeks prior to the test. It is still not known how long oospores can remain viable in soil. However, germination of 43 percent of oospores from material kept in dry storage for 20 years does indicate their potential longevity (Verma and Petrie, 1975).

Three distinct types of germination were observed. In the most common type, the oospore content was divided into numerous zoospores which were then extruded into a globular, thin-walled vesicle. Zoospores subsequently escaped from the vesicle. Initiation of a vesicle to zoospore escape was completed in 3.0-5.2 minutes with an average elapsed time of 4.1 minutes. Between 40 and 60 zoospores were formed per vesicle (Verma and Petrie, 1975).

In the second germination type, observed only infrequently, a germ tube was produced from the germinating oospore and zoospores which were differentiated in the oospore were discharged through the tube into a so-called "terminal vesicle" formed at the end of the tube. Zoospores subsequently escaped from the vesicle (Verma and Petrie, 1975).

A less commonly observed mode of germination was by a germ tube. The germ tube may be simple or branched. Occasionally up to three branches were observed on a germ tube (Verma and Petrie, 1975).

### **Oospores as primary source of inoculum**

In the absence of reliable methods of germination, the role of oospores both over-wintering agent, as well as incitants of primary infection have largely been speculated. Therefore, one of our next objectives was to determine whether the zoospores from germinating oospores would infect rapeseed plants. The most likely infection site in the field is the emerging cotyledons. Therefore, for pathogenicity studies, plants of *B. rapa* cv. Torch grown in the growth chamber, under conditions described earlier, were kept at cotyledon stage by

removing the growing points. Cotyledons of 10-day-old plants were drop-inoculated with zoospore suspension derived from germinating oospores. Plants were kept under a mist for 3 days. Ten days after inoculation nearly every inoculated plant showed heavy infection in the form of white pustules on the underside of cotyledons. These infection studies suggest that zoospores from germinating oospore are most likely the actual infecting units for initiation of primary infection (Verma *et. al.*, 1975).

The importance of oospore as a source of primary inoculum was also explored in a field experiment conducted under irrigated and dry land conditions (Verma and Petrie, 1980). The treated plots were seeded with seeds of susceptible *B. rapa* cv. Torch mixed with an equal weight of oospore powder. The control plots received no oospore powder. Both number of pustules per infected leaf, and the percentage of plants with stagheads were significantly higher in oospore-infected than those in the non-infected plots. These results convinced suggest that oospores over-wintered in soil, or carried on the seed are most likely the primary source of infection.

### **Staghead in flower-bud inoculated plants**

A number of plant pathologists believe that the hypertrophies or stagheads are produced as a result of early infection of young seedlings and systemic development of the fungus in the plant. This theory was rejected when we routinely obtained stagheads by artificially inoculating flower buds of plants grown under growth chamber and greenhouse conditions (Verma and Petrie, 1980; Goyal *et. al.*, 1996). These, and results of our several field experiments (Verma and Petrie, 1979, 1980) conclusively prove that a large percentages of stagheads in the field are produced as a result of secondary infection of flower buds rather than a systemic development of the fungus in the plant. We now routinely use the flower bud inoculation technique at growth stage 3.1 (Goyal *et.al.*, 1996) for screening advanced breeding lines. Results of these studies have also proved useful in determining actual time of application of both protectant and systemic fungicides.

### Detached-leaf culture technique

Screening lines of *B. rapa* for resistance to race 7 of *A. candida* has been restricted by a lack of growth chamber facilities. To overcome this problem, detached leaf techniques were investigated (Verma and Petrie, 1978). Healthy leaves from the rosette of 12-14-day-old *B. rapa* seedlings were detached and transferred to petri dishes containing 20-25 ml of autoclaved medium consisting of 0.5 ppm benzyl adenine and 0.8% agar. Leaves placed in the dishes with their lower surface on the medium usually within 15 minutes of detachment. Four leaves were placed in a plate and at least 20 leaves were used per treatment. Leaves were drop-inoculated with a zoospore suspension (75,000-100,000 zoospore/ml) derived from zoosporangia of *A. candida* race 7. Control leaves were treated with distilled water. A clean but generally non-sterile technique was used and no attempt was made to manipulate leaves aseptically or to sterilize the inoculum. Leaves were kept under 100% relative humidity for 72-h with day-night temperatures of 21 and 16°C, respectively. Following an initial 24-h dark period, an 18-h day ( $312\mu\text{EM}^{-2}\text{S}^{-1}$ ) was maintained for the duration of the experiment. Observations were recorded 14 days after inoculation.

Plant susceptibility ratings of various *Brassica* species and breeding lines on the inoculated detached leaves were essentially the same as when intact plants were used as the host. In addition, the detached-leaf technique has several advantages to the pathologist and plant breeder. The method facilitates the establishment and maintenance of single zoospore cultures and should enable almost complete isolation from extraneous inoculum, including other races of *A. candida*. Detached-leaf culture also results in greater uniformity of experimental units, more economic use of growth and mist chamber space and allows greater use of environmental control. From the plant breeder's point of view, the program efficiency is increased, since the breeder can select resistant material for inter-crossing from among a vigorous growing plant population rather than a weak group of resistant plants that have survived the unfavourable environment necessary to obtain differential infection on potted plants.

### Temperature effects on disease development:

The detached-leaf culture technique was used to study the influence of temperature on the temporal progression of white rust, the development of disease on leaves of different ages, and the development of disease on leaves detached at the end of light and dark periods (Verma *et al.*, 1983). This information was necessary so that detached-leaf culture technique could be used in the screening of rapeseed cultivars for resistance against *A. candida*.

Temperature, leaf age, time of leaf detachment, and the interaction of these factors had a significant effect on the temporal development of *A. candida* race 7 on detached leaves. Of the temperatures tested (3-32°C), 21°C gave the best disease development, with 18.5°C being the calculated optimum. The disease did not develop at 3°, 29°, and 32°C and was slow to develop at 9°, 12°, and 27°C. there was a highly significant ( $p < 0.01$ ) interaction between length of incubation period and temperature. Unlike intact plants, detached leaves developed pustules on both surfaces. Infection occurred on leaves of all ages, but medium aged leaves supported the maximum number of pustules, followed by the younger leaves. Leaves detached at the end of a dark period developed more pustules than those detached at the end of light period. When using the detached leaf culture technique for screening germplasm for resistance to white rust, we advise an adaxial surface inoculation of cotyledons, or medium aged leaves, and an incubation temperature of 18-22 °C.

### Temperature effects on oospore development:

Epidemiological studies on *A. candida* have focused on the production, viability and germination of zoosporangia (Melhus, 1911; Endo and Linn, 1960; Lakra *et al.*, 1989), and the influence of host age and time of leaf detachment on development of the disease (Verma *et al.*, 1983).

Little is known about the sexual reproduction and genetics of the fungus due to the difficulty in determining the factors responsible for induction of the sexual reproductive phase. The effect of temperature on *in vitro* germination of oospore has

been reported (Verma and Petrie, 1975), however, information on the optimum temperature and the time required for production of oogonia, antheridia and mature oospore in leaf tissue would assist in designing experiments for the study of oogenesis, fertilization and karyogamy. The detached leaf culture technique was used to determine effect of temperature and incubation period on progressive development of oospores of *A. candida* race 2V in *B. juncea* leaves (Goyal *et. al.*, 1996).

The progressive development of *A. candida* oospores in detached leaves of *B. juncea* was largely dependent on incubation temperature. Oogonia and oospore production occurred over the entire range of incubation temperatures of 10-27 °C. The earliest development of oogonia was observed at 25 °C, 7 days after inoculation and incubation. The largest number of oogonia for the 21°, 23°, 24° and 25 °C treatments was observed 12 days post inoculation and numbers decreased after that. At lower and higher temperature development of oogonia occurred later. Maximum numbers of oogonia were recorded at 17 days for the 15 °C treatment at the end of the experiments.

Mature oospores were observed 12 days after incubation at 23° and 24°C. The number of mature oospores was still increasing at 17 days post inoculation at all temperatures. Mature oospores developed later and more slowly at lower and higher incubation temperatures.

The production of *A. candida* oospores in leaf tissues could be important in disease perpetuation. Hypertrophied tissues (staghead) are quite resistant to decomposition and the release of oospores could take to 3-4 years. Leaf tissues are quick to decompose, and thus oospore release from such material could be expected the following year. In naturally infected leaves, oospores are produced in the later part of the season when temperatures are warm (Verma, 1989). Warm temperatures hasten leaf senescence, which in turn enhances tissue decomposition and early release of oospores.

The knowledge of an optimum temperature and time for the development of oospores in detached leaves in our study make it possible to compare the

sequential events of oogenesis, fertilization and karyogamy in various *Albugo* species at the earliest stages of their development. These comparative investigations in *Albugo* species could also be useful in fungal taxonomy. The detached leaf culture technique for oospore development could also be used to determine the heterothallic nature of *A. candida*.

### **Temporal development of *A. candida* infection in cotyledons:**

Progression of white rust infection was studied in cotyledons of susceptible (*B. rapa*, *B. juncea*), moderately resistant (*B. hirta*), and immune (*B. napus*) hosts (Verma *et. al.*, 1975). Cotyledons of all the four *Brassica* species were inoculated with zoospores of *A. candida* produced from germinating oospores or zoosporangia. At different times after inoculation, whole cotyledons were fixed in 95% ethanol-acetic acid (v/v) solution, cleaned in 70% lactic acid at 40°C for 3-4-days and stained with cotton blue in lactophenol. The preparation was examined under the compound microscope.

Generally, the sequence of events from zoospore encystment to formation of the first haustorium was the same in all hosts, although under field conditions, *B. hirta* is moderately resistant and *B. napus* is essentially “immune”. In *B. juncea* the first haustorium was observed 16-18 h after inoculation, while in *B. rapa*, *B. hirta* and *B. napus* the first haustorium was observed about 48 h after inoculation.

In the susceptible hosts, after the formation of the first haustorium, the hyphae grew rapidly and produce variable number of haustoria in each cell. The profusely branched, nonseptate mycelium appeared to fill all available intercellular spaces. And in five to six days after inoculation, the club-shaped zoosporangia develop from a dense layer of mycelium.

In the immune host, usually only one haustorium was formed, after which the hyphae ceased to elongate. At about 72 h after inoculation, a fairly thick, densely staining encapsulation was usually detected around each haustorium, and later only “ghost” outlines of hyphae and haustoria were observed.

Encapsulations were not observed around haustoria of susceptible hosts.

From our observations (Verma *et. al.*, 1975) it seems probable that zoospores derived from germinating oospores constitute the primary inoculum for infection of cotyledons of susceptible *Brassica* species. No evidence of direct infection by the germ tubes was seen (Verma and Petrie, 1975).

The establishment and maintenance of compatible relationship between *A. candida* and its hosts hinges on the successful formation of the first haustorium. A similar sequence of events in both susceptible and immune hosts upto this point suggests that there appears to be no morphological barrier to zoospore encystment, germination and subsequent penetration through stomata. In the incompatible combination it is not clear whether the parasite fails to produce a functional haustorium or whether a viable haustorium is formed within the host cell and is subsequently killed by the host's defence mechanism. The fairly dense, thick encapsulation observed around haustorium of immune host tissue suggests that the later may be the case. In any event it does seem that the decision between compatibility and incompatibility is made within 48 h after inoculation.

Studies using whole mounts (Verma *et.al.*, 1975) can provide a rapid and useful quantitative means of measuring fungal development and can be useful in screening for disease resistance or testing the effects of environmental changes or fungicide treatments. Whole mounts may also provide a useful perspective for ultrastructural studies where the total amount of fungal thallus present in a susceptible host is not always appreciated. Certainly, the massive amount of intercellular mycelium, particularly the much-branched sporangiophore "base", which the host is capable of supporting while still actively photosynthesizing, emphasizes the highly integrated and delicate control occurring in the type of parasitism that has evolved in *A. candida*.

### **Development of a resistant variety and inheritance of resistance:**

Research towards development of a resistant variety was initiated in July 1973, with the

identification of a host-specific *A. candida* race 7 which infects *B. rapa*. Several thousand *B. rapa* lines from different parts of the world were screened using 12-14-day-old plants, detached leaves and flower-bud inoculation techniques. A source of resistance was identified in a line from Mexico. A gene for resistance was transferred to a commercial variety and highly resistant variety name Tobin was licensed in 1980. Several resistant varieties of both *B. rapa* and *B. juncea* have since been licensed.

The inheritance of resistance to *A. candida* race 2 and 7 was studied in crosses involving resistant and susceptible cultivars. The reaction of the  $F_1$  was like the resistant parent, indicating that resistance is dominant and controlled by nuclear genes. Backcrosses of  $F_1$  to the susceptible parent segregated in 1:1 ratio of resistant and susceptible. The  $F_2$  segregation of resistant and susceptible plants gave a good fit to a 3:1 ratio. The studies revealed that resistance is monogenic and could be easily transferred to adapted genotypes via backcrossing (Tewari *et. al.*, 1988).

### **Effect of fungicides on germination of *A. candida* oospores *in vitro*:**

*Albugo candida* oospores occur commonly on *Brassica* seed samples (Petrie, 1975). According to this report the inoculum levels on seeds may be considerably higher than actually required for initiation of infection considering that upon germination a single oospore releases 40-60 zoospores (Verma and Petrie, 1975). Germination of oospores following a period of washing in water (Verma and Petrie, 1975), infection of *Brassica* cotyledons by zoospores from germinating oospores (Verma *et. al.*, 1975), and field experiments showing more foliar and staghead infection in oospore-treated plots than in the controls, support the view that seed-borne oospores constitute primary inoculum for infection of *Brassica* species. Thus treatment even by a protectant fungicide could be important in controlling white rust infections either by inhibiting oospore germination or by killing the zoospores on emergence.

An oospore germination technique was used to study

the effectiveness of 27 fungicides in inhibiting germination at various stages (Verma and petri, 1979). Among the chemicals tested, the three mercurial fungicides, mersil, PMA-10 and pathogen, were the best inhibitors of oospore germination. The total inhibition with any of these fungicides at a concentration of 500 ppm active ingredient was about 75%. Among the non-mercurial compounds, mancozeb and ethazol were the most effective giving total inhibition of about 60%. The inhibition provided by bromosan and pyroxychlor was about 50%. Since none of the fungicides tested in this study was 100% effective, the search for a completely effective, preferably systemic, fungicide needs to be continued.

#### **Efficacy of protectant fungicides in controlling both the foliar and staghead phase of white rust disease:**

Of the nine protectant fungicides tested in the growth chamber, application of either chlorothalol or mancozeb, at 250 or 500 ppm, respectively, 6 h before inoculation and then a week later, controlled the disease effectively (Verma and Petrie, 1979). In view of their mainly protectant action, failure to control white rust by either fungicide applied 24 h and 7 days after inoculation was not surprising, as establishment of *A. candida* infection of rapeseed cotyledons and perhaps leaves would normally be completely within 24 h of inoculation (Verma *et. al.*, 1975).

Two foliar spraying of chlorothalonil (Bravo) in June when the plants were 3-4 weeks old significantly reduced both foliar and staghead infections in the field (Verma and Petrie, 1979). However, in view of the growth room studies on successful initiation of stagheads (Verma and Petrie, 1980), a third application at the time of flowering is also advised. Multiple applications, however, may not be economically feasible under commercial rapeseed production.

#### **Efficacy of metalaxyl in controlling both the foliar and staghead phase of white rust disease:**

Among the systemic chemicals, metalaxyl is probably the best fungicide currently available for

white rust control. Metalaxyl was active against *A. candida* race 7 in *B. rapacv.* Torch (Stone *et. al.*, 1987a, b). Treating the seed with metalaxyl at 5.0g a.i./kg controlled foliar infection in the growth chamber up to the sixth leaf stage, 22 days after planting. When sprayed on the plants up to 4 days after inoculation, metalaxyl reduced foliar infection by 95%. Foliar infection was also controlled when applied as a soil drench but phytotoxicity was evident. Foliar spray application at 2.0 kg a.i./ha or higher reduced foliar infections in three years of field studies. Foliar applications also reduced staghead infections when applied at growth stages 3.2 or 4.1.

Growth chamber and field studies (Stone *et. al.*, 1987a) showed that metalaxyl possesses both protective and eradivative activity against *A. candida*. Control of disease in tissues remote from the site of application indicated that the fungicide moves systemically in rape plants. Disease control was obtained on the foliage, either by seed treatment or soil drenching, and disease eradication was successful when the fungicide was sprayed within 4 days of inoculation, a further evidence of systemicity (Stone *et. al.*, 1987b).

Seed treatment results were promising but in field situation it provided adequate protection only in the early stages of plant growth. The decline in the activity of metalaxyl with increasing age of plants in seed treatment experiments may have been the result of fungicide dilution as the volume of plant tissue increased. Accordingly, infection of flower buds by wind-borne zoosporeangia was not controlled by seed dressing.

In the growth chamber metalaxyl was active as a foliar eradicator for up to 4 days, but when applied 5 or 6 days after inoculation, the fungicide did not prevent sporulation (Stone *et. al.*, 1987a). It would appear, therefore, that after 4 days the fungus had reached a stage of development when fungicide treatment could not completely arrest growth, although pustule size and development were restricted with these late applications.

Results of our studies (Verma and Petrie, 1979, 1980; Stone *et al.*, 1987) suggest that *A. candida* does not require early infections to develop systemically

but can produce stagheads from infections of young flower buds by zoospores arising from wind-borne zoosporangia after plant growth stage 2.6. Successful disease control with metalaxyl, therefore, requires that a sufficient quantity of the fungicide be available well into the growing season. Seed dressings only provide protection for a limited period of time and if conditions favour disease development throughout the season, staghead development will not be controlled. By proving early disease control, however, seed treatment could reduce the secondary inoculum potential in the crop and thereby limit initiation of stagheads from newly infected flower buds.

Bioassay and gas chromatographic analysis of plant tissue extract confirmed the presence of metalaxyl in tissue remote from the site of the treatment (Stone *et. al.*, 1987b). Both bioassay and chemical analysis of plants grown in metalaxyl-drenched soil showed that the fungicide was readily taken up by plants from the soil solution, that the greatest accumulation was in the lower leaves, and that metalaxyl was found in decreasing amount in leaves furthest from the roots and in only small concentrations in the stem and inflorescence. These results indicate that root absorption is an efficient means of metalaxyl was applied to a single leaf it was not detected in the leaves below or above the treated leaf; thus, it is concluded that negligible symplastic translocation occurs.

### **In vitro callus cultures of *A. candida***

The growth of *A. candida* in *B. juncea* (Goyal *et. al.*, 1995) and *B. rapa* (Goyal *et. al.*, 1996) callus cultures was achieved on MS medium (Murashige and Skoog, 1962) supplemented with 1.0mg L<sup>-1</sup>-naphthalene acetic acid and 1.0 mgL<sup>-1</sup>benzylamino-purine. There is a possibility that the growth regulators and sucrose used in the culture medium for host callogenesis played a role in haustorial production. This was the first report of the growth of *A. candida* race 7C on leaf callus tissues of *B. rapa* and of the establishment of haustorial connections between the fungus and host callus cells in this species (Goyal *et. al.*, 1996). Pathogenicity tests with *in vitro*-produced zoospores and oospores confirmed the viability and the virulence of *A. candida* in dual cultures.

The *A. candida*-*B. rapa* dual culture system reported by us has potential for sexual studies of the fungus. Because it was possible to trace the development of antheridia and oogonia from the mycelium, our results support the view that isolates of *A. candida* race 7V are homothallic. This dual culture system could also prove useful *in vitro* selection studies for recovering resistant cells and plants.

In the late 1975, or early 1976, as the saying goes “we received the biggest bang for our buck”. In our routine screening of germplasm for resistance, I noted that plants in two pots were free of white rust pustules. At first, I assumed that these plants probably did not receive enough inoculum. However, the most memorable and laughable thing was what Dr. Downey told me at that moment “Verma you are becoming too expensive for me, because I have to buy you a second free cup of coffee within a year”.

On that day, the project on white rust took a happy turn, and Dr. Al. Klassen, a Plant Breeder, and I started studies towards development of a resistant variety. Research on inheritance of resistance to *A. candida* race 7 in *B. rapa* showed that resistance is governed by a single dominant gene, which can easily be transferred to an adapted susceptible genotype via backcrossing. In 1980, the Agriculture Canada Saskatoon Research Station proudly licensed a highly resistant *B. rapa* variety “Tobin”, and I am pleased to say that *B. rapa* crops in Canada have been almost completely free of white rust for almost 32 years.

### **Suggestions for future research**

1. Information regarding production of oospores inside the seeds, and their possible importance in the survival of the pathogen is lacking.
2. Role of simple or branched germ tube from germinating oospores need to be studied.
3. Single zoospore cultures from germinating sporangia and from germinating oospores must be prepared and their pathogenicity compared.
4. After screening lines for resistance against foliar infections, some select advanced lines must



also be screened for production of stagheads using flower-bud inoculation technique.

5. Using Pound and Williams's differential hosts, although I identified biological races of *A. candida* occurring on different *Brassica* crops in India, I still think that a comprehensive study is needed. Without down playing the importance of several studies from India, I still suggest that the Indian researchers must use International set of differential hosts, like Pound and Williams' rather than probably heterogeneous hosts of their own; without the use of same differential hosts, we are probably comparing apples and oranges.
6. Occasionally, both *A. candida* and *Hyloperonospora brassicae* are present in hypertrophied inflorescence. Our studies have very conclusively proved that *A. candida* alone produces typical hypertrophies. What is not known, however, a) whether *H. brassicae* alone can cause hypertrophies, b) whether *A. candida* and *H. brassicae* synergistically produce bigger or different kind of hypertrophies, and c) whether *A. candida* creates avenues for penetration and growth of *H. brassicae*.
7. The term systemic infection to me implies that *A. candida* after infecting cotyledons or lower leaves grows throughout the plant, and after reaching the pathway inflorescence produces hypertrophies. Unless there is more than one this theory does not hold true, because in a completely healthy plant, we can produce hypertrophies, at will, by inoculating flower buds. Although, it is not that important, the question of systemic infection can very easily be settled by following presence of mycelium after inoculation of only cotyledons or lower leaves.

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