A METHODOLOGY FOR GENETIC STUDIES WITH *ALBUGO CANDIDA*

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Qi Liu

In Partial Fulfilment of the

Requirements for the Degree

of

Doctor of Philosophy

The Department of Plant Science

Winnipeg, Manitoba

ISBN 0-315-77945-4

A METHODOLOGY FOR GENETIC STUDIES WITH *ALBUGO CANDIDA*

BY

QI LIU

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

GENERAL ABSTRACT

Genetic studies with *Albugo candida* have been hampered by a poor understanding of its sexual processes, the difficulty in germinating oospores, and lack of selectable markers. The purpose of this study was to develop a methodology for genetic studies with *A. candida*. Oospores were produced in senescent cotyledons and stagheads of infected plants after inoculation at the cotyledon or early bud stage. High percent (≥80%) germination of oospores were consistently attained by exposing oospores from stagheads to a 1-2% mixture of β-glucuronidase and aryl sulfatase for up to 24 h followed by washing for 3 days on a rotary shaker and chilling for 15 h at 15°C. Isolates pathogenic to four different *Brassica* species were examined for sexual systems. Single-pustule isolates (SPI) were established and cultured either singly or in paired combinations on their homologous hosts. Isolates from *Brassica juncea* (race 2) and *B. rapa* (race 7) were self-fertile (homothallic), since SPI derived from them were capable of sexual reproduction when cultured singly. In contrast, isolates from *B. oleracea* (race 9) and *B. carinata* were self-sterile (heterothallic) as SPI derived from them produced no or few oospores in infected host tissues. Metalaxyl-insensitive isolates of *A. candida* race 2 and race 7 were obtained from field plots treated with metalaxyl. Subsequent laboratory tests showed that both the isolates sporulated profusely on cotyledons of *B. juncea* or *B. rapa* treated with 200 μg a.i. mL^{-1} of metalaxyl whereas metalaxyl-sensitive isolates did not sporulate in the presence of 80 μg a.i. mL^{-1} of metalaxyl. Preliminary genetic analysis suggested that metalaxyl insensitivity in *A. candida* was

controlled by a single dominant gene. The occurrence of cross-fertilization between homothallic isolates of *A. candida* was investigated using metalaxyl sensitivity and variation in pathogenicity as genetic markers. In the F_1, hybrid isolates were metalaxyl-insensitive and virulent only to common suscepts, and thus different from both the parental isolates. In the F_2, isolates with recombinant phenotypes were identified. This study demonstrates that cross-fertilization can occur between isolates pathogenic to different *Brassica* species and that sexual recombination can be a mechanism for generating pathogenic variation in *A. candida*.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. S.R. Rimmer for his guidance and encouragement throughout the course of this research. I would also like to thank Drs. C.C. Bernier, D.E. Harder, R. Scarth, and P.R. Verma for their valuable advice. Special thanks are also extended to Drs. L. Buchwaldt and K. van den Berg for their interest in my work and critical review of some of the manuscripts, and to Ms. P.S. Parks for her technical assistance.

The financial support from the University of Manitoba Graduate Fellowship is also gratefully acknowledged.

FORWARD

This thesis is written in a manuscript style. Four manuscripts are presented, including abstract, introduction, materials and methods, results, and discussion. A general abstract, a general introduction, and a literature review precede the manuscripts. A general discussion and a literature cited terminate the thesis. The first and the third manuscripts (Chapters 3 and 5) will be submitted to Canadian Journal of Plant Pathology. The last manuscript (Chapter 6) will be submitted to Plant Pathology. The second manuscript (Chapter 4) will not be submitted for publication.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

GENERAL INTRODUCTION

White rust, caused by *Albugo candida* (Pers.) Kuntze, is one of the most important diseases of *Brassica* species in Canada and other parts of the world. In western Canada, average yield losses on oilseed turnip rape *Brassica rapa* L. (syn. *Brassica campestris* L.) were between 1.2 and 9% in the 1970s (Berkenkamp 1972, Petrie 1973). In heavily infected fields, yield losses ranging from 30 to 60% have been reported (Bernier 1972). The disease on *B. rapa* and *Brassica juncea* (L.) Czern. & Coss. has been controlled by use of resistant cultivars. However, new pathotypes which are virulent to the resistant cultivars have recently been isolated. Their distribution and significance is as yet unknown. Canadian cultivars of *Brassica napus* L. are highly resistant to all isolates of *A. candida* present in North America (Petrie 1975, 1988; Pidskalny & Rimmer 1985, Verma et al. 1975), but are susceptible to some Ethiopian isolates from *Brassica carinata* (Liu & Rimmer 1991) as well as a European isolate from *Brassica oleracea* (S.R. Rimmer *unpublished data*).

In common with biotrophs in other taxa, *A. candida* is highly specialized. At least ten "biological races" of *A. candida* have been identified and classified based on specificity to different crucifer species (Delwiche & Williams 1977, Hill et al. 1988, Petrie 1988, Pound & Williams 1963, Verma et al. 1975). Although they are most virulent on the host from which they were originally isolated, races of *A. candida* are also capable of infecting some genotypes of related species. Several studies have suggested that *Brassica-Albugo* specificity occurs at both the

species and cultivar level (Hill et al. 1988, Petrie 1988, Pidskalny & Rimmer 1985, Pound & Williams 1963, S.R. Rimmer *unpublished data*). Information regarding the genetics of host-pathogen interactions between *Brassica* species and *A. candida* has been generated solely from studies on the inheritance of resistance in the host. In most cases, resistance to *A. candida* is controlled by single or duplicated dominant genes (Delwiche & Williams 1974, Ebrahimi et al. 1976, Fan et al. 1983, Liu & Rimmer 1991, Tiwari et al. 1988, Verma & Bhowmik 1989). To have a better understanding of the relationship between *Brassica* species and isolates of *A. candida*, parallel studies on the genetics of resistance in *Brassica* species and the genetics of virulence in *A. candida* are required because consideration of only one partner requires an assumption of the number and action of genes being involved in the other. To date, no complementary studies have been made on the inheritance of virulence in *A. candida*. Such studies have been hampered by a poor understanding of sexual processes of this pathogen, difficulties in obtaining consistently high germination of oospores, and lack of selectable markers.

There has only been circumstantial evidence that isolates of *A. candida* from shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.) are heterothallic, but self-fertilization may be induced by secondary infection by *Peronospora parasitica* (Sansome & Sansome 1974), a pathogen frequently associated with *A. candida* on crucifers in nature. Oospores resulting from sexual reproduction are difficult to germinate (de Bary 1886, Vanterpool 1959), but Verma and Petrie (1975) have developed methods to germinate oospores collected from hypertrophic floral parts of infected plants in the field. Studies on sexual recombination in *A. candida*

require easily scored phenotypic markers. Natural variation in pathogenicity to *Brassica* species among isolates of *A. candida* is at present the only character that is phenotypically selectable. No attempts have been made to develop markers for genetic studies with *A. candida*. However, native variation in sensitivity to the systemic fungicide metalaxyl has been reported for several members of the Peronosporales, including *A. candida* race 1 (Valdes & Edgington 1983). Metalaxyl insensitivity in *Phytophthora infestans* (Change & Ko 1990, Shattock 1988) and in *Bremia lactucae* (Crute 1987, Crute & Harrison 1988) has been demonstrated to be controlled by a single nuclear gene with complete or incomplete dominance, thus providing a suitable phenotypic marker for genetic studies with these diploid organisms.

The purpose of this research was to develop a methodology for genetic studies with *A. candida*. The specific aims were: 1) to develop a reliable procedure for production and germination of oospores, 2) to examine the sexual system of isolates of *A. candida* from different *Brassica* species, 3) to select for and characterize isolates with insensitivity to metalaxyl, and 4) to develop procedures for obtaining sexual recombinants in the progeny from crosses between races of *A. candida* using metalaxyl insensitivity and variation in pathogenicity as genetic markers.

Chapter II

LITERATURE REVIEW

Albugo candida (Pers.) Kuntze is a diploid biotroph belonging to the family Albuginaceae of the order Peronosporales in the class Oomycetes. It causes white rust of Cruciferae. This disease caused considerable yield losses of *Brassica rapa* L. (syn. *Brassica campestris* L.) in western Canada during the 1970s (Berkenkamp 1972, Bernier 1972, Petrie 1973). The disease on *B. rapa* and *Brassica juncea* Czern. & Coss. has been controlled by incorporation of resistance into widely grown cultivars. However, new pathotypes of *A. candida* which are virulent on resistant cultivars have been isolated recently although their distribution and significance is presently unknown.

Disease cycle

Albugo candida overwinters mainly as resting spores (oospores) on plant debris or in the soil. As temperatures rise in the spring, oospores germinate and release biflagellate zoospores from sessile or terminal vesicles (Vanterpool 1959, Verma & Petrie 1975). The liberated zoospores swim, encyst and then germinate with the production of germ tubes. Infection is established when germ tubes penetrate host cotyledons and leaves through stomata. They grow intercellularly as a downward spiral and form small, knob-like haustoria in mesophyll cells. Masses of mycelia beneath the adaxial host epidermis produce short, club-shape sporangiophores which give rise to asexual spores (zoosporangia) in basipetal succession. The pressure exerted by the growing pathogen causes

rupture of the host epidermis; zoosporangia are thus liberated and dispersed by air-currents and rain-splash onto host surfaces. Under the favourable condition of free water and mild temperatures, zoosporangia germinate and release zoospores, which initiate secondary infection. The asexual cycle may repeat many times during a growing season, and therefore zoosporangia play an important role in the spread of the disease.

Sexual reproduction occurs when gametangia are formed in hypertrophic stems and floral parts (stagheads). Oospores carried by seeds and overwintered in the soil are the source of primary inoculum in the field (Petrie 1975, Petrie & Verma 1974, Verma et al. 1975, Verma & Petrie 1980). Thus, oospores of *A. candida* are of crucial importance for both initiation of the disease and the survival of the pathogen in the absence of the host.

Spore germination

Zoosporangial germination. Two modes of zoosporangial germination have been reported: 1) the production of zoospores and 2) the direct production of germ tubes (Melhus 1911, Napper 1933). The second mode occurs rarely and usually does not lead to infection (Liu et al. 1989, Pidskalny 1983).

Zoosporangia were reported to germinate within the range of 0-23°C, with an optimum temperature of 10°C (Melhus 1911). Napper (1933) stated that zoosporangia germinated readily between 1-18°C, but not above 20°C. Endo and Linn (1960) observed that zoosporangia of *A. candida* from horseradish germinated at pH of 3.5-9.5 with an optimum of 6.5 at 15-20°C. Melhus (1911) found that chilling stimulated germination of zoosporangia.

Napper (1933) observed that germination required reduction of the water content of zoosporangia to about 30%. However, Lakra et al. (1988) demonstrated that chilling and dehydration were not essential for germination of zoosporangia because up to 75% of zoosporangia germinated without such treatments. Lakra et al. (1988) observed that germination of zoosporangia was slightly delayed at a light intensity of $150\mu Em^{-2}s^{-1}$. However, others showed that zoosporangial germination was not affected by light (Melhus 1911). The inconsistency in experimental results of zoosporangial germination is probably due to the use of different *A. candida* isolates collected from different geographic regions. Williams (1985) developed a technique for germination of zoosporangia by incubating fresh or frozen stored zoosporangia in distilled water at 12-16°C for 2-3 h. This technique is simple and reliable, and has been adopted in other laboratories.

Oospore Germination. Oospores of many species of the Peronosporales are notoriously difficult to germinate (Shaw 1983a). Limited information is available on the germination of oospores of *A. candida*. de Bary (1866) was the first to report that oospores germinated via the production of sessile vesicles. This was later confirmed by Vanterpool (1959), who observed that oospores could also germinate through formation of a discharge tube with a terminal vesicle. When vesicles burst, 40-60 zoospores can be released. Verma and Petrie (1975) reported a third mode of oospore germination by direct production of germ tubes. According to them, germination by sessile vesicles is the most common and the presence of the other two modes of germination is indicative of the high adaptability of the pathogen to environmental stresses.

In the early literature, germination of oospores of *A. candida* was reported to be poor and irregular (<4%, de Bary 1866, Vanterpool 1959). Verma and Petrie (1975) developed some techniques to improve oospore germination. The most efficient way to induce oospore germination was to wash them for up to 7 days on a rotary shaker at 18-20°C followed by one day of still-culture at 13°C. Using this method, oospore germination was increased to 67%. They also obtained good germination of fresh oospores collected from hypertrophic floral parts of naturally infected plants of *B. rapa*. They concluded that oospores of *A. candida* do not have a dormant period. Although there are reports indicating immediate germination of fresh oospores of other oomycetes (Ribeiro 1983), most studies show that oospores are dormant at maturity and that high percentages of germination are obtained only after specific treatments (e.g., Ann and Ko 1988, Förster et al. 1983, Ruben et al. 1980, Salvatore et al. 1973, Shattock et al. 1986, Stanghellini & Russell 1973). As virtually nothing is known about the nature of oospore dormancy or the actual mechanism regulating oospore germination, the interpretation of the experimental results regarding oospore germination should be viewed with caution.

Sexual systems

Little is known of the sexual system of *A. candida*. To date, there has been only one report on sexual activity of this organism. In their study on the life cycle of isolates of *A. candida* attacking *Capsella bursa-pastoris* (L.) Medic., Sansome and Sansome (1974) observed that these isolates were self-sterile but became self-fertile when the host was also infected by *Peronospora parasitica* (Pers. Fr.) Fr., a downy mildew

pathogen frequently associated with *A. candida* on cruciferous crops in nature. They postulated that isolates of *A. candida* from *C. bursa-pastoris* are heterothallic and that oospore production is a result of selfing induced by chemical stimuli released from *P. parasitica*.

Detailed studies have been made on the sexual system of other members of the Peronosporales. In *Phytophthora*, many species are homothallic as single-spore isolates can produce oospores promptly and abundantly. Other species are heterothallic as single-spore isolates are either of the A1 or A2 mating type, and sexual reproduction requires the pairing of two isolates with compatible mating types. However, single isolates of either A1 or A2 mating type can be induced to self by cohabiting species of a compatible mating type (Shen et al. 1983, Skidmore et al. 1983), by diffusible chemical substances (Ko 1978), or by adverse external conditions (Shaw 1983a). Also, self-fertile strains have frequently been recovered in the sexual progeny of predominantly heterothallic species of *Phytophthora* (Sansome 1980), and of downy mildews, including *P. parasitica* (Sherriff & Lucas 1989b). The term "secondary homothallism" is used to describe these strains as they become self-fertile due to having an extra chromosome involved in the reciprocal translocation on which mating type determinants are presumably carried (Michelmore & Sansome 1982, Sansome 1980, Sherriff & Lucas 1989a).

Host specificity

Physiological specialization in *A. candida* has long been recognized. As early as 1904, Eberhardt recognized two specialized forms of *Albugo*: one infecting *Capsella, Lapidium* and *Arabis*, and the other infecting

Brassica, *Sinapis* and *Diplotaxis*. Since then, the existence of distinct biological forms has been reported by many workers (e.g., Melhus 1911, Hiura 1930, Napper 1933, Togashi & Shibasaki 1934, Biga 1955). However, these early workers seemed to be hesitant to use the terminology "biological forms" or "races" for this pathogen, and the extent of specialization was not explored systematically.

In 1963, Pound and Williams studied isolates of *A. candida* collected from six cruciferous hosts representing six different genera. They described the isolate from each genus as a distinct race: race 1 on *Raphanus sativus* L., race 2 on *B. juncea*, race 3 on *Armoracia rusticana* Gaetn., Mey., & Scherb., race 4 on *C. bursa-pastoris*, race 5 on *Sisymbrium officinale* (L.) Scop., race 6 on *Rorippa islandica* (Oeder) Borba's. This classification has subsequently been enlarged to include isolates occurring predominantly on oilseed turnip rape (*B. rapa*) in western Canada (Pidskalny & Rimmer 1985, Verma et al. 1975), on *Brassica nigra* (L.) Koch (Delwiche & Williams 1977), on *Brassica oleracea* L., and on *Sinapis arvensis* L. (Hill et al. 1988). They are designated as race 7, race 8, race 9 and race 10, respectively.

While isolates of *A. candida* cause most severe symptoms on the host species from which they were originally isolated, they are usually capable of colonizing some genotypes of related species (Liu & Rimmer 1991, Pound & Williams 1963, Petrie 1988). This pattern of host specificity has been reported for other fungi attacking *Brassica* species, e.g., *P. parasitica* (Sherriff & Lucas 1990) and *Plasmodiophora brassicae* (Crute et al. 1983), and may be interpreted based on the genome relationship among *Brassica* species established by U (1935, Fig. 2.1). In a recent review on the

genetic control of species specificity of fungal plant pathogens, Newton and Crute (1989) stated that different *Brassica* species having a common genome may be more equivalent to the status of "cultivar" within a single species when host-parasite association is concerned.

The concept of races in *A. candida*, as first proposed by Pound and Williams (1963), is based on the genus or species relationships. Recent studies have clearly shown variation among isolates of *A. candida* in virulence to cultivars within a *Brassica* species (Petrie 1988, S.R. Rimmer *unpublished data*), thus casting doubt on the validity of the basis on which the present "races" are grouped.

Genetics

Genetic markers. Genetic studies of any organism require easily scored phenotypic markers. The utilization of a range of naturally occurring variations and induced mutations as markers has resulted in the development of several Ascomycete fungi as model systems for genetic studies. Genetic studies with members of the Oomycetes have lagged behind, mainly due to the difficulty of manipulating sexual processes and lack of suitable markers.

Physiological and morphological variations (e.g., loci controlling mating type, spore colour, and colony development) have been observed in *Phytophthora* species, but few of these segregated in a Mendelian fashion (Galindo & Zentmyer 1967, Shaw 1988, Shaw & Elliott 1968). Several members of the Peronosporales, including *A. candida*, are biotrophs with hyaline zoosporangia (or conidia); therefore the use of colony morphology or spore colour as visible markers is ruled out. Nutritional mutations,

like auxotrophs, are usually conditioned by single recessive alleles and are useful in selecting for complementing auxotrophs in forcing heterokaryon formation. Auxotrophic markers have successfully been used to study the genetics of several homothallic species of *Phytophthora* (Elliott & MacIntyre 1973, Long & Keen 1977). The selection for auxotrophic mutants for *A. candida* is not possible because it is an obligate parasite. Resistance to antibiotics and fungicides may, however, be induced and selected in a diploid biotroph. Resistance to the systemic fungicide metalaxyl (Ridomil) in *Phytophthora infestans* and *Bremia lactucae* is known to be controlled by a single nuclear gene with complete (Chang & Ko 1989) or incomplete dominance (Crute 1987, Crute & Harrison 1988, Shattock 1988), and has been used as a selectable marker in genetic studies with these diploid organisms. Natural variation among isolates of *A. candida* in pathogenicity may also provide suitable markers for genetic studies, as this pathogen is highly specialized (Delwiche & Williams 1977, Petrie 1988, Pound & Williams 1963, Verma et al. 1975), and resistance to homologous and heterologous isolates of *A. candida* in cruciferous species is usually controlled by single dominant genes (Reviewed in Liu & Rimmer 1991).

Isozymes and restriction fragment length polymorphisms have been developed and used as markers for studying genetics of two oomycetes, *P. infestans* and *B. lactucae*, respectively (Hulbert & Michelmore 1988, Shattock et al. 1986, Spielman et al. 1989, 1990). Molecular markers have several advantages over conventional markers (reviewed by Michelmore & Hulbert 1987) and are no doubt a powerful complement to existing markers in genetic studies.

Genetics of host-parasite interactions. Flor, working with flax (*Linum usitatissimum* L.) and the flax-rust fungus (*Melamspora lini* Desm.), was the first to study simultaneously the genetics of both partners in a host-parasite association (Flor 1971). His results led him to develop the gene-for-gene theory. This states that for each gene conditioning specific resistance in the host there is a corresponding gene conditioning pathogenicity in the parasite (Flor 1956, 1971). Subsequent studies showed that gene-for-gene relationships envisaged by Flor may operate in many host-parasite systems (Day 1974, Crute 1985). In many cases, however, the gene-for-gene relationship has been proposed based solely on the genetics of resistance in the host. Even in the most extensively investigated host-parasite systems, limited data have been obtained from genetic analysis of virulence in the parasite.

The importance of parallel studies of resistance and virulence to determine the genetic basis of host-parasite interaction has been clearly stated by Flor (1959):

> "At least two organisms, the host and the parasite, are involved in infectious diseases. The genes in the host that condition reaction can be identified only by their interaction with specific cultures of the parasite; the genes in the parasite that condition pathogenicity can be identified only by their interaction with specific varieties of the host. Thus, parallel studies of the genetics of disease reaction in the host and of genetics of pathogenicity in the parasite are a requisite to the determination of host-parasite interaction."

Information regarding the genetics of host-parasite interactions

between *Brassica* species and isolates of *A. candida* has been exclusively generated from studies on the inheritance of resistance in the host. Monogenic dominant resistance to race 1 has been reported in several radish (*R. sativus*) cultivars (Bonnet 1981, Humaydan & Williams 1976, Williams & Pound 1963). Resistance to race 2 in *B. nigra, Brassica carinata Braun, B. rapa and B. juncea* has also been found to be controlled by a single dominant gene (Delwiche & Williams 1974, 1981; Ebrahimi et al. 1976, Tiwari et al. 1988). Edwards and Williams (1987) have reported quantitative resistance in a rapid-cycling population of *B. rapa* to *A. candida* race 2. Verma and Bhowmik (1989) have demonstrated that resistance in the *B. napus* line, BN-Sel, to the *B. juncea* pathotype of *A. candida* from India is governed by duplicate dominant genes. Inheritance of resistance to *A. candida* race 7 in the *B. napus* cultivar Regent is conditioned by triplicate dominant genes, two of which were thought to be in a homogeneous condition (Fan et al. 1983). So far, no complementary genetic studies have been made on the inheritance of virulence in *A. candida*.

Amongst oomycetes, only the *Lactuca sativa* (lettuce)-*B. lactucae* (downy mildew) association has been genetically well-characterized (Michelmore et al. 1988). Specificity between the host and pathogen is generally regulated by a gene-for-gene relationship, with 13 dominant genes conditioning specific resistance in the host matched by 14 avirulence genes in the pathogen. Avirulence to R11 (designated as DM11) is determined by two independent genes. The avirulence genes are usually inherited at single loci (Norwood et al. 1983, Michelmore et al. 1984, Norwood & Crute 1984), whereas all the complementary resistance genes so

far characterized are clustered in only four linkage groups (Hulbert and Michelmore 1985, Farrara et al. 1987).

Although a gene-for-gene relationship is generally sufficient to account for the interaction between the specific resistance genes in lettuce cultivars and their matching avirulence genes in *B. lactucae*, there are some cases in which the host-pathogen interaction appears to be governed by a more complex relationship. In analyzing one backcross progeny from matings between an Israeli and an English isolate, Norwood et al. (1983) found that virulence was determined by dominant alleles. Later, it was demonstrated that a non-allelic interaction between an avirulence allele (A) and a dominant inhibitor allele (I) at an independent locus in the pathogen would result in the expression of virulence. Three inhibitor loci have been identified to suppress avirulence to three specific resistance genes in the host (Michelmore et al. 1984, Norwood & Crute 1984).

The host-parasite interaction phenotypes can also be modified by genes with minor effects. For example, avirulence to specific resistance genes R4 and R6 (designated as Dm4 and Dm6, respectively) is controlled by single dominant genes, but intermediate interaction phenotypes in the form of sparse sporulation and macroscopically visible necrosis were frequently observed on the host cultivars carrying Dm4 or Dm6 (Ilott et al. 1989, Michelmore et al. 1984, Norwood & Crute 1984). It was suggested that the range of intermediate reactions was a result of segregation of modifier genes which are highly sensitive to environmental conditions (Michelmore et al. 1984).

There is also evidence for the effect of gene dosages on the outcome

of interactions between resistance and avirulence alleles. Assessment of pathogen development on the cultivar carrying Dm6 revealed that the expression of both resistance and avirulence depended on gene dosage. A general trend of decreasing colonization and sporulation in incompatible genotype combinations was Aa/Rr, AA/Rr, Aa/RR and AA/RR (Crute & Norwood 1986).

A gene-for-gene relationship is also thought to operate in the interaction of *Solanum tuberosum* and *P. infestans* (Person 1959, Shaw 1983a). The genetic basis of resistance to races of *P. infestans* in potato has been well documented (Gallegly 1968), but the genetic control of virulence is still unclear. Segregation for virulence among F_1 progeny was observed in early studies (Gallegly 1970), so it was tentatively concluded that virulence/avirulence to each resistance gene in the host was determined at a single locus and that one parent was homozygous and the other was heterozygous at the locus concerned. Using isozymes as genetic markers, Al-Kherb (1988) analyzed crosses between European and Egyptian isolates and concluded that virulence to four specific resistance genes (R1, R2, R3 and R4) in potato was controlled at single loci, with avirulence dominant to virulence. However, conflicting results were obtained from crosses involving Mexican isolates by Spielman et al. (1989). They showed that virulence to R2 and R4 was conditioned by dominant alleles, whereas virulence to R3 was determined by recessive alleles. Segregation for virulence frequently deviated from Mendelian segregation patterns, suggesting that in some cases a more complex relationship might be operating (Spielman et al. 1989, 1990).

These studies have revealed some constraints associated with genetic

analysis of the Oomycetes. The understanding of the genetic basis of host-parasite interactions in the *S. tuberosum-P. infestans* association and other members of the Peronosporales must await the development of additional markers and further characterization of the genetic basis of resistance in the host. The aberrant segregation ratios of avirulence and virulence phenotypes observed in early genetic studies with *B. lactucae* were later found to be caused by the presence of previously uncharacterized resistance genes in the host or by polyploidy in some isolates (Hulbert & Michelmore 1985, Ilott et al. 1989). Therefore, the importance of conducting parallel studies on the inheritance of resistance in the host and of virulence in the parasite cannot be overemphasized.

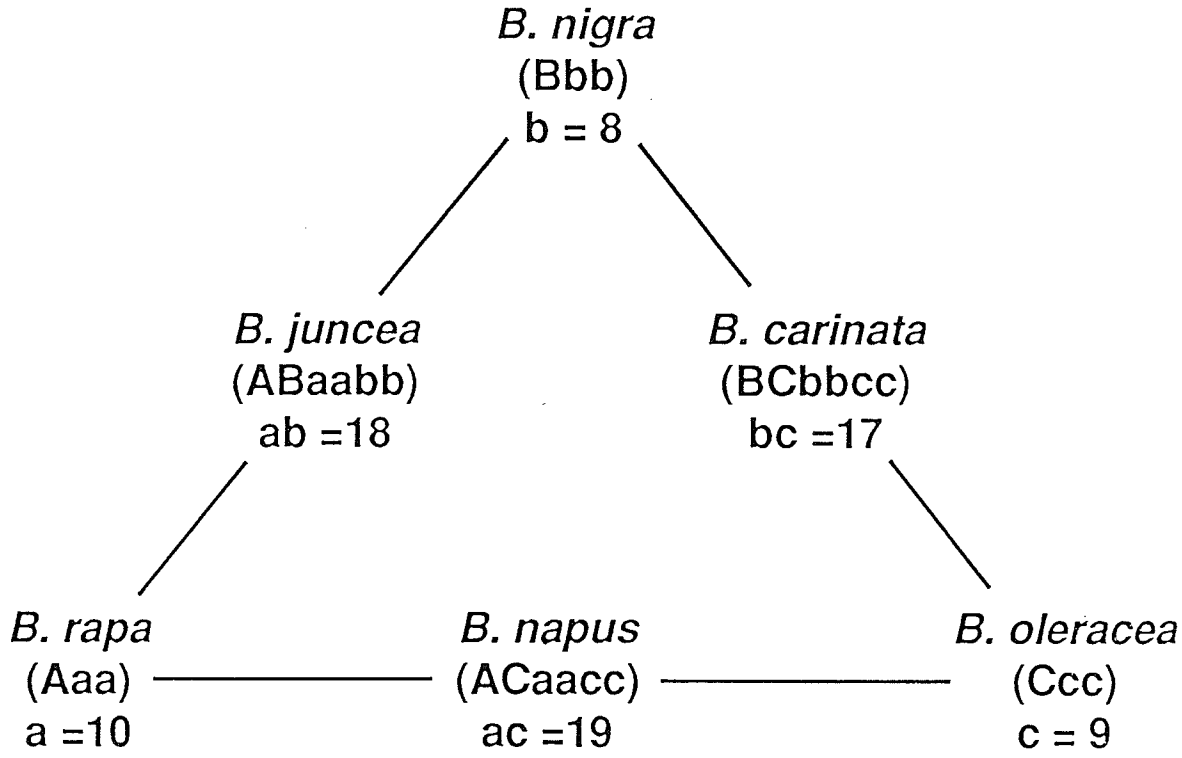


Fig. 2.1. Genome relationship of *Brassica* species (according to U 1935). Capital letter refers to cytoplasm; small letters refer to nuclear genome.

Chapter III

PRODUCTION AND GERMINATION OF OOSPORES OF ALBUGO CANDIDA

Abstract

To facilitate genetic studies with *Albugo candida*, a procedure was developed to obtain good production and germination of oospores under controlled environmental conditions. Oospores were produced in senescent cotyledons and in hypertrophic stems and floral parts (stagheads) of *Brassica juncea* and *B. rapa* by inoculating plants with either race 2 or race 7 at the seedling and early bud stage, respectively. High percent (≥80%) germination of oospores was consistently attained by agitating oospores produced in stagheads in sterile distilled water containing a 1-2% mixture of β-glucuronidase and aryl sulfatase for up to 24 h followed by 3 days of washing on a rotary shaker at room temperature and 15 h of chilling at 13°C. The effect of the enzymes on stimulation of oospore germination depended on stages of oospore maturity. Aging of mature stagheads for two weeks increased oospore germination. Chilling was also a key factor for stimulating oospore germination.

Introduction

Genetic studies on *Albugo candida* (Pers.) Kuntze, the causal agent of white rust of crucifers, have been hampered by the difficulty in controlling sexual reproduction and germinating oospores. Oospores of *A. candida* race 2 (Ac2) and race 7 (Ac7) are produced regularly and abundantly in hypertrophic stems and floral parts (stagheads) of infected plants in the field, but little is known of external factors and host conditions affecting oospore formation. There has been only two reports describing production of oospores in infected host tissues after inoculation of flower buds or detached leaves of *Brassica rapa* L. (syn. *Brassica campestris* L.) with zoospores derived from zoosporangia of an isolate of Ac7 (Verma & Petrie 1980, Verma et al. 1983). No studies have been reported on the germinability of the oospores produced under controlled environmental conditions.

In the earlier literature, germination of oospores of *A. candida* was described as poor and irregular (<4%, de Bary 1866, Vanterpool 1959). Verma and Petrie (1975) reported some methods for germination of oospores collected from naturally infected plants and obtained high percent germination by washing oospores on a rotary shaker for up to 7 days followed by one day of still-culture at 13°C. However, percentages of germinated oospores varied markedly in different oospore samples, ranging from 8.8 to 67%. Studies on *Phytophthora* and *Pythium* species have shown that percent germination of oospores can be considerably increased by passage of oospores through the digestive tract of aquatic and terrestrial snails, or by exposure them to snail gut enzymes (Banihashemi & Mitchell 1976, Jimenez & Lockwood 1982, Salvatore et al. 1973, Shaw 1967,

Stanghellini & Russell 1973).

The objective of the present study was to develop a procedure for production and germination of oospores of *A. candida* at consistently high frequencies so as to meet the basic requirement for genetic studies. Effects of chilling and a mixture of commercial snail gut enzymes, β-glucuronidase and aryl sulfatase, on germination of oospores at different stages of maturity were investigated.

Materials and methods

Pathogen and host. Two local isolates of *A. candida* were used; one was collected from diseased plants of brown mustard (*Brassica juncea* (L.) Czern. & Coss) and the other was from oilseed turnip rape (*B. rapa*). The two collections were tested on differential host cultivars and accessions, and were confirmed to be Ac2 and Ac7, respectively (Pidskalny & Rimmer 1985). The Canadian cultivars Burgundy (*B. juncea*) susceptible to Ac2 and Torch (*B. rapa*) susceptible to Ac7 were used as hosts to maintain and increase the isolates. Seeds were planted 0.5 cm deep in Jiffypots (Jiffy Products Ltd., Shippegan, NB) containing Metro-Mix (W.R. Grace & Co. Canada Ltd., Ajax, Ontario).

Production of oospores in cotyledons. Zoospore suspensions were prepared according to the method described by Williams (1985). Zoosporangia were placed in Erlenmeyer flasks containing an appropriate amount of double-distilled water. The flasks were covered with Parafilm and shaken gently for even distribution of zoosporangia. The resulting suspensions were incubated at 12-16°C for about 3 h to induce zoosporogenesis. Six days after seeding, cotyledons of *Brassica* plants

were inoculated with a zoospore suspension (1-2 × 10^5 zoospores mL^{-1}) of Ac2 or Ac7. A 10 μL droplet of inoculum was delivered on the adaxial surface of each half cotyledon using an Eppendorf Repeater Pipette (Brinkmann Instruments Inc., Rexdale, Ontario). The inoculated seedings were incubated for 24 h in a mist chamber at 20°C. Afterwards, they were transferred to a growth chamber and grown under 18-h photoperiod at day/night temperatures of 22/17°C.

Two methods were used to produce oospores in inoculated cotyledons. With the first method, infected cotyledons were detached 5-7 days after inoculation, rinsed with distilled water to remove asexual spores (zoosporangia), and then placed on moist filter paper in 9-cm petri dishes. The petri dishes were put back to the growth chamber. Detached cotyledons were moistened by adding distilled water regularly with a pipette. After 10-14 days of culture, cotyledons were fixed and cleared using the method described previously (Liu et al. 1989) and examined for the presence of sexual spores (oospores) using light microscopy.

With the second method, the inoculated cotyledons were allowed to senesce naturally on seedlings grown under the same conditions as described above. Brownish cotyledons were collected at the time of abscission and stored in envelopes at room temperature. Segments of dried cotyledons were crushed in a drop of distilled water on a microscope slide and examined for the presence of oospores by light microscopy.

Production of oospores in stagheads. Preliminary experiments had been conducted to optimize the procedure for production of stagheads under controlled environmental conditions. Plants of *B. rapa* cv. Torch were inoculated with a zoospore suspension of Ac7 at the seedling, two-leaf,

six-leaf, or early bud stage (growth stage (GS) 1.0, 2.2, 2.6, or 3.1, Appendix 1). Three different inoculation methods were tested: 1) drop inoculation of cotyledons or leaves, 2) spray inoculation of whole plants, and 3) injection of stems and flower buds. Stagheads were produced by inoculating plants at all four growth stages, but the highest percentage of plants with stagheads was obtained after plants were inoculated at GS 3.1. The three inoculation methods were equally effective in inducing staghead formation. In order to hasten staghead formation, development and maturity, the rapid-cycling lines of *B. juncea* CrGC4-2 and *B. rapa* CrGC1-19 (Williams & Hill 1986) were used as hosts. Plants were grown in 15-cm diameter plastic pots filled with Metro-Mix. Based on results obtained from the preliminary experiments, leaves and flower buds of CrGC4-2 and CrGC1-19 were spray-inoculated with a zoospore suspension of an isolate of Ac2 or Ac7 when plants were at GS 3.1. Each isolate was inoculated onto 20 plants, and the experiment was repeated once. The inoculated plants were incubated in a mist chamber for 72 h and transferred to the growth chamber where they grew under continuous daylight at 20±2°C for 4 wk. Plants with stagheads and/or hypertrophied stems were then moved to the greenhouse and grown to maturity.

Germination of oospores. Cotyledons containing oospores and stagheads produced under controlled environmental conditions were stored at room temperature for at least 2 wk to kill mycelia and zoosporangia. They were then ground to fine powder with a mortar and pestle. Four collections of oospores representing different stages of maturity were examined in this study: oospores 1) from senescent, dried cotyledons, 2) from light-brown stagheads formed on inoculated plants collected at

maturity, 3) from dark-brown stagheads also formed on inoculated plants but collected 2 wk after maturation, and 4) from stagheads formed on naturally infected plants of *B. rapa* and stored at -15°C for over 10 years. Oospore suspensions were prepared by suspending small amounts of the oospore-containing powder in 50-mL Erlenmeyer flasks with 20 mL sterile distilled water (SDW) containing 0, 0.5, 1.0, 1.5, or 2.0% mixture of β-glucuronidase and aryl sulfatase (obtained from *Helix pomatia*, Sigma Chemical Co., St. Louis, MO). The oospore suspensions were cultured on a rotary shaker (200 rpm) at room temperature for up to 24 h. Afterwards, oospores were washed three times with SDW by pelleting in a bench centrifuge and re-suspending with 20 mL SDW. The final oospore suspensions were returned to the rotary shaker. After 72 h, 10 mL aliquots of each spore suspension were transferred to empty flasks and chilled at 13°C for about 20 h.

Germination of oospores was recognized by formation of sessile vesicles and the disappearance of the central reserve globule and granular contents. Counts of germinated oospores were made using light microscopy at 400× magnification. The experiment was conducted in a complete randomized design with three replicates and was repeated once. Results of germination of oospores produced in *B. rapa* are presented. Analysis of variance and trend comparison by polynomial contrasts were performed using the SAS package (SAS Institute Inc. 1985).

The effect of chilling on oospore germination was further evaluated in a separate experiment. Oospores from light-brown stagheads formed on inoculated plants of a rapid-cycling *B. juncea* (CrGC4-2) were suspended in SDW containing 1% of the enzyme mixture. Oospores were washed and

incubated as already described. Observations of oospore germination were made between 0 and 34 h of chilling at 4 h intervals. The experimental design and data analysis were as described above.

Results

Production of oospores in cotyledons. Oospores were produced in inoculated cotyledons of the *B. juncea* and *B. rapa* cultivars through either detached-cotyledon culture or natural senescence. Proportions of inoculated cotyledons containing oospores are shown in Table 3.1. Oospores were first observed in detached cotyledons 18 days after inoculation. Oospores and zoosporangia were frequently observed in the same section of an infected cotyledon, indicating that sexual sporulation and asexual sporulation were not antagonistic processes. Microscopic examination at 400× magnification revealed that many of the oospores produced in cotyledons were colourless, lacking granular contents and a thick oospore wall and probably were immature.

Stagheads were formed on 12.1-43.2% of inoculated plants with a mean of 31.8%. Swelling and deformation of individual florets and terminal inflorescences were visible within 2 wk after inoculation. Deformed inflorescences developed into full stagheads in another 2 wk (Fig. 3.1). These stagheads were identical to those formed on infected plants of *Brassica* species in the field. White pustules were frequently seen on the surface of green stagheads. Stagheads turned brownish at maturity and were largely composed of oospores. Numerous oospores were also produced in hypertrophied stems and individual florets of inoculated plants.

Germination of oospores. Analysis of variance indicates that the

effect of stages of oospore maturity, enzyme mixture and chilling on oospore germination as well as the interactions between and among these factors are highly significant ($P<0.01$). The effect of β-glucuronidase and aryl sulfatase on stimulation of oospore germination was found to depend on the stage of oospore maturity (Fig. 3.2). When no enzyme was applied, only 6% of oospores from light-brown stagheads formed on inoculated plants germinated, but the percentage of germination was increased to 70% by treatment with 1.5-2.0% of the enzyme mixture for 20 h followed by washing and chilling (Fig. 3.2A). Oospores from dark-brown stagheads, which were also formed on inoculated plants but harvested 2 wk after maturation, germinated at a significantly higher percentage than those from light-brown stagheads when no enzyme was applied ($P<0.001$). The percentage of germination of oospores from dark-brown stagheads was further increased with the application of 1-2% of the enzyme mixture ($P<0.05$). Oospores collected from stagheads formed on naturally infected *B. rapa* plants responded to enzyme treatment in a way similar to those from dark-brown stagheads formed on inoculated plants. Ten years of storage in a freezer did not considerably reduce their germinability. Chilling of oospores at 13°C for about 20 h significantly increased percent germination for all the three types of oospores produced in stagheads (compare Fig. 3.2A with Fig. 3.2B). With or without the chilling treatment, percentages of oospore germination were not significantly different at enzyme concentrations between 1-2%, although trend analysis of the enzyme effect using polynomial contrasts indicates that the response of oospores from dark-brown stagheads and those from field stagheads to enzyme treatment still fit a linear model ($P<0.05$).

Despite the sequential treatments employed in this study, oospores produced in senescent cotyledons germinated poorly (Fig. 3.2). Similar results were obtained from experiments using oospores collected from infected tissues of *B. juncea*.

Oospores from light-brown stagheads formed on the inoculated *B. juncea* plants were used to investigate further the effect of chilling on oospore germination. Although some oospores germinated prior to the chilling treatment, the percentage of germinated oospores increased significantly with 6 h of chilling at 13°C following exposure to 1% of the enzyme mixture and washing on a rotary shaker (Fig. 3.3). Continuous chilling permitted more oospores to germinate until the maximum percent germination was obtained with about 15 h of chilling.

Discussion

Oospores are known to be produced late in the growing season in stagheads and senescent leaves of naturally infected plants of *B. rapa* (Verma & Petrie 1980, Verma et al. 1983). The ease of inducing oospore production in cotyledons demonstrated in this study suggests that oospores may develop throughout the growing season in senescent cotyledons and leaves, as well as in hypertrophic stems and floral parts of infected plants. Production of oospores by *Peronospora parasitica* (a pathogen frequently associated with *A. candida* on cruciferous crops in nature) in naturally infected cotyledons and leaves of oilseed rape (*Brassica napus* L.) has also been documented (Sherriff & Lucas 1989b).

Although a large number of oospores was produced in infected senescent cotyledons under controlled environmental conditions, induction

of oospore germination was difficult even after the sequential treatments employed in this study and after other treatments used in preliminary experiments (e.g., heat shock for varying length of time, high-low temperature alteration, washing for a prolonged period of time, and exposure to 0.01-0.20% $KMnO_4$ for 15-20 min). By contrast, oospores produced in lettuce cotyledons by *Bremia lactucae* (a biotrophic member of the Peronosporales) can be germinated after sonication in the presence of glass beads (Michelmore & Ingram 1981) or after >4 wk of culture on a shaker at 5°C (Michelmore et al. 1988). The present study did not allow an adequate explanation for unsuccessful germination of oospores of *A. candida* produced in *Brassica* cotyledons, but immaturity of the oospores could be the cause. Blackwell (1943) reported that oospores of *Phytophthora cactorum* would not germinate until they matured. Jiang et al. (1989) observed nuclear changes in *Phytophthora* species during oospore maturation and germination. They found that the percentage of germination was positively correlated to the percentage of oospores in which nuclei had fused. Nuclear conditions might be useful criteria for evaluating oospore maturity. Future studies involving determination of degree of maturity based on nuclear conditions as well as on morphological features of *A. candida* oospores might help unravel this puzzling question.

Inoculation of rapid-cycling *Brassica* plants at GS 3.1 was found to be efficient for inducing staghead formation. This method is simple and reliable. Large numbers of mature oospores can be obtained within 8-9 wk after planting. Our results also support previous workers' suggestion that under natural conditions stagheads may develop as a result of meristematic infection caused by wind-borne zoosporangia (Verma & Petrie

1980).

Oospores produced in stagheads germinated well after exposure to a 1-2% mixture of β-glucuronidase and aryl sulfatase followed by a period of washing and chilling. Chilling appeared to be of primary importance for germination of oospores of *A. candida*. Without chilling, germination was reduced to approximately 50% for the three types of oospores from stagheads. Chilling is also essential for germination of zoosporangia of *A. candida* (Melhus 1911, Williams 1985).

There have been several reports describing induction of oospore germination in other members of the Peronosporales by either passing oospores through the digestive tract of snails, or by using snail gut enzymes (e.g., Banihashemi & Mitchell 1976, Jimenez & Lockwood 1982, Salvatore et al. 1973, Shaw 1967, Stanghellini & Russell 1973). It has been suggested that the enzymes may enhance permeability of the oospore wall, permitting the diffusion of nutrients required for germination (Stanghellini & Russell 1973). However, the actual mechanism for the effect of β-glucuronidase and aryl sulfatase on stimulation of oospore germination is as yet unknown.

In the present study, oospores from dark-brown stagheads harvested 2 wk after maturation germinated at a consistently higher frequency than other types of oospores. Aging or after ripening may be responsible for the increased percent germination. Other investigators working on *Phytophthora* species have also reported that oospore germination increased with increased age of oospores (e.g., Ann & Ko 1988, Ayer & Lumsden 1975, Förster et al. 1983, Kaosiri et al. 1980).

The development of a procedure for good production and germination

of oospores of *A. candida* under controlled environmental conditions is an important step towards genetic studies with this oomycetous organism. This procedure has been used in our laboratory to investigate sexual systems of isolates pathogenic to four different *Brassica* species and to study sexual recombination in *A. candida*. It should also facilitate physiological and biochemical studies on sexual processes of *A. candida*.

Table 3.1. Proportions of infected cotyledons containing oospores of *Albugo candida*

Method I*		Method II†	
Race 2 in Burgundy	Race 7 in Torch	Race 2 in Burgundy	Race 7 in Torch
Experiment 1			
72.4 (6.3)§	69.8 (5.2)	89.5 (3.1)	85.3 (2.1)
Experiment 2			
67.8 (7.1)	73.1 (6.0)	88.1 (4.9)	92.2 (3.3)

* Production of oospores through detached-cotyledon culture; values represent the mean of 10 plates with 15 cotyledons per plate.

† Production of oospores through natural senescence; values represent the mean of 3 flats with 100 cotyledons per flat.

§ Values in brackets are standard deviations.

Fig. 3.1. Hypertrophic floral parts induced by inoculation of *Brassica* plants at growth stage 3.1. A, swelling and deformation of inflorescence; B, an immature staghead with white pustules on the surface; C, a distorted floret; D, mature and dried stagheads.


A
B
C
D

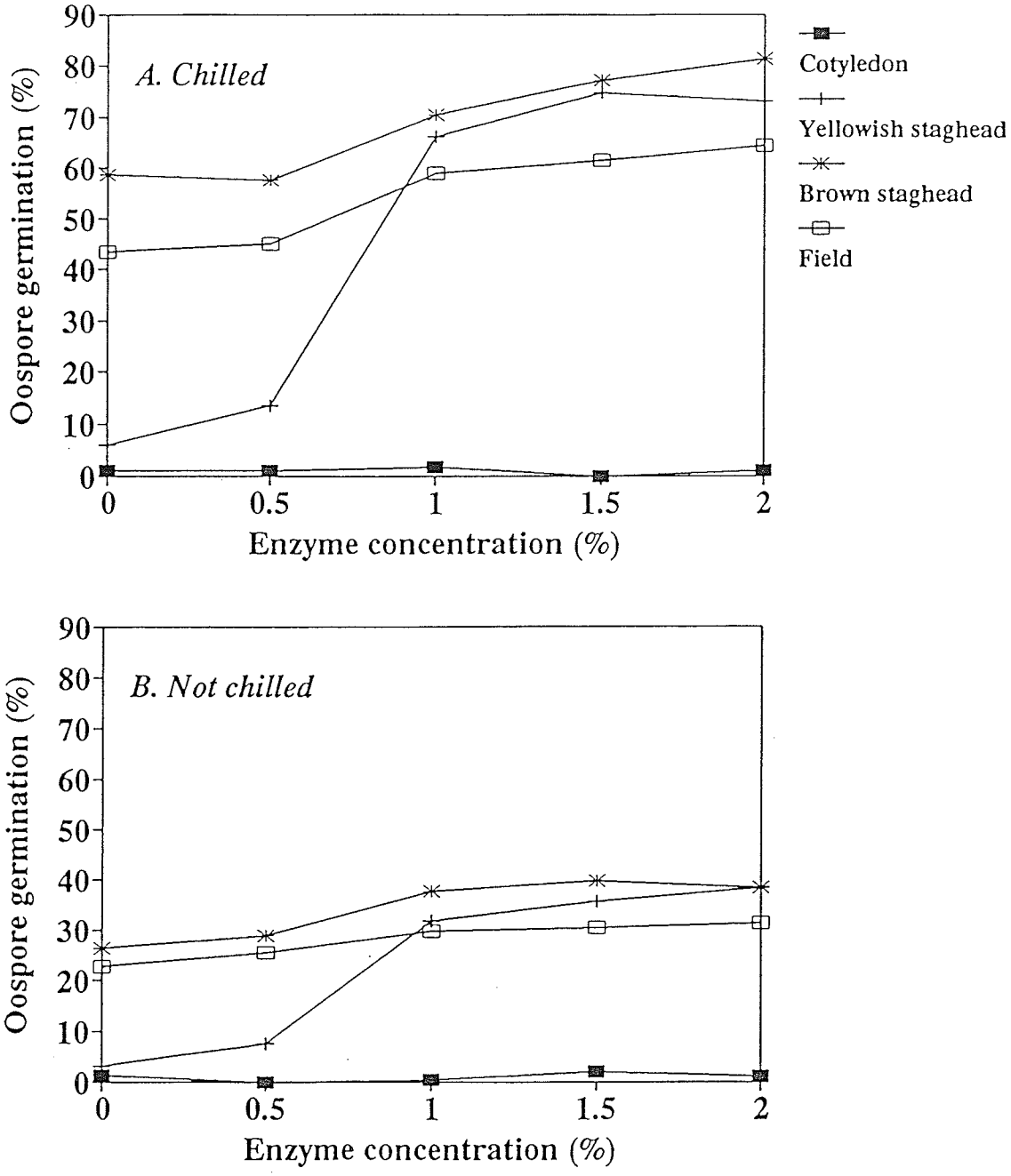


Fig. 3.2. Effects of snail gut enzymes (β-glucuronidase and aryl sulfatase) and chilling on germination of *Albugo candida* oospores at different stages of maturity. Following the enzyme treatment, oospores were washed three times using a desk centrifuge, cultured on a rotary shaker for 3 days, and chilled at 13°C for 20 h (A) or not chilled (B). Points represent the mean of three replicates with 100 oospores per replicate.

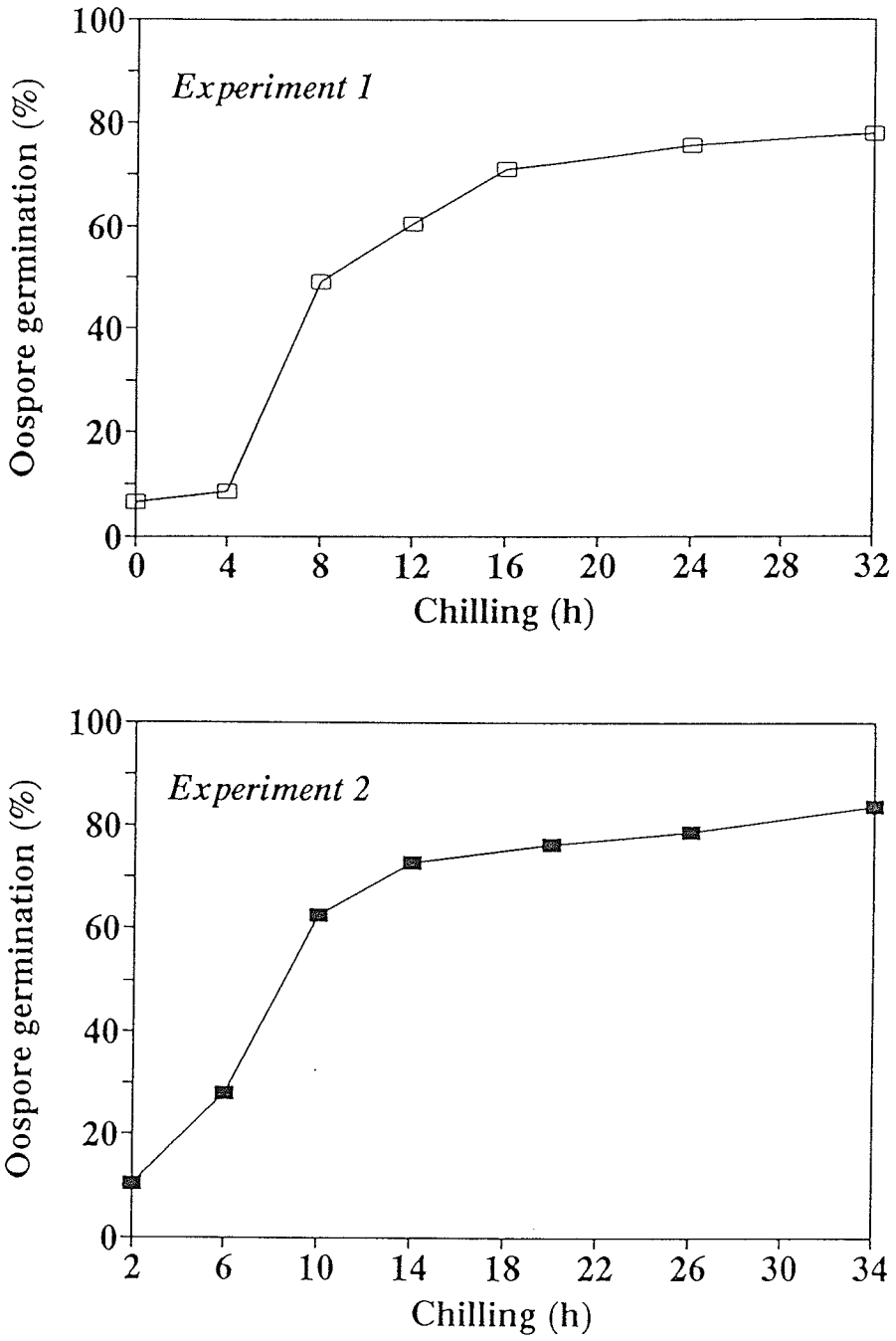


Fig. 3.3. Effect of chilling on germination of *Albugo candida* oospores from light-brown stagheads produced under controlled environmental conditions. See Fig. 3.2 for the sequential treatments. Points represent the mean of three replicates with 100 oospores per replicate.

Chapter IV

HOMOTHALLISM AND HETEROTHALLISM IN ALBUGO CANDIDA

Abstract

Techniques for producing oospores of *Albugo candida* in senescent cotyledons as well as in hypertrophic floral parts of infected *Brassica* plants were used to determine the sexual system of *A. candida*. Single-pustule isolates (SPI), each presumably derived from a single uninucleate zoospore, were established for isolates pathogenic to four different *Brassica* species. They were inoculated either singly or in paired combinations on their homologous hosts at the seedling and early bud stage (growth stage 3.1). Two Manitoba isolates, pathogenic to *Brassica juncea* (Ac2) and to *B. rapa* (Ac7) respectively, were considered to be homothallic (self-fertile) because SPI derived from them were capable of producing oospores abundantly when cultured singly on their homologous hosts. In contrast, a European isolate pathogenic to *B. oleracea* (Ac9) and three Ethiopian isolates pathogenic to *B. carinata* (Accar1, 6 and 7) were considered to be heterothallic (self-sterile) because SPI derived from them produced no or few oospores in infected host tissues. No or very few oospores were produced when two self-sterile SPI derived from Ac9 were cultured together with each of the four self-sterile SPI derived from Accar1, 6 or 7 on cotyledons of common susceptible cultivars (*Brassica napus*).

Introduction

Albugo candida (Pers.) Kuntze, the causal agent of white rust of crucifers, is a biotrophic member of the order Peronosporales in the Oomycetes. This class of organisms is diploid with meiosis occurring in the gametangia (Caten & Day 1977). Fertilization occurs by passage of a single haploid nucleus from an antheridium into the oosphere where it fuses with the egg nucleus of the oogonium, resulting in the formation of a zygote, the oospore (Webster 1980).

In nature, sexual reproduction of *A. candida* occurs in hypertrophic stems and floral parts (stagheads), and in senescent leaves of infected plants. Oospores are the form by which the pathogen overwinters in the soil (Petrie 1975, Petrie & Verma 1974, Verma & Petrie 1980, Verma et al. 1975). Despite the importance of oospores as the source of primary inoculum and of genetic variation, the sexual system of *A. candida* is largely unknown. There has only been circumstantial evidence that isolates of *A. candida* from Shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.) are heterothallic with sexual reproduction being stimulated by secondary infection by *Peronospora parasitica* (Sansome and Sansome 1974), a pathogen frequently associated with *A. candida* on cruciferous crops in nature. No further investigations have been made to identify mating types of the isolates pathogenic to *C. bursa-pastoris*. Studies determining sexual systems of *A. candida* isolates pathogenic to other cruciferous species are also lacking. Therefore, the inability to control sexual crosses of *A. candida* remains a major barrier to genetic analysis of this pathogen.

Detailed studies on the sexual system have been made on other

members of the Peronosporales. Both homothallism and heterothallism have been demonstrated in *Phytophthora* species (Savage et al. 1968, Shaw 1983b), and in several downy mildews, e.g., *P. parasitica* (Sherriff & Lucas 1989a,b) and *Bremia lactucae* (Michelmore & Ingram 1980, 1982).

The present study was undertaken to examine the sexual system of isolates of *A. candida* pathogenic to four different *Brassica* species.

Materials and Methods

Isolates. One isolate of *A. candida* pathogenic to *Brassica juncea* (L.) Czern. & Coss. (Ac2), one isolate pathogenic to *Brassica rapa* L. (Ac7), one isolate pathogenic to *Brassica oleracea* L. (Ac9), and three isolates pathogenic to *Brassica carinata* Braun (Accar1, Accar6, and Accar7) were examined in this study. The isolates of Ac2 and Ac7 were obtained in Manitoba from naturally infected brown mustard and oilseed turnip rape, respectively. The isolate of Ac9 was kindly supplied by I.R. Crute, Institute of Horticultural Research, East Malling, Maidstone, England. The isolates of Accar1, 6, and 7 were supplied by Y. Semeane, Holetta Research Centre, I.A.R. Addis Ababa, Ethiopia. Pathogenicity phenotypes of these isolates had been checked prior to this study on differential host cultivars or accessions belonging to six major *Brassica* species.

Procedures for obtaining single-pustule isolates. Single-pustule isolates (SPI) were established for each isolate of *A. candida* on cotyledons of the following cultivars or University Manitoba (UM) accessions: Ac2 on the *B. juncea* cv. Burgundy, Ac7 on the *B. rapa* cv. Torch, Ac9 on the *B. oleracea* accession UM5051, and Accar1, 6, and 7 on

the *B. carinata* accession UM4029. Methods for raising plants and preparing zoospore suspensions are described in Chapter 3. Inoculation was performed 6 days after seeding when cotyledons had expanded for 24-28 h. A 10 μL droplet of inoculum was placed on the adaxial surface of each half cotyledon using an Eppendorf Repeater Pipette (Brinkmann Instruments Inc., Rexdale, Ontario). Inoculum concentrations of 1×10^2, 2×10^2, 4×10^2, 6×10^2, 8×10^2, and 1×10^3 zoospores mL^{-1} were used for each isolate to assure development of single pustules on individual cotyledons. The inoculated seedlings were incubated as previously described (Liu et al. 1989).

Single pustules were collected from individual cotyledons before the epidermis of infected host tissues ruptured. Each pustule was assumed to be produced by a single uninucleate zoospore. Single-pustule isolates were subcultured individually on cotyledons of their homologous hosts for three generations, using a single pustule to initiate each generation. Isolates derived from the third generation were then subcultured for two more generations to obtain sufficient zoosporangia for determination of sexual systems. Single-pustule isolates were designated by letters in combination with Arabic numeral(s), and stored individually in gelatin capsules (Parke-Davis Size No. 00) in glass screw-cap vials at -15°C.

Determination of sexual systems. The sexual system of the *A. candida* isolates pathogenic to four different *Brassica* species was determined in separate experiments, each involving at least five SPI. Zoospore suspensions of SPI were prepared separately, quantified with a haemocytometer, and the concentration adjusted to $1\text{-}2 \times 10^5$ zoospores mL^{-1}. Single-pustule isolates derived from each of the original isolates were

used to inoculate cotyledons of their homologous hosts either singly or in paired combinations. In paired combinations, zoospore suspensions were mixed in equal volume prior to inoculation. About 80 cotyledons were inoculated with each SPI or combination of SPI. The original isolates were included for comparison. Interaction phenotypes (IP) were scored 8 to 12 days after inoculation on a 0-9 scale based on the intensity of asexual sporulation (Williams 1985).

Infected cotyledons were collected at the time of abscission from the host plants and stored in envelopes. Segments of dried cotyledons were examined microscopically for the presence of oospores using the method described in Chapter 3. To quantify oospore production, counts of oospores were made in 10 cotyledons containing oospores for each SPI or combination of SPI. Cotyledons were ground individually in a mortar with 1 mL of distilled water, and the number of oospores formed in a cotyledon was estimated using a haemocytometer. Standard deviations were computed to give an indication of variation in numbers of oospores produced in cotyledons that had been inoculated with the same SPI or combination of SPI.

The isolates were also examined for their ability to produce oospores in hypertrophic stems and floral parts of infected plants. Rapid-cycling *B. juncea* (CrGC4-2), *B. rapa* (CrGC1-19), *B. oleracea* (CrGC3-4), and *B. carinata* (CrGC6-1), were obtained from the Crucifer Genetics Cooperative, University of Wisconsin, Madison, WI, and their reaction to *A. candida* had been determined in preliminary experiments. CrGC4-2, CrGC1-19 and CrGC3-4 were uniformly susceptible to their homologous isolates, whereas CrGC6-1 segregated for reaction to the *B. carinata*

isolates. The rapid-cycling *Brassica* lines were inoculated with their respective homologous isolates at the early bud stage (growth stage (GS) 3.1, Appendix 1) and incubated to induce staghead formation under the conditions described in Chapter 3. Brownish, mature stagheads were collected and ground finely for microscopic examination.

Results

High intensity of asexual sporulation was observed for each SPI and combination of SPI on cotyledons of their respective homologous hosts. Due to seed shortage, the accession UM5051 used for developing SPI of Ac9 was substituted with two hybrid cabbage cultivars, Bartolo and Renova, to determine the sexual system of Ac9. Asexual sporulation by SPI from Ac9 on Bartolo and Renova was as profuse as that on UM5051 although the time between inoculation and the onset of asexual sporulation on these two cultivars was prolonged by 2-3 days as compared with that on UM5051.

The isolate of Ac2 and the isolate of Ac7 were considered to be homothallic as all the SPI tested were capable of producing oospores in senescent cotyledons when inoculated singly (Table 4.1). Although the number of oospores produced varied from one cotyledon to another, cotyledons which supported heavier asexual sporulation by *A. candida* generally contained more oospores, indicating that the intensity of sexual sporulation was positively correlated with that of asexual sporulation. Further evidence for homothallism of these two isolates came from a subsequent experiment, in which about one-third of plants inoculated at GS 3.1 formed typical stagheads. Microscopic observation revealed that these stagheads contained numerous oospores.

Neither the isolate of Ac9 nor its single-pustule derivatives were capable of sexual reproduction. The three Ethiopian isolates from *B. carinata* produced oospores in inoculated cotyledons, but the SPI derived from them did not produce any oospores when cultured singly. Oospores were occasionally observed in cotyledons of *B. carinata* UM4029 when SPI Accar1-4 was used in combination with SPI Accar1-3 or with SPI Accar1-6 (Table 4.2). When the rapid-cycling lines of *B. oleracea* CrGC3-4 and *B. carinata* CrGC6-1 were inoculated at GS 3.1 with SPI derived from Ac9 or Accar1, swelling and distortion of floral tissues occurred at low frequencies, but no oospores were observed in distorted host tissues.

An additional experiment was conducted to see if oospores would be produced when self-sterile SPI from the *B. oleracea* isolate were cultured together with those from *B. carinata* isolates on common susceptible hosts. *Brassica napus* cultivars Stellar and Westar, susceptible to both the *B. oleracea* and *B. carinata* isolates, were chosen for this experiment. Two SPI derived from the isolate of Ac9 were cultured together with each of the four SPI from *B. carinata* isolates Accar1, Accar6 or Accar7, but only a few oospores were observed in cotyledons co-inoculated with SPI Ac9-6 and SPI Accar1-8 (Table 4.3).

Discussion

The present study suggests that *A. candida* has both homothallic and heterothallic isolates. Single-pustule isolates derived from the Manitoba isolates of Ac2 and Ac7 produced oospores abundantly in senescent cotyledons and stagheads of infected plants, and were thus considered to be homothallic or habitually self-fertile. These two Manitoba isolates

had been subcultured on cotyledons of their homologous hosts in a growth chamber for several to many asexual generations before they were used in this study. The establishment of SPI, each presumably derived from a single uninucleate zoospore, did not reduce them to single mating types. This indicates that self-fertility in these two isolates was a relatively stable property, and was not due to a mixture of heterothallic isolates of compatible mating types.

However, our study does not exclude the possibility that *A. candida* is predominantly heterothallic and that self-fertility found in the isolate of Ac2 and Ac7 is due to secondary homothallism. Self-fertility due to secondary homothallism has been reported in predominantly heterothallic species of *Phytophthora* (Mortimer et al. 1977) and in downy mildews, e.g. *P. parasitica* (Sherriff & Lucas 1989b) and *B. lactucae* (Michelmore & Ingram 1982). Cytological studies suggest that self-fertile isolates are trisomic for one of the four chromosomes involved in a reciprocal translocation on which mating type determinants are presumably located, and thus exhibit a form of secondary homothallism (Michelmore & Sansome 1982, Sansome 1980, Sherriff & Lucas 1989a). In contrast, no reciprocal chromosome translocation was found in truly homothallic species of *Phytophthora* (Sansome 1980). Therefore, cytological studies may aid in determining whether the two self-fertile isolates of *A. candida* are truly homothallic.

The absence of oospores in most inoculated cotyledons and in all distorted floral tissues of *B. oleracea* and *B. carinata* indicated that the *B. oleracea* and *B. carinata* isolates of *A. candida* examined in this study were probably heterothallic. Isolates of *A. candida* pathogenic to *C.*

bursa-pastoris were also considered to be heterothallic, since formation of oospores in infected host tissues was stimulated by a secondary infection by *P. parasitica* (Sansome & Sansome 1974). In the present study, pairing of SPI from the three *B. carinata* isolates with those from the isolate of Ac9 did not result in good oospore production and therefore, evidence in support of Sansome and Sansome's hypothesis regarding induced selfing is still lacking. For heterothallic species of *Phytophthora*, there has been strong evidence of selfing induced by cohabiting species of compatible mating type (Shen et al. 1983, Skidmore et al. 1984) or by external stimuli (Shaw 1983a).

Only one *B. oleracea* isolate and three *B. carinata* isolates were examined in this study. The relative frequencies of mating types of a heterothallic species in natural populations could be more or less equal or strongly biased with one mating type being rare and the other widely distributed (Shaw 1988). The three original Ethiopian isolates from *B. carinata* produced oospores, whereas SPI derived from them did not. A possible explanation is that the original collections are a mixture of isolates of compatible mating types with one at a low frequency. This is actually not an uncommon situation in fungi (e.g., *Phytophthora* species) with prolific asexual sporulation (Shaw 1988). It should be mentioned here that Accar6, Accar7 and probably other *B. carinata* isolates are heterogeneous for their reaction to *B. carinata* cultivars or accessions as some SPI derived from them lost pathogenicity on *B. carinata*. The pathogenicity and virulence of the Ethiopian isolates should be further characterized, so that their sexual system can be further investigated to identify isolates of compatible mating types.

The understanding of the sexual system and the ability to control sexual crosses between isolates of *A. candida* are prerequisites to genetic studies with this pathogen. The homothallic behaviour of the isolate of Ac2 and Ac7 reported here, the isolation of metalaxyl-insensitive mutants (Chapter 5), and the availability of differential host cultivars provide opportunities to determine if sexual recombination occurs in the progeny from crosses between these two isolates.

Table 4.1. Oospore production in cotyledons of *Brassica juncea* cv. Burgundy and *Brassica rapa* cv. Torch following inoculation with 5 single-pustule isolates of *Albugo candida* race 2 or race 7, singly and in paired combinations

Single-pustule isolate and combination of isolates	No. of oospores ($\times 10^3$) per cotyledon*			
	Race 2 in Burgundy		Race 7 in Torch	
	Mean	±SD	Mean	±SD
Ac2/Ac7-1	4.4	3.06	16.9	10.56
2	15.8	6.89	15.5	7.96
3	6.1	4.53	19.3	7.15
4	13.3	8.03	14.9	9.71
5	13.8	5.63	15.7	8.63
1 + 2	4.9	3.07	24.2	10.04
1 + 3	5.2	3.16	11.9	8.13
1 + 4	4.4	1.96	16.0	9.36
1 + 5	5.1	2.42	34.6	12.80
2 + 3	10.5	4.79	8.8	7.16
2 + 4	12.1	6.01	23.0	7.72
2 + 5	9.1	6.70	18.4	20.18
3 + 4	13.9	6.67	8.9	4.65
3 + 5	13.4	6.85	20.3	15.11
4 + 5	13.0	5.03	8.1	7.37

* Data were based on the microscopic observation of 10 cotyledons in each case.

Table 4.2. Infection severity and oospore production in cotyledons of *Brassica carinata* accession UM4029 following inoculation with single-pustule isolates of an Ethiopian isolate of *Albugo candida* from *B. carinata*, singly and in paired combinations

Single pustule isolate	Accar1				
	2	3	4	6	10
Experiment 1					
Accar1-2	7.8*(-)	8.3 (-)	8.2 (-)	7.4 (-)	8.0 (-)
3		8.3 (-)	8.3 (±)	7.5 (-)	7.6 (-)
4			8.8 (-)	8.1 (±)	8.2 (-)
6				8.0 (-)	7.5 (-)
10					8.2 (-)
Experiment 2					
Accar1-1				8.7 (-)	7.8 (-)
5				8.4 (-)	9.0 (-)
8				8.5 (-)	8.3 (-)
9				8.7 (-)	9.0 (-)
Experiment 3					
Accar6-4					6.8 (-)
6					5.8 (-)
9					7.2 (-)

* Infection severity (scale 0-9) reflects the intensity of asexual sporulation.

- = no oospore observed; ± = oospores were occasionally observed.

Table 4.3. Infection severity and oospore production in cotyledons of *Brassica napus* cultivars, Stellar and Westar, following co-inoculation of single-pustule isolates (SPI) from three *Brassica carinata* isolates and two SPI from one *Brassica oleracea* isolate of *Albugo candida*

Single pustule isolate	Accar1		Accar6	Accar7
	8	10	2	7
Ac9-6	6.7* (±)	7.5 (-)	8.1 (-)	7.5 (-)
Ac9-10	7.8 (-)	6.7 (-)	7.4 (-)	7.9 (-)

* Infection severity (scale 0-9) reflects the intensity of asexual sporulation.

- = no oospore observed; ± = oospores were occasionally observed.

Chapter V

IDENTIFICATION AND CHARACTERIZATION OF METALAXYL-INSENSITIVE ISOLATES OF ALBUGO CANDIDA

Abstract

Isolates of *Albugo candida* were collected from field plots treated with metalaxyl. Evaluation of dose-response under controlled environmental conditions indicated that these isolates were insensitive to 200 μg a.i. mL^{-1} of metalaxyl when it was applied as a foliar spray 2 days after inoculation. In contrast, sensitive isolates of *A. candida* did not sporulate on cotyledons of *Brassica* plants treated with ≥80 μg a.i. mL^{-1} of metalaxyl. Phytotoxicity was observed at leaf margins when metalaxyl was applied as a foliar spray at concentrations of ≥300 μg a.i. mL^{-1} and as a soil drench at all the concentrations (40-200 mg m^{-2}) used. Single-pustule isolates, each presumably derived from a single uninucleate zoospore, were established from the sexual progeny of a selfed metalaxyl-insensitive (MI) isolate originating from the *Brassica juncea* cv. Burgundy. Patterns of segregation in S_1 and S_2 progenies suggested that metalaxyl insensitivity was controlled by a single dominant gene. These findings are discussed in relation to the occurrence of MI isolates in the crop which had never been treated with metalaxyl and to the possibility of using metalaxyl insensitivity as a selectable marker for genetic studies on *A. candida*.

Introduction

Albugo candida (Pers.) Kuntze is the diploid, oomycetous organism causing white rust of crucifers. Physiological specialization in *A. candida* has long been recognized, and at least ten "biological races" have been identified (Delwiche & Williams 1977, Hill et al. 1988, Pound & Williams 1963, Verma et al. 1975). Of these, race 2 and race 7 are of great importance for *Brassica* crop production. Race 2 causes the most severe symptoms on brown mustard (*Brassica juncea* (L.) Czern. & Coss.), but can infect many genotypes of other *Brassica* species, including oilseed turnip rape (*Brassica rapa* L.) (Petrie 1988, Pound & Williams 1963). Race 7 is largely restricted to *B. rapa*, but can also attack *Brassica napus* varieties from China (Fan et al. 1983) and some genotypes of *B. juncea* (Petrie 1988). Therefore, it is of interest to know whether the two races would exchange genetic information through sexual recombination when they colonize the same host plant. Since both race 2 and race 7 were determined to be homothallic (Chapter 4), an easily scored phenotypic character should be used along with variation in pathogenicity as genetic markers to distinguish hybrid from selfed progeny in the oospore population produced by co-inoculation of the two races on a common suscept.

Isolates of *A. candida* are not morphologically distinguishable, and it is not possible to obtain auxotrophic mutants because *A. candida* is an obligate parasite. Insensitivity to fungicides may, however, be induced or selected in a diploid organism because such a character is often dominant or semidominant (Shaw 1983b). The acylalanine fungicide metalaxyl (Ridomil) is very effective in controlling plant diseases caused

by oomycetes (Cohen & Coffey 1986). However, widespread occurrence of metalaxyl-insensitive (MI) isolates in the field has been frequently reported (reviewed by Crute et al. 1987) since the fungicide was first commercially used in the 1970s. Because native variation in sensitivity to metalaxyl has been reported for several members of the Peronosporales (Coffey et al. 1984, Hunger et al. 1982, Shew 1984, Stack & Millar 1985), including *A. candida* race 1 (Ac1) attacking radish plants (Valdes & Edgington 1983), it was thought that search for metalaxyl insensitivity based on spontaneous mutation would be rewarding.

This paper reports the presence of insensitivity to metalaxyl in two populations of *A. candida* and the potential of using metalaxyl insensitivity as a genetic marker.

Materials and methods

The pathogen and host. One isolate of *A. candida* race 2 (Ac2) and one isolate of race 7 (Ac7) were used in this study. They were collected from susceptible cultivars of *B. juncea* or *B. rapa* grown in Manitoba. The *Brassica* hosts used in this study are listed in Table 5.1.

Growth chamber selection for metalaxyl insensitivity. Seeds of the *B. juncea* cv. Burgundy and *B. rapa* cv. Torch were planted 0.5 cm deep in individual cells of 12-pack multipot (Koral Products Ltd., Bramalea, Ontario) filled with Metro-Mix (W.R. Grace & Co. Canada Ltd., Ajax, Ontario). The multipots were drenched with metalaxyl (Ridomil, 240 g L^{-1} EC, Ciba-Geigy Canada Ltd.) at concentrations of 0, 40, 80, 120, 160, and 200 mg a.i. m^{-2}) before planting or 2-4 days after planting. Cotyledons of cultivars Burgundy and Torch were inoculated with a zoospore suspension

of Ac2 or Ac7 six days after planting. Procedures for inoculation and incubation are described in Chapter 3.

Field selection for metalaxyl insensitivity. Two plots were established at the Experimental Station on the Fort Garry Campus, the University of Manitoba in the summer of 1988. Seeds of the cultivars Burgundy and SV03202 were treated with metalaxyl at the rate of 0.5 g a.i. per kg of seed using the method described by Stone et al. (1987). The treated seeds were placed on paper towels and sown on the next day. Seeding rate was 1.6 g per metre of row, with row spacing of 30 cm. Metalaxyl was also applied as foliar sprays at 4-6 day intervals using a 15-L backpack sprayer. The spray schedule and concentrations used are shown in Table 5.2.

Untreated seeds of cultivars Burgundy and SV03202 were mixed with small quantities of fine powder containing oospores of Ac2 and Ac7, respectively. They were sown in three rows at both ends and two rows in the centre of the plot to serve as spreader rows. These spreader rows were subsequently inoculated with zoospore suspensions of either Ac2 or Ac7 when plants were at growth stage (GS) 2.1, 2.5, 3.1, and 4.1, respectively (Appendix 1). After inoculation, spaces between rows were moistened, and two thirds of the spreader rows were covered with plastic tents overnight for dew formation. Plants protected with metalaxyl were checked regularly for development of white rust symptoms. Zoosporangia were collected from about 20 metalaxyl-treated leaves of cv. Burgundy or SV03202 and stored in gelatin capsules (Parke-Davis Size 00) as two separate collections at -15°C.

Testing field isolates for response to metalaxyl. The sensitivity to

metalaxyl of isolates collected from the treated field plots was compared to that of metalaxyl-sensitive (MS) isolates of Ac2 and Ac7 in the growth chamber. Methods for raising plants and for inoculation were as described above. Metalaxyl was applied as a foliar spray 2 days after inoculation at concentrations of 0, 20, 40, 60, 80, and 100 μg a.i. mL^{-1}. For each concentration, 45-50 seedlings grown in multipots were sprayed, and the experiment was repeated once. Isolates were classified as insensitive and sensitive to metalaxyl based on their ability to sporulate on cotyledons 12 days after inoculation.

Determining levels of metalaxyl insensitivity. Zoosporangia that developed on cotyledons of cv. Burgundy or Torch receiving 80 or 100 μg a.i. mL^{-1} of metalaxyl were collected and used to inoculate the homologous hosts treated with 200 μg a.i. mL^{-1} of metalaxyl. Zoosporangia thus obtained were used as base populations for establishment of single-pustule isolates (SPI) using the method described in Chapter 4. To determine levels of insensitivity, metalaxyl was applied as a foliar spray 2 days after inoculation at concentrations of 0, 200, 300, 400, 500, 600, 700, and 800 μg a.i. mL^{-1}. Examination for pustule formation commenced 8 days after inoculation. Dose-response of MI SPI of Ac2 and Ac7, MIAc2-A5 and MIAc7-A2, to metalaxyl will be presented. MS SPI of Ac2 and Ac7, MSAc2-A3 and MSAc7-A1, were included for comparison.

Testing selfed progenies for virulence and response to metalaxyl. Five MI SPI of Ac2 were tested for virulence in a preliminary experiment. The SPI MIAc2-A5, which was as virulent as the MS isolate of Ac2 on the *B. juncea* cv. Burgundy, was selected and passed through a sexual cycle (selfed) on the rapid-cycling *B. juncea* CrGC4-2 by inoculating plants at

GS 3.1. Single-pustule isolates were established from first-generation (S_1) and second-generation (S_2) oospore progeny. They were tested for virulence on differential host cultivars and two common suscepts (Table 5.1). Experiments were laid out in a randomized complete block design with two replicates. Each replicate consisted of 12 seedlings per host-isolate combination. Interaction phenotypes were scored 8 days after inoculation using a 0-9 scale (Appendix 2). Data were subjected to analysis of variance using SAS package (SAS Institute Inc. 1985).

Single-pustule isolates derived from selfed oospore populations were also assessed for sensitivity to 200 μg a.i. mL^{-1} of metalaxyl. The experimental design was the same as above. The parental isolate MIAc2-A5 as well as MS SPI, MSAc2-A3 and MSAc7-A1, were included as controls.

Results

Selection for metalaxyl-insensitive isolates. No MI isolates were obtained from the growth chamber study in which metalaxyl was applied as a soil drench. Sporulation of MS isolates of Ac2 and Ac7 was completely inhibited on cotyledons of their homologous hosts grown in the presence of 40-200 mg a.i. m^{-2} of metalaxyl, no matter when the fungicide was applied. Chlorosis and brittleness occurred at leaf margins at all the concentrations used, indicating phytotoxicity. The number of seedlings with phytotoxic symptoms increased with increasing concentrations of metalaxyl.

In both field plots, inoculated plants of spreader rows were heavily infected with *A. candida*. Many of these plants formed stagheads in early August. Despite the presence of high inoculum pressure, only a small

number of plants repeatedly treated with metalaxyl had pustules on their leaves.

Testing field isolates for response to metalaxyl. Zoospore suspensions were prepared with some of the zoosporangia collected from about 20 leaves of the *B. juncea* cv. Burgundy or of the *B. rapa* cv. SV03202 treated with metalaxyl. They were used to inoculate cotyledons of the homologous hosts sprayed with up to 100 μg a.i. mL^{-1} of metalaxyl. Field isolates were found to be capable of sporulating profusely on cotyledons of cv. Burgundy or Torch receiving 100 μg a.i. mL^{-1} of metalaxyl 2 days after inoculation. In contrast, MS isolates of Ac2 and Ac7 did not sporulate at 80 μg a.i. mL^{-1} of metalaxyl and therefore, were distinguishable from MI isolates of Ac2 and Ac7 (Fig. 5.1). In the absence of metalaxyl, MI isolates sporulated as readily (within 7 days) as did MS isolates of Ac2 and Ac7 on cotyledons of their homologous hosts. In the presence of metalaxyl, however, sporulation was usually delayed 2-3 days, and pustules generally developed at the basal part of cotyledons (Fig. 5.2).

Determining levels of metalaxyl insensitivity. Table 5.3 shows levels of metalaxyl insensitivity of SPI derived from the field isolates. MI SPI of Ac2 (MIAc2-A5) and Ac7 (MIAc7-A2) sporulated well on their respective homologous hosts treated with up to 200 μg a.i. mL^{-1} of metalaxyl. Leaf margins became chlorotic and brittle when $\geq$300 μg a.i. mL^{-1} of metalaxyl was applied.

Testing selfed progenies for virulence and response to metalaxyl. The S_1 progeny were obtained by selfing MIAc2-A5 on the common suscepts. Eight SPI established from the S_1 oospore population remained virulent to

Burgundy, UM3512 and CrGC1-18 (Table 5.4). Thus, the parental isolate and its sexual progeny were of *B. juncea* pathotype (i.e., race 2). Two of the eight S_1 progeny isolates failed to sporulate on cotyledons treated with 200 μg a.i. mL^{-1} of metalaxyl (Table 5.4). This suggests that insensitivity is dominant to sensitivity and that the parental isolate MIAc2-A5 is heterozygous at the locus regulating metalaxyl insensitivity. Three MI S_1 isolates (MIAc2-B1, MIAc2-B5 and MIAc2-B7) were selected and selfed to obtain the S_2 generation. Fifteen SPI were established from each of the three S_2 oospore populations. All the S_2 SPI derived from the selfed S_1 isolates, MIAc2-B1 and MIAc2-B5, were insensitive to metalaxyl, virulent to Burgundy and avirulent to Torch, suggesting that the two isolates were homozygous for the alleles controlling these three characters. All the 15 S_2 SPI derived from the selfed S_1 isolate, MIAc2-B7, were uniformly virulent to Burgundy and avirulent to Torch, but three of them were sensitive to metalaxyl, indicating that this isolate was homozygous for virulence to Burgundy and avirulence to Torch, but heterozygous for insensitivity to metalaxyl.

Discussion

In western Canada, metalaxyl has never been commercially used to control white rust. Therefore, MI isolates of *A. candida* obtained in this study were naturally occurring variants in the population of Ac2 and Ac7. Mutants of *A. candida* race 1 with insensitivity to 150 μg a.i. mL^{-1} of metalaxyl were isolated from one of three radish plots sprayed with 200 g a.i. ha^{-1} of metalaxyl at 7-10 day intervals during a single growing season (Valdes & Edgington 1983). Hunger et al. (1982) and Coffey et al. (1984)

reported natural variation in sensitivity to metalaxyl between and within species of *Phytophthora*. Stack and Millar (1985) isolated a MI mutant from a wild-type isolate of *Phytophtora megasperma*, which had never been exposed to metalaxyl. This mutant was stable in response to metalaxyl and did not differ significantly from the wild type for zoospore production, germination of zoosporangia, or pathogenicity. Data from these and other studies (Bruin & Edgington 1981, 1982; Holmes & Channon 1984, Shew 1984) support the hypothesis that metalaxyl-insensitivity occurs spontaneously in natural populations of oomycetes.

This study has shown that the mutants of Ac2 and Ac7 obtained from field plots treated with metalaxyl were insensitive to 200 μg a.i. mL^{-1} of metalaxyl whereas MS isolates of Ac2 and Ac7 did not sporulate in the presence of 80 μg a.i. mL^{-1} of metalaxyl. Metalaxyl, applied either as a foliar spray at $\geq$300 μg a.i. mL^{-1} or as a soil drench at all the concentrations used was toxic to *Brassica* plants. Phytoxicity was also observed on radish leaves sprayed with $\geq$150 μg a.i. mL^{-1} of metalaxyl (Valdes & Edgington 1983) and on oilseed turnip rape drenched with 1.25-5 $\times 10^4$ g a.i. ha^{-1} of metalaxyl (Stone et al 1987). The obligate parasitism of *A. candida* in combination with sensitivity of *Brassica* plants to metalaxyl appeared to exclude the possibility of obtaining isolates with higher levels of insensitivity to metalaxyl.

The pattern of segregation for metalaxyl insensitivity among S_1 progeny indicates that insensitivity is dominant to sensitivity and that the parental isolate MIAc2-A5 was heterozygous for the locus conditioning metalaxyl insensitivity. The S_2 data further indicate that metalaxyl insensitivity in *A. candida* is controlled by a single dominant gene, but

the genetic basis of this character needs to be further established by crossing a MI isolate to a MS isolate and critically testing segregation ratios in the F_2. Metalaxyl insensitivity in other oomycetes e.g., *Phytophthora infestans* and *Bremia lactucae* has been demonstrated to be controlled by a single nuclear gene with complete (Chang & Ko 1990) or incomplete dominance (Crute 1987, Crute & Harrison 1988, Shattock 1988).

This study has shown that metalaxyl insensitivity occurs naturally within *A. candida*, and demonstrated that this character can be expressed in an heterozygous condition and therefore, might be a suitable marker for genetic studies of the diploid organism *A. candida*.

Table 5.1. Origins of *Brassica* hosts used in this study and their interaction with race 2 (Ac2) and race 7 (Ac7) of *Albugo candida*

Host	Origin	H-P interaction*		Supplier
		Ac2	Ac7	
Brassica juncea				
Burgundy	Canada	+	-	Univ. of Manitoba
CrGC4-2	USA	+	-	P.H. Williams, WI
UM3512†	USA	+	+	P.H. Williams, WI
Brassica rapa				
Torch	Canada	-	+	Univ. of Manitoba
SV03202	Sweden	-	+	G. Olsson, Svalof
CrGC1-18	USA	+	+	P.H. Williams, WI

* Host-pathogen interactions; + = compatible (susceptible); - = incompatible (resistant).

† A selection from rapid-cycling *Brassica juncea*.

Table 5.2. Schedule for metalaxyl treatment and concentrations used in field studies to isolate metalaxyl-insensitive isolates of *Albugo candida*

Date of treatment	Growth stage	Mode of application	Metalaxyl concentration
June 16	0.0	Seed dressing	5 g a.i. kg^{-1} seed
July 7	2.4	Foliar spray	150 g a.i. ha^{-1}
July 13	3.1	Foliar spray	100 g a.i. ha^{-1}
July 18	3.2	Foliar spray	150 g a.i. ha^{-1}
July 22	4.1	Foliar spray	300 g a.i. ha^{-1}

Table 5.3. Levels of metalaxyl insensitivity of single-pustule isolates of *Albugo candida* determined in growth chamber tests*

Isolate	Host	% seedlings with pustules							
		0	200	300	400	500	600	700	800
		(μg a.i. mL^{-1} metalaxyl)							
MIAc2-A5	Burgundy	97†	96	59	55	26	15	5	3
MSAc2-A3	Burgundy	95	0	0	0	0	0	0	0
MIAc7-A2	Torch	93	78	36	11	0	0	0	0
MSAc7-A1	Torch	94	0	0	0	0	0	0	0

* Metalaxyl was applied by spraying cotyledons of *Brassica* plants 2 days after inoculation.

† For each concentration, 45-50 seedlings were inoculated with an homologous isolate and treated with metalaxyl; values are the mean of two replicates.

Table 5.4. Virulence phenotype and response to metalaxyl of S_1 progenies from a metalaxyl-insensitive isolate MIAc2-A5 of *Albugo candida*

Single pustule isolate	*Brassica juncea*		*Brassica rapa*		Response to metalaxyl*
	UM3512	Burgundy	CrGC1-18	Torch	
MIAc2-A5	7.3†	8.6	6.6	1.9	I
MIAc2-B1	9.0	7.1	7.0	2.9	I
-B4	7.7	6.4	5.7	0.4	S
-B5	9.0	8.6	8.4	2.0	I
-B7	9.0	6.8	6.4	2.1	I
-B8	8.8	7.1	6.8	3.0	S
-B9	7.0	6.4	6.0	1.4	I
-B10	7.0	6.3	6.2	1.1	I
-B11	8.9	6.5	5.5	1.6	I
MSAc2-A3§	7.4	8.1	6.9	0.8	S
MSAc7-A1‡	5.4	0.0	7.1	8.5	S
Standard error = 0.52					

* I = insensitive, S = sensitive.

† Based on a 0-9 scale (Appendix 2). Values represent the mean of ratings on 24-30 seedlings.

§ Metalaxyl-sensitive race 2 check.

‡ Metalaxyl-sensitive race 7 check.

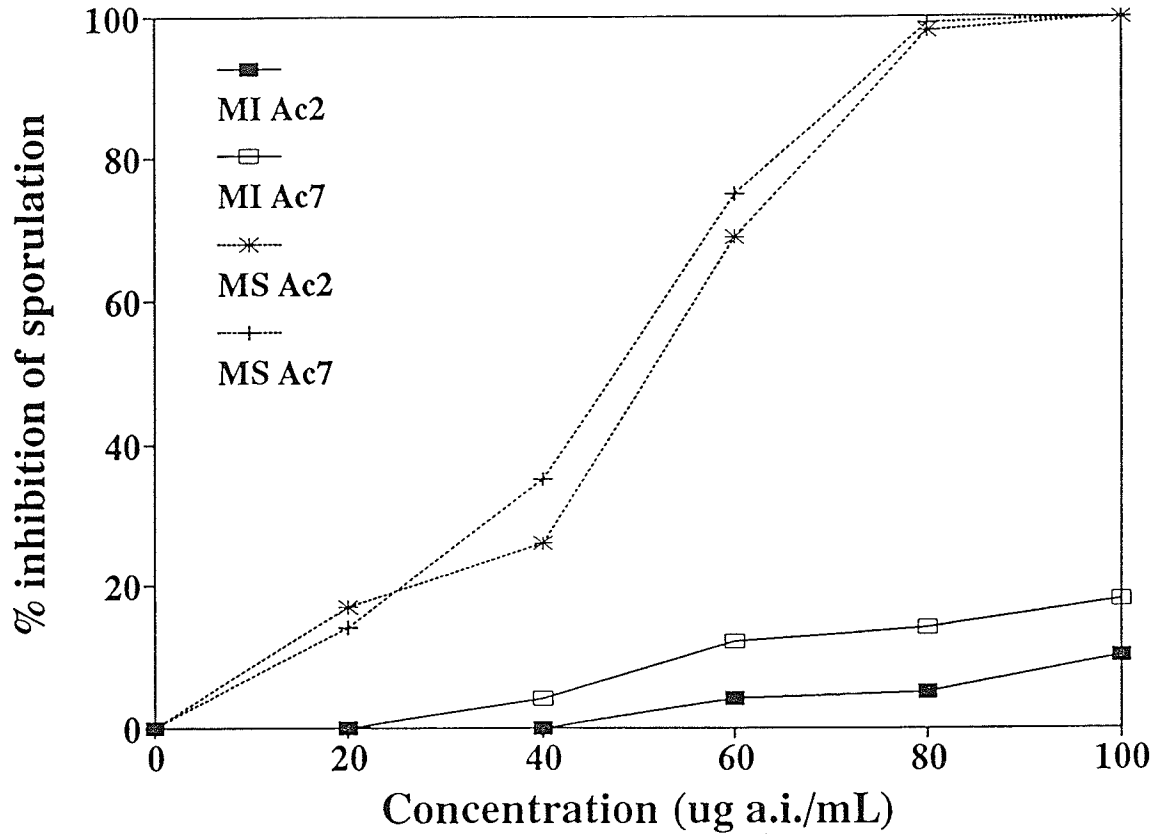


Fig. 5.1. Response of sensitive and insensitive isolates of *Albugo candida* to metalaxyl when applied as a foliar spray 2 days after inoculation. Points represent the mean of two replicates.

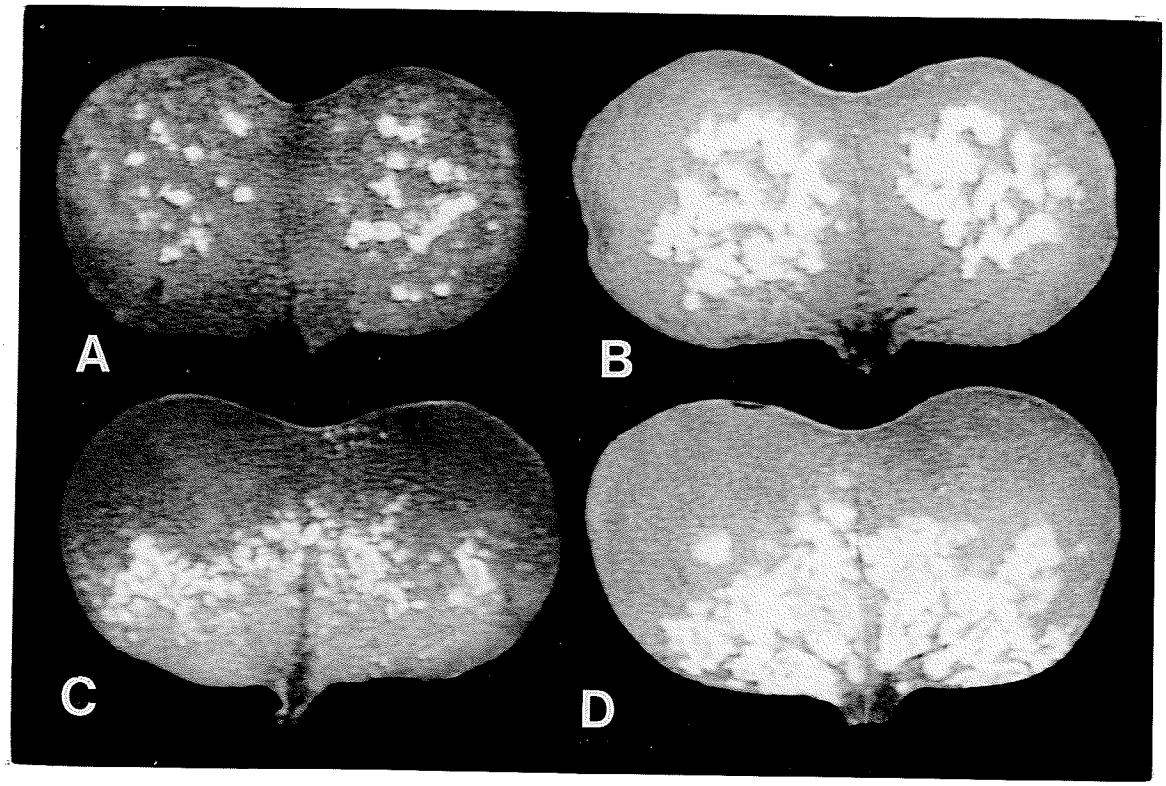


Fig. 5.2. Asexual sporulation of metalaxyl-sensitive race 7 of *Albugo candida* on the adaxial (A) and abaxial (B) surface of cotyledons of a common susceptible host (UM3512) in the absence of metalaxyl; asexual sporulation of metalaxyl-insensitive race 2 on the adaxial (C) and abaxial (D) surface of cotyledons sprayed with 200 μg a.i. mL^{-1} of metalaxyl 2 days after inoculation.

Chapter VI

EVIDENCE FOR SEXUAL RECOMBINATION IN ALBUGO CANDIDA

Abstract

The occurrence of cross-fertilization between homothallic isolates of *Albugo candida* was investigated using metalaxyl insensitivity and variation in pathogenicity as genetic markers. Two metalaxyl-insensitive (MI) isolates pathogenic to brown mustard (*Brassica juncea*) were crossed with one metalaxyl-sensitive isolate pathogenic to oilseed turnip rape (*Brassica rapa*). Twelve single-pustule isolates derived from the sexual progeny of each cross were tested for virulence and response to metalaxyl. Six of 12 MI isolates from Cross 1 and four of 12 MI isolates from Cross 2 were either avirulent to the *B. juncea* cv. Burgundy and *B. rapa* cv. Torch or caused low infection on Burgundy only, and were thus different from both parental isolates. MI isolates avirulent to both Burgundy and Torch from Cross 1 were selfed on common suscepts, and the selfed progeny produced by one isolate, CR1-12, were examined. The recovery of isolates with recombinant phenotypes suggests that cross-fertilization can occur between isolates pathogenic to different *Brassica* species and that sexual recombination can be a mechanism for pathogenic variation in *A. candida*.

Introduction

A. candida (Pers.) Kuntze is the biotrophic oomycete which causes white rust of crucifers. Physiological specialization in this obligate parasite has long been recognized (Melhus 1911, Hiura 1930, Napper 1933), and ten "biological races" have been identified and classified based on compatibility to different cruciferous species (Delwiche & Williams 1977, Hill et al. 1988, Pound & Williams 1963, Verma et al. 1975). Although they are well adapted to their species of origin (homologous host species), isolates of *A. candida* are also capable of infecting some genotypes of closely related species (heterologous host species), especially those sharing a genome with the homologous host. There is evidence that host-pathogen specificity in the *Brassica-A. candida* association occurs at the level of genus, species within a genus, and cultivars within a species (Hill et al. 1988, Petrie 1988, Pidskalny & Rimmer 1985, Pound & Williams 1963, S.R. Rimmer *unpublished data*).

Elucidation of the genetics of interactions between *Brassica* species and isolates of *A. candida* requires complementary studies on the genetics of host and pathogen. The inheritance of resistance to *A. candida* in *Brassica* species has been studied by a number of workers, and resistance is usually conferred by single or duplicate dominant genes (Delwiche & Williams 1974, 1981; Ebrahimi et al. 1976, Fan et al. 1983, Liu & Rimmer 1991, Tiwari et al. 1988, Verma & Bhowmik 1989). However, there is no information on the inheritance of virulence in *A. candida*. The development of a reliable procedure for production and germination of oospores (Chapter 3) and the availability of isolates with genetic markers (Chapter 5) provide an opportunity to study sexual compatibility and

recombination between races of *A. candida* pathogenic to different *Brassica* species.

This paper reports the identification of sexual recombinants in the oospore progeny from crosses between homothallic isolates of *A. candida* using metalaxyl insensitivity and variation in pathogenicity as genetic markers.

Materials and Methods

Parental isolates. Three metalaxyl-insensitive (MI) isolates of *A. candida* race 2 (Ac2), MIAc2-B1, MIAc2-B5 and MIAc2-B7, and one metalaxyl-sensitive (MS) isolate of race 7 (Ac7), MSAc7-A1, were passed through a sexual cycle (selfed) on their respective homologous hosts. MIAc2-B1 and MIAc2-B5 were crossed to MSAc7-A1 by co-inoculation on common suscepts (see below). Isolate CR1-12 derived from the cross MIAc2-B1 × MSAc7-A1 was selfed. All the isolates were single-pustule isolates (SPI), each presumably produced by a single uninucleate zoospore (Chapter 4). Details of the isolates are given in Table 6.1.

Hosts. Two differential cultivars were used for virulence tests. Burgundy (*B. juncea*) is susceptible to Ac2, but resistant to Ac7. Torch (*B. rapa*) is susceptible to Ac7, but resistant to Ac2. Also used were UM3512 (a selection from rapid-cycling *B. juncea*) and CrGC1-18 (a rapid-cycling *B. rapa*) obtained from Crucifer Genetics Cooperative, University of Wisconsin, Madison, WI. They are susceptible to both Ac2 and Ac7, but are generally more susceptible to their respective homologous isolates. Homogeneity of these two lines was assured by self-pollinating or sib-mating plants susceptible to both Ac2 and Ac7 for six generations. For

production of oospores in hypertrophic stems floral parts (stagheads), plants were grown in 15-cm diameter plastic pots filled with Metro-Mix (W.R. Grace & Co. Canada Ltd., Ajax, Ontario) in a growth chamber at day/night temperatures of 22/17°C under 18-h photoperiod. Controlled release fertilizer (Nutricote, 14-14-14, Plant Products Co. Ltd., Brampton, Ontario) was applied at seeding. For determination of virulence and response to metalaxyl, plants were grown in Jiffy-pots (Jiffy Products Ltd., Shippegan, NB) under similar growth conditions.

Establishment of sexual progeny. Plants approaching to or at the beginning of the bud stage (growth stage (GS) 3.1, Harper & Berkenkamp 1975) were spray-inoculated with zoospore suspensions (1-2 $\times 10^5$ zoospores mL^{-1}) of SPI or of parental isolates mixed in equal volume. About 50 plants were inoculated with each isolate or combination of isolates. Mature stagheads containing numerous oospores were obtained within 6-7 wk after inoculation. Oospores were germinated using the method described in Chapter 3. Suspensions of zoospores released from germinated oospores were filtered through Miracloth (Calbiochem Corporation, La Jolla, CA) and used to inoculate cotyledons at concentrations of 2 $\times 10^2$, 4 $\times 10^2$, and 6 $\times 10^2$ zoospores mL^{-1}. Ten to twelve SPI were established from each of the sexual progeny using the method described in Chapter 4.

Procedures for identifying sexual recombinants. Both Ac2 and Ac7 are known to be homothallic from the previous study (Chapter 4) and therefore, the oospore progeny from crosses between MIAc2-B1 × MSAc7-A1 (Cross 1) and MIAc2-B5 × MSAc7-A1 (Cross 2) are expected to consist of selfed as well as hybrid isolates. Two procedures were used to identify sexual recombinants. With the first procedure (Fig. 6.1), cotyledons of the

common suscepts were inoculated with zoospores released from germinated oospores. Seedlings were sprayed with 200 μg a.i. mL^{-1} of metalaxyl 2 days after inoculation to kill the selfed progeny of the MS parental isolate, MSAc7-A1. To separate the hybrid progeny from selfed progeny of the other parent, SPI were established from the zoosporangia collected from treated cotyledons and tested for virulence to the differential host cultivars and common suscepts. Hybrids from cross-fertilization between Ac2 and Ac7 are expected to be virulent only to the common suscepts if avirulence is dominant to virulence, or virulent to the *B. juncea* cv. Burgundy and *B. rapa* cv. Torch as well as the common suscepts if virulence is dominant to avirulence. Non-parental pathotypes (putative hybrids) were selected and selfed on the common suscepts. Ten SPI were established from the oospore progeny produced by isolate CR1-12 derived from Cross 1 and tested for virulence and response to metalaxyl.

With the second procedure (Fig. 6.2), oospore progenies (referred to as first-generation oospore progeny) from Cross 1 and Cross 2 were first passed through an asexual cycle on the common suscepts in the presence of 200 μg a.i. mL^{-1} of metalaxyl to eliminate the selfed progeny from the MS parent MSAc7-A1. No attempt was made at this stage to establish SPI. Zoospore suspensions were prepared with the zoosporangia collected from treated cotyledons, and were used to inoculate the common suscepts at GS 3.1 to produce oospores. Single-pustule isolates were then established from the selfed progeny (referred to as second-generation oospore progeny), and tested for response to metalaxyl and virulence. As the parental isolates MIAc2-B1 and MIAc2-B5 were known to be homozygous for the alleles controlling insensitivity to metalaxyl, recovery of both MI

and MS isolates in second-generation oospore progeny would indicate occurrence of cross-fertilization.

Testing for virulence and response to metalaxyl. Single-pustule isolates from sexual progenies were tested for virulence on fully expanded cotyledons of the differential host cultivars and common suscepts. Procedures for raising plants and inoculation have been described in Chapter 3. At least 12 seedlings were used per cultivar and SPI combination, and each SPI was tested at least twice. Asexual sporulation was evident within 7 days after inoculation. Interaction phenotypes (IP) were scored on a 0-9 scale 8-10 days after inoculation (Williams 1985, Appendix 2). Cotyledons which showed no symptoms or small necrotic flecks on the adaxial surface without sporulation were scored IP 0 or 1 and considered resistant, whereas those showing scattered or coalescing pustules on the abaxial and/or adaxial surface were scored IP 7 or 9 and considered susceptible. Intermediate IP (3 & 5) were sometimes observed on either of the differential host cultivars but not on common suscepts.

Single-pustule isolates from selfed progenies were tested for response to metalaxyl on cotyledons of the common suscepts in the presence of 200 μg a.i. mL^{-1} of metalaxyl. Parental isolates were included as controls. The inoculated cotyledons were examined for asexual sporulation 8-12 days after inoculation. Since it was difficult and time-consuming to establish and characterize large numbers of SPI, the test of hypothesis was based on recovery of progeny isolates showing genetic recombination between markers of the parental isolates. Analysis of data for Mendelian segregation ratios was not attempted.

Results

Selfed progeny. All the S_2 SPI derived from the selfed S_1 isolates, MIAc2-B1 and MIAc2-B5, were insensitive to metalaxyl, virulent to the *B. juncea* cv. Burgundy, and avirulent to the *B. rapa* cv. Torch, indicating that these two isolates were homozygous for the alleles controlling the three characters. All the 15 S_2 SPI from the S_1 isolate MIAc2-B7 were uniformly virulent to Burgundy and avirulent to Torch, but three of them were sensitive to metalaxyl, indicating that isolate MIAc2-B7 was homozygous for virulence to Burgundy and avirulence to Torch, but heterozygous for insensitivity to metalaxyl. All the SPI obtained by selfing the MS isolate MSAc7-A1 were sensitive to metalaxyl, avirulent to Burgundy, and virulent to Torch, indicating that this isolate is homozygous for the three characters in question.

Sexual crosses between Ac2 and Ac7. Isolates MIAc2-B1 and MIAc2-B5 known to be homozygous for insensitivity to metalaxyl, virulence to Burgundy, and avirulence to Torch, were selected as parents to cross to the MS isolate MSAc7-A1 with contrasting virulence phenotypes. Response to metalaxyl and variation in pathogenicity were used as genetic markers to detect hybrids (Fig. 6.1). Interaction phenotypes of MI SPI obtained after application of metalaxyl are presented in Tables 6.2 and 6.3. Of 12 MI SPI derived from the oospore progeny from Cross 1, six were either avirulent to the two differential cultivars, or showed reduced virulence to Burgundy but were avirulent to Torch, and were thus different from both the parental isolates; the remaining six did not differ in interaction phenotypes from the parental isolate MIAc2-B1 (virulent to Burgundy but avirulent to Torch), suggesting that they probably resulted from self-

fertilization (Table 6.2). MI SPI avirulent to both Burgundy and Torch were also recovered from the oospore progeny from Cross 2 (Table 6.3). No isolates virulent to both Burgundy and Torch were recovered from the two crosses, suggesting that virulence is controlled by recessive alleles.

The SPI avirulent to both Burgundy and Torch could arise through sexual recombination. To test this hypothesis, three SPI avirulent to both Burgundy and Torch were selected from Cross 1 and selfed on the common suscepts. Single-pustule isolates were established from the germinated oospores produced by isolate CR1-12. MI isolates (CR1-12-1 and CR1-12-9) avirulent to both Burgundy and Torch, a MI isolate (CR1-12-5) virulent to Torch but avirulent to Burgundy, and a MS isolate (CR1-12-6) avirulent to Torch but with reduced virulence to Burgundy were recovered, confirming that the isolate CR1-12 derived from Cross 1 was a true hybrid (Table 6.4). Also recovered were MI isolates exhibiting reduced virulence to Burgundy and/or Torch. One isolate did not produce enough zoosporangia for determination of response to metalaxyl.

Additional evidence for the occurrence of cross-fertilization between isolates of Ac2 and Ac7 was provided with the second procedure (Fig. 6.2). Two of 15 SPI derived from second-generation oospore progeny of Cross 1 and one of 13 SPI from Cross 2 failed to sporulate on the common suscepts treated with 200 μg a.i. mL^{-1} of metalaxyl. Since the parental isolates MIAc2-B1 and MIAc2-B5 used in Cross 1 and Cross 2, respectively were known to be homozygous for alleles determining insensitivity to metalaxyl from the previous experiment, segregation for metalaxyl insensitivity among SPI in second-generation oospore progeny indicated that cross-fertilization occurred when MSAc7-A1 was inoculated

together with MIAc2-B1 or MIAc2-B5 on the common suscepts. A subsequent test indicated that the two MS isolates from the second-generation oospore progenies of Cross 1 had the virulence phenotype similar to those of the MI parental isolate MIAc2-B1, whereas the MS isolate from Cross 2 had the virulence phenotype identical to the MS parental isolate MSAc7-A1.

Discussion

Sexual recombinants of *A. candida* were obtained using metalaxyl insensitivity and variation in pathogenicity as genetic markers. The result suggests that in nature cross-fertilization can occur between isolates of Ac2 and Ac7 when they simultaneously infect a common susceptible host and that pathotypes virulent to both *B. juncea* and *B. rapa* cultivars can arise through segregation and recombination. The significance of sexual reproduction and recombination in generating pathogenic variation in natural populations of *A. candida* is presently unknown.

The hybrid isolate from the cross between Ac2 and Ac7 was avirulent to the *B. juncea* cv. Burgundy carrying no known resistance gene to Ac2 and avirulent to the *B. rapa* cv. Torch carrying no known resistance gene to Ac7. This suggests that avirulence is dominant to virulence and provides evidence for the existence of avirulence alleles corresponding to resistance alleles. These results are in general agreement with those of others that avirulence in specialized biotrophic fungi is usually determined by dominant alleles (Ellingboe 1976, Crute 1985). Resistance in *Brassica* species to homologous and heterologous isolates of *A. candida* is, in most cases, controlled by single or duplicate dominant genes

(Delwiche & Williams 1974, 1981; Ebrahimi et al. 1976, Fan et al. 1983, Liu & Rimmer 1991, Tiwari et al. 1988, Verma & Bhowmik 1989). Also, *Brassica* species sharing genomes are thought to be more equivalent to the status of "cultivars" within a species when host-pathogen association is concerned (Newton & Crute 1989). Based on these facts, *Brassica-A. candida* specificity is probably under simple genetic control.

The genetic control of host species specificity in specialized biotrophic fungi has been reviewed by Newton and Crute (1989). The fact that new genes for specific avirulence can be revealed by hybrids from crosses between formae speciales of rusts and of powdery mildews has led them to conclude that host species specificity and cultivar specificity within a host species may have an identical genetic basis and may even involve the same gene. Recent studies seem to support their hypothesis. Tosa (1989) has provided evidence that the host-pathogen interaction between gramineous genera and formae speciales of *Erysiphe graminis* is governed by a gene-for-gene relationship. Valent et al. (1991) have demonstrated that an isolate of *Magnaporthe grisea* pathogenic to weeping lovegrass but non-pathogenic to rice carries three major genes that confer specific avirulence to three rice cultivars when transferred through repeated backcrosses to an isolate pathogenic to rice. Results from host range studies on *A. candida* have shown that host-pathogen specificity in the *Brassica-A. candida* association occurs at both the species and cultivar level (Delwiche & Williams 1977, Hill et al. 1988, Petrie 1988, Pidskalny & Rimmer 1985, Pound & Williams 1963, Verma et al. 1975). Further work using *Brassica* cultivars possessing known resistance genes and homologous and heterologous isolates of *A. candida* is required to

determine whether the genetic control of host species specificity is similar to that of cultivar specificity.

In this study, a number of SPI derived from crosses between Ac2 and Ac7 exhibited reduced virulence on either of the differential host cultivars. There are possibilities of infection of cotyledons by isolates derived from more than one zoospore. However, this seemed to be very unlikely because isolates were established by collecting single pustules that developed on different cotyledons and because each SPI was subcultured for three generations using a single pustule to initiate each generation (Chapter 4). Isolates showing intermediate infection types have also been obtained in hybridization between formae speciales or pathotypes of fungal pathogens in other taxa (Green 1971, Hiura 1978, Kolmer & Ellingboe 1988). Such a phenomenon may be explained by assuming that the expression of specific virulence alleles is influenced by genetic backgrounds (Newton & Crute 1989).

Our study reveals some constraints on genetic work with *A. candida*. For example, establishment of SPI is very laborious and time-consuming. The small number of SPI established from the sexual progeny made the verification of Mendelian segregation patterns impossible. Therefore, the conclusion that cross-fertilization occurred between the isolates of Ac2 and Ac7 was based on the appearance of isolates showing recombinant phenotypes. Further genetic analysis using larger numbers of individuals from each progeny is required to determine segregation ratios of the factors controlling virulence and metalaxyl insensitivity.

Another major constraint associated with genetic analysis of *A. candida* and other diploid biotrophs in the class Oomycetes is the lack of

a range of well-characterized markers. Metalaxyl insensitivity in *A. candida* was shown to be a dominant character and so can be selected directly in a diploid. However, the genetic basis of this character remains uncertain. Segregation ratios should be critically tested in future genetic studies. Variation in pathogenicity can provide appropriate phenotypic markers for genetic studies with *A. candida* when sets of differentials that are genetically pure have been constructed. Our study shows that the *B. rapa* cv. Torch is heterogeneous for its reaction to isolates of Ac2 and some SPI from the crosses, probably due to the outcrossing nature of this species. For detailed genetic studies on *A. candida*, other easily scored markers should be developed; the importance of using many markers has been demonstrated and emphasized by Newton (1987). Isozymes and restriction fragment length polymorphisms have been developed and used in genetic studies with other oomycetes, e.g., *Phytophthora infestans* (Shattock et al. 1986, Spielman et al. 1989, 1990) and *Bremia lactucae* (Hulbert & Michelmore 1988). Molecular markers are generally co-dominant and would thus be of particular value for genetic studies on the diploid biotroph *A. candida*.

Classical genetic analysis by means of sexual crosses is important in establishing the genetic basis of pathogenic variation by the pathogen. The results presented here are the first evidence that cross-fertilization between isolates pathogenic to different *Brassica* species can occur and that sexual recombination can be one of the mechanisms that cause pathogenic variation in *A. candida*. Our experiments represent a preliminary stage of genetic analysis of *A. candida*. Further studies are needed to examine the genetics of virulence of *A. candida* to different

genotypes within a *Brassica* species when sets of differential host cultivars for each *Brassica* species and isolates with genetic markers are available. Genome organization differences among isolates of *A. candida* pathogenic to different *Brassica* species can be determined through DNA analyses (e.g., electrophoretic karyotyping) to aid in determining the genetic relatedness of these isolates. Such studies complementary to those on the inheritance of resistance in the host should lead to a better understanding of the genetic relationship between genotypes of *Brassica* species and isolates of *A. candida*.

Table 6.1. Origin, virulence phenotype, and response to metalaxyl of isolates of *Albugo candida*

Isolate	Virulence phenotype				Response to metalaxyl	Origin
	UM3512	Burgundy	Torch	CrGC1-18		
MIAc2-A5	+	+	-	+	I	SPI from field isolate
MIAc2-B1	+	+	-	+	I	S_1 from MIAc2-A5
MIAc2-B5	+	+	-	+	I	S_1 from MIAc2-A5
MIAc2-B7	+	+	-	+	I	S_1 from MIAc2-A5
MSAc7-A1	+	-	+	+	S	S_1 from MS race 7
CR1-12	+	-	-	+	I	F_1 (MIAc2-B1 × MSAc7-A1)

+ = virulent, - = avirulent.

I = insensitive, S = sensitive.

Table 6.2. Virulence phenotype of metalaxyl-insensitive progenies from the cross between isolates MIAc2-B1 and MSAc7-A1 of *Albugo candida*[*]

Single pustule isolate	*Brassica juncea*		*Brassica rapa*	
	UM3512	Burgundy	CrGC1-18	Torch
Parents				
MIAc2-B1	7.5[†]	7.4	7.2	1.3
MSAc7-A1	6.9	0.0	7.4	8.9
MIAc2-B1 × MSAc7-A1 or selfed MIAc2-B1				
CR1-1	8.4	7.2	6.7	0.7
-4	8.5	4.0	7.5	1.5
-5	6.3	7.1	5.5	0.1
-6	6.7	7.1	6.1	1.6
-7	7.3	7.2	5.1	0.0
-9	8.3	1.0	6.2	0.8
-10	7.5	7.2	7.0	0.8
-11	8.4	0.1	6.4	0.4
-12	8.1	0.3	6.8	0.4
-13	8.9	4.2	8.3	1.4
-14	8.0	2.2	7.0	0.0
-15	7.5	7.2	6.3	0.8
Standard error = 0.56				

[*] UM3512 and CrGC1-18 are susceptible to both Ac2 and Ac7; Burgundy is susceptible to Ac2 but resistant to Ac7; Torch is resistant to Ac2 but susceptible to Ac7.

[†] Based on a 0-9 scale (Appendix 2). Values represent the mean of ratings on 24-30 seedlings.

Table 6.3. Virulence phenotype of metalaxyl-insensitive progenies from the cross between isolates MIAc2-B5 and MSAc7-A1 of *Albugo candida*[*]

Single pustule isolate	*Brassica juncea*		*Brassica rapa*	
	UM3512	Burgundy	CrGC1-18	Torch
Parents				
MIAc2-B5	8.3[†]	8.2	7.2	0.9
MSAc7-A1	7.3	0.0	7.7	8.7
MIAc2-B5 × MSAc7-A1 or selfed MIAc2-B5				
CR2-1	8.5	7.3	7.1	1.0
-2	8.5	7.6	7.2	0.6
-3	8.9	8.0	6.9	0.0
-5	7.5	0.6	8.2	0.1
-6	9.0	8.7	7.2	1.5
-7	8.0	3.2	5.8	0.4
-8	7.5	1.4	6.6	0.7
-9	8.1	8.4	7.3	0.9
-11	8.9	7.8	7.1	0.8
-12	8.7	1.0	7.8	0.4
-16	8.6	8.7	8.5	1.0
-17	8.4	8.8	9.0	1.0
Standard error = 0.48				

[*] UM3512 and CrGC1-18 are susceptible to both Ac2 and Ac7; Burgundy is susceptible to Ac2 but resistant to Ac7; Torch is resistant to Ac2 but susceptible to Ac7.

[†] Based on a 0-9 scale (Appendix 2). Values represent the mean of ratings on 24-30 seedlings.

Table 6.4. Virulence phenotype and response to metalaxyl of selfed progenies produced by isolate CR1-12 derived from the cross between isolates MIAc2-B1 and MSAc7-A1 of *Albugo candida*[*]

Single pustule isolate	*Brassica juncea*		*Brassica rapa*		Response to metalaxyl[†]
	UM3512	Burgundy	CrGC1-18	Torch	
CR1-12	8.7[§]	1.0	7.8	0.4	I
CR1-12-1	8.2	1.6	7.1	0.7	I
-2	8.8	8.6	7.1	1.9	I
-3	8.1	8.7	7.6	4.3	I
-4	6.8	7.8	6.7	0.8	I
-5	6.8	0.2	7.7	6.9	I
-6	7.5	4.0	7.1	0.8	S
-7	8.1	3.1	6.9	7.2	I
-9	8.6	1.6	7.2	1.9	I
-11	8.6	2.2	6.6	4.2	I
-13	8.1	0.0	7.6	7.9	ND
Standard error = 0.82					

[*] UM3512 and CrGC1-18 are susceptible to both Ac2 and Ac7; Burgundy is susceptible to Ac2 but resistant to Ac7; Torch is resistant to Ac2 but susceptible to Ac7.

[†] I = insensitive, S = sensitive, ND = not determined.

[§] Based on a 0-9 scale (Appendix 2). Values represent the mean of ratings on 24-30 seedlings.

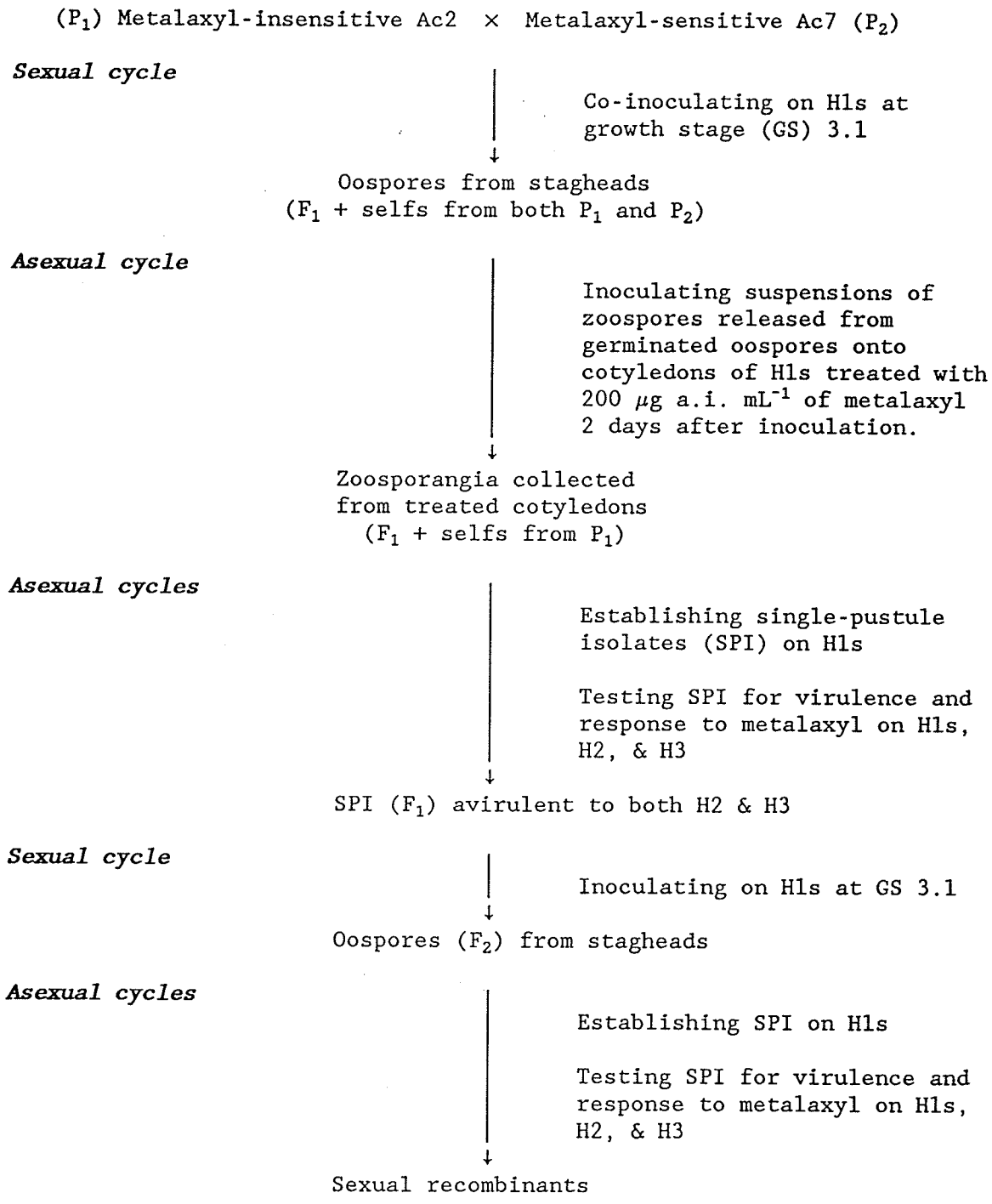


Fig. 6.1. Procedure 1 for identifying sexual recombinants. H1s, common suscepts UM3512 and CrGC1-18; H2, *Brassica juncea* cv. Burgundy susceptible to Ac2 but resistant to Ac7; H3, *Brassica rapa* cv. Torch resistant to Ac2 but susceptible to Ac7.

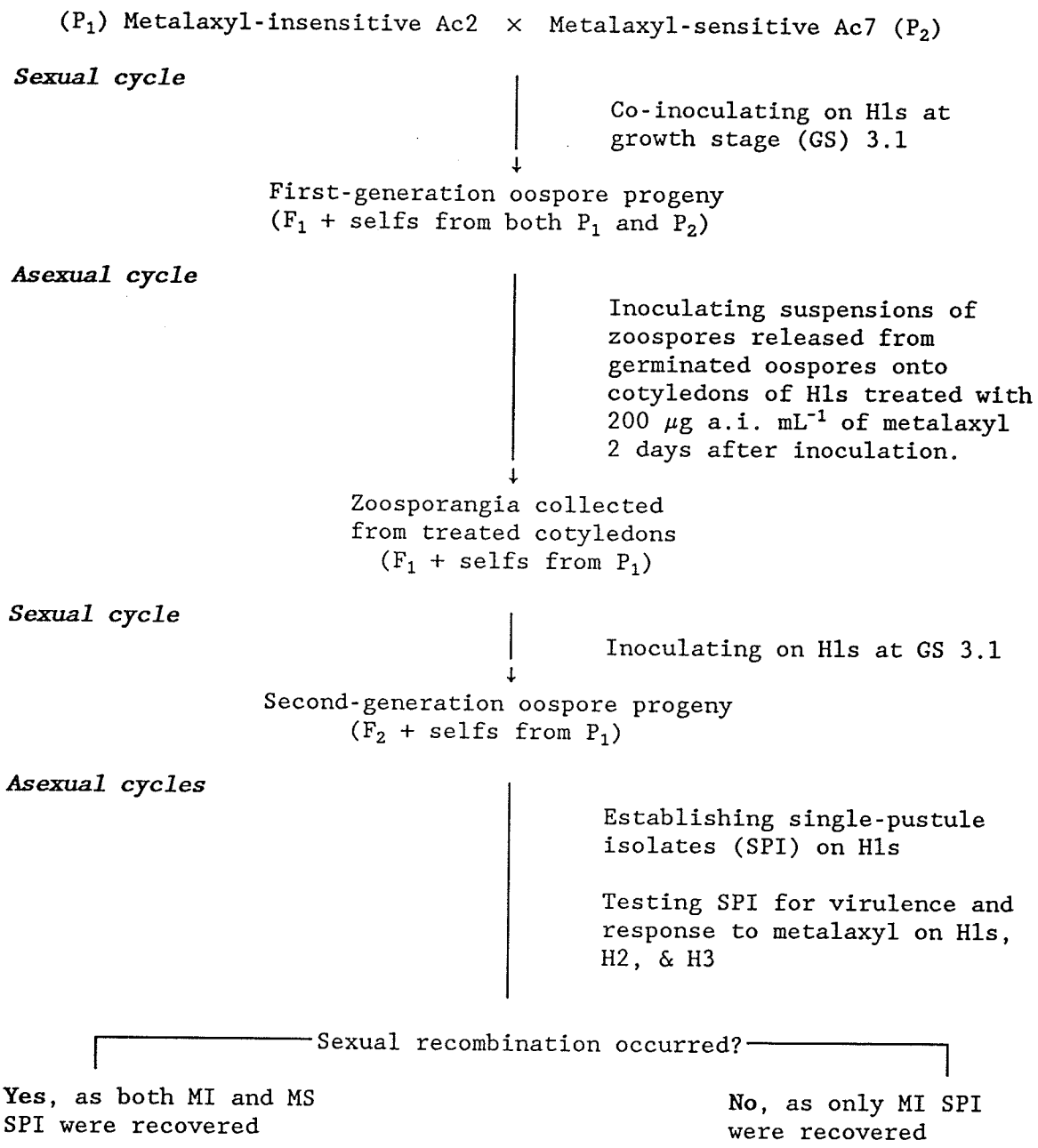


Fig. 6.2. Procedure 2 for identifying sexual recombinants. H1s, common suscepts UM3512 and CrGC1-18; H2, *Brassica juncea* cv. Burgundy susceptible to Ac2 but resistant to Ac7; H3, *Brassica rapa* cv. Torch resistant to Ac2 but susceptible to Ac7.

Chapter VII

GENERAL DISCUSSION

The potential for genetic studies with *A. candida* was demonstrated in this study. Oospores were produced in hypertrophic floral parts of rapid-cycling *B. juncea* and *B. rapa* after inoculation of plants at GS 3.1. The rapid-cycling *Brassica* lines have a short life cycle (Williams & Hill 1986), and mature oospores of Ac2 and Ac7 can be obtained about two months after planting. This method is efficient because even for the oomycetes (e.g., *Phytophthora* and *Pythium* species) that can be cultured on a defined medium, 2-3 months is required to obtained mature oospores *in vitro*. High percent germination (≥80%) of oospores was consistently attained by agitating oospores in a 1-2% mixture of β-glucuronidase and aryl sulfatase followed by washing and chilling. High percent germination of oospores is essential for genetic work with *A. candida* to ensure that the progeny isolates derived from germinated oospores can be considered as a random sample of the population.

This study also confirmed the earlier findings (Verma and Petrie 1980, Verma et al. 1983) that oospores of *A. candida* could be produced in inoculated, senescent leaves as well as in hypertrophic stems and floral parts of *Brassica* plants. However, oospores produced in senescent cotyledons germinated poorly in the present study. Since oospores produced by heterothallic isolates of *B. lactucae* in lettuce cotyledons do germinate after certain treatments (Michelmore 1988, Michelmore & Ingram 1981), it may be possible to produce germinable oospores of *A. candida* in senescent cotyledons of oilseed rape and mustard. Further experiments to

optimize environmental conditions for oospore production and maturation in inoculated cotyledons would be worthwhile.

The finding that Manitoba isolates of Ac2 and Ac7 are homothallic may have some epidemiological and genetical implications. Homothallism allows oospore production at high frequencies as sexual reproduction does not require association of isolates with compatible mating types. Indeed, oospores are produced regularly and abundantly in hypertrophic stems and floral parts of naturally infected *B. juncea* and *B. rapa* in western Canada. Oospores carried with seeds and overwintered in the soil are the source of primary infection of oilseed rape (Petrie 1975, Petrie & Verma 1974, Verma & Petrie 1980, Verma et al. 1975). Furthermore, homothallism results in inbreeding and may reduce the amount of variation in the population. This might partially explain the apparent low capacity of Ac2 and Ac7 to overcome the resistance in Canadian *B. juncea* and *B. rapa* cultivars, which has remained effective for about 10 years, and to adapt to Canadian *B. napus* cultivars which have maintained resistance for fifty years. However, the isolates of Ac2 and Ac7 examined in this study were sexually compatible. This implies that in nature simultaneous infection of a common host by Ac2 and Ac7 could provide a means for generating pathotypes virulent to both *B. juncea* and *B. rapa* cultivars via sexual recombination.

Metalaxyl insensitivity occurred naturally in two populations of *A. candida*. Metalaxyl insensitivity in this pathogen was probably controlled by a single dominant gene, but more work is needed to draw a definite conclusion about the genetic basis of this character. Once characterized, metalaxyl insensitivity can be used as a selectable marker in crosses with

other races to identify pathotype recombinants. It would also be desirable to have additional markers in such studies. Isozymes and restriction fragment length polymorphisms have recently been used as markers in genetic studies with other oomycetes (Hulbert & Michelmore 1988, Shattock et al. 1986, Spielman et al. 1989, 1990). These and other molecular markers are generally co-dominant and could thus be ideal for genetic studies with the diploid fungus *A. candida*.

The use of metalaxyl insensitivity and variation in pathogenicity enabled the identification of sexual recombinants from crosses between different races of *A. candida*. This study has provided a framework for studying the inheritance of virulence in *A. candida* and demonstrated the potential for developing the *Brassica-A. candida* association into a model system for studying the genetics of host-pathogen interactions.

LITERATURE CITED

Al-Kherb, S.M. 1988. The inheritance of host-specific pathogenicity in *Phytophthora infestans*. Ph.D. Thesis, University College of North Wales, Bangor (cited by L.J. Spielman, B.J. McMaster and W.E. Fry, 1989).

Ann, P.J., and W.H. Ko. 1988. Induction of oospore germination of *Phytophthora parasitica*. Phytopathology 78:335-338.

Ayers, W.A., and R.D. Lumsden. 1975. Factors affecting production and germination of oospores of three *Pythium* species. Phytopathology 65:1094-1100.

Banihashemi, Z., and J.E. Mitchell. 1976. Factors affecting oospore germination in *Phytophthora cactorum*, the incitant of apple collar rot. Phytopathology 66:443-448.

Berkenkamp, B. 1972. Diseases of rapeseed in central and northern Alberta in 1971. Can. Plant Dis. Surv. 52:62-63.

Bernier, C.C. 1972. Diseases of rapeseed in Manitoba in 1971. Can. Plant Dis. Surv. 52:108.

Biga, M.L.B. 1955. Riesamnazione della specie del genere *Albugo* in base alla morfologia dei conidi. Sydowia 9:339-358 (cited by Pound & Williams 1963).

Blackwell, E. 1943. Presidential address. On germinating the oospores of *Phytophthora cactorum*. Trans. Br. Mycol. Soc. 26:93-103.

Bonnet, A. 1981. Resistance to white rust in radish (*Raphanus sativus* L.). Cruciferae Newsletter 6:60.

Bruin, G.C.A., and L.V. Edgington. 1981. Adaptive resistance in Peronosporales to metalaxyl. Can. J. Plant Pathol. 3:201-206.

Bruin, G.C.A., and L.V. Edgington. 1982. Induction of fungal resistance to metalaxyl by ultraviolet irradiation. Phytopathology 72:476-480.

Caten, C.E., and A.W. Day. 1977. Diploidy in plant pathogenic fungi. Ann. Rev. Phytopathol. 15:295-318.

Chang, T.T., and W.H. Ko. 1990. Resistance to fungicides and antibiotics in *Phytophthora parasitica*: Genetic nature and use in hybrid determination. Phytopathology 80:1414-1421.

Coffey, M.D., L.J. Klure, and L.A. Bower. 1984. Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. Phytopathology 74:417-422.

Cohen, Y., and **M.D. Coffey**. 1986. Systemic fungicides and the control of Oomycetes. Ann. Rev. Phytopathol. 24:311-338.

Crute, I.R. 1985. The genetic bases of relationships between microbial parasites and their hosts. Pages 80-142 *in*: R.S.S. Fraser ed., Mechanisms of Resistance to Plant Diseases. Nijhoff and Junk, Dordrecht.

Crute, I.R. 1987. Genetic studies with *Bremia lactucae* (lettuce downy mildew). Pages 207-219 *in*: P.R. Day and G.J. Jellis eds., Genetics and Plant Pathogenesis. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne.

Crute,I.R., and **J.M. Harrison**. 1988. Studies on the inheritance of resistance to metalaxyl in *Bremia lactucae* and on the stability and fitness of field isolates. Plant Pathol. 37:231-250.

Crute, I.R., and **J.M. Norwood**. 1986. Gene-dosage effects on the relationship between *Bremia lactucae* (downy mildew) and *Lactuca sativa* (lettuce): the relevance to a mechanistic understanding of host-parasite specificity. Physiol. Mol. Plant Pathol. 29:133-145.

Crute, I.R., J.M. Norwood, and **P.L. Gordon**. 1987. The occurrence, characteristics and distribution in the United Kingdom of resistance to phenylamide fungicides in *Bremia lactucae* (lettuce downy mildew). Plant Pathol. 36:297-315.

Crute, I.R., K. Phelps, A. Barnes, S.T. Buczacki, and **P. Crisp**. 1983. The relationship between genotypes of three *Brassica* species and collections of *Plasmodiophora brassicae*. Plant Pathol. 32:405-420.

Day, P.R. 1974. Genetics of Host-parasite Interaction. W.M. Freeman, San Francisco, 238pp.

de Bary, A. 1866. Morphologie und physiologie der Pilze, Flechten und Myxomyceten. Wilhelm Engelmann, Leipzig (cited by P.R. Verma and G.A. Petrie 1975).

Delwiche, P.A., and **P.H. Williams**. 1974. Resistance to *Albugo candida* race 2 in *Brassica* species. Proc. Amer. Phytopathol. Soc. 1:66. (Abstract).

Delwiche, P.A., and **P.H. Williams**. 1977. Genetic studies in *Brassica nigra* (L.) Koch. Cruciferae Newsletter 2:39.

Delwiche, P.A., and **P.H. Williams**. 1981. Thirteen marker genes in *Brassica nigra*. J. Hered. 72:289-290.

Eberhardt, A. 1904. Contribution a l'etude de *Cystopus candidus* Lev. Zentr. Baskteriol. Parasitenk. 12:235-249, 426-439, 614-631, 714-725, (cited by Pound and Williams 1963).

Ebrahimi, A.G., P.A. Delwiche, and P.H. Williams. 1976. Resistance in *Brassica juncea* to *Peronospora parasitica* and *Albugo candida* race 2. Proc. Amer. Phytopathol. Soc. 3:273. (Abstract).

Edwards, M.D., and P.H. Williams. 1987. Selection for minor gene resistance to *Albugo candida* in a rapid-cycling population of *Brassica campestris*. Phytopathology 77:527-532.

Ellingboe, A.H. 1976. Genetics of host-parasite interactions. Pages 761-778 *in*: R. Heitefuss and P.H. Williams, eds., Encyclopedia of Plant Physiology, New Series, Vol. 4, Physiological Plant Pathology. Springer-Verlag, Berlin.

Elliott, C.G., and D. MacIntyre. 1973. Genetic evidence on the life-history of *Phytophthora*. Trans. Br. Mycol. Soc. 60:311-316.

Endo, R.M., and M.B. Linn. 1960. The white rust disease of horseradish. Illinois Agric. Exp. Stat. Bull. 655, 56pp.

Fan, Z., S.R. Rimmer, and B.R. Stefansson. 1983. Inheritance of resistance to *Albugo candida* in rape (*Brassica napus* L.). Can. J. Genet. Cytol. 25:420-424.

Farrara, B.F., T.W. Ilott, and R.W. Michelmore. 1987. Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* and *L. serriola*). Plant Pathol. 36:499-514.

Flor, H.H. 1956. The complementary genetic systems in flax and flax rust. Adv. Genet. 8:29-54.

Flor, H.H. 1959. Genetic controls of host-parasite interactions in rust diseases. Pages 137-144 *in*: C.S. Holton, G.W. Fischer, R.W. Fulton, H. Hart, and S.E.A. McCallan eds., Plant Pathology Problems and Progress 1908-1958. University of Wisconsin Press, Madison WI.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol. 9:275-296.

Förster, H., O.K. Ribeiro, and D.C. Erwin. 1983. Factors affecting oospore germination of *Phytophthora megasperma* f. sp. *medicaginis*. Phytopathology 73:442-448.

Galindo, J.G., and G.A. Zentmyer. 1967. Genetical and cytological studies of *Phytophthora* strains pathogenic to pepper plants. Phytopathology 57:1300-1304.

Gallegly, M.E. 1968. Genetics of pathogenicity of *Phytophthora infestans*. Ann. Rev. Phytopathology 6:375-396.

Gallegly, M.E. 1970. Genetics of *Phytophthora*. Phytopathology 60:1135-

1141.

Green, G.J. 1971. Hybridization between *Puccinia graminis tritici* and *Puccinia graminis secalis* and its evolutionary implications. Can J. Bot. 49:2089-2095.

Harper, F.R., and **B. Berkenkamp.** 1975. Revised growth stage key for *Brassica campestris* and *B. napus*. Can. J. Plant Sci. 55:657-658.

Hill, C.B., I.R. Crute, C. Sherriff, and **P.H. Williams.** 1988. Specificity of *Albugo candida* and *Peronospora parasitica* pathotypes toward rapid-cycling crucifers. Cruciferae Newsletter 13:112-113.

Hiura, M. 1930. Biologic forms of *Albugo candida* (Pers.) Kuntze on some cruciferous plants. Japan. J. Bot. 5:1-20.

Hiura, U. 1978. Genetic basis of formae speciales in *Erysiphe graminis* DC. Pages: 101-128 *in*: D.M. Spensor ed., The Powdery Mildews. Academic Press, London, New York, San Francisco.

Holmes, S.J.I., and **A.G. Channon.** 1984. Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. Plant Pathol. 33:347-354.

Hulbert, S.H., and **R.W. Michelmore.** 1985. Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*). Theor. Appl. Genet. 70:520-528.

Hulbert, S.H., and **R.W. Michelmore.** 1988. DNA restriction fragment length polymorphism and somatic variation in the lettuce downy mildew fungus, *Bremia lactucae*. Mol. Plant-Microbe Interact. 1:17-24.

Humaydan, H.S., and **P.H. Williams.** 1976. Inheritance of seven characters in *Raphanus sativus* L. Hortic. Sci. 11:146-147.

Hunger, R.M., P.B. Hamm, C.E. Horner, and **E.M. Hansen.** 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. 66:645-649.

Ilott, T.W., Hulbert, S.H., and **R.W. Michelmore.** 1989. Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. Phytopathology 79:888-897.

Jiang, J., L.W. Stephenson, D.C. Erwin, and **J.V. Leary.** 1989. Nuclear changes in *Phytophthora* during oospore maturation and germination. Mycol. Res. 92:463-469.

Jimenez, B., and **J.L. Lockwood.** 1982. Germination of oospores of *Phytophthora megasperma* f. sp. *glycinea* in the presence of soil. Phytopathology 72:662-666.

Kaosiri, T., G.A. Zentmyer, and **D.C. Erwin.** 1980. Oospore morphology and germination in the *Phytophthora palmivora* complex from cacao. Mycologia 72:888-907.

Ko, W.H. 1978. Heterothallic *Phytophthora*: Evidence for hormonal regulation of sexual reproduction. J. Gen. Microbiol. 107:15-18.

Kolmer, J.A., and **A.H. Ellingboe.** 1988. Genetic relationships between fertility and pathogenicity and virulence to rice in *Magnaporthe grisea*. Can. J. Bot. 66:891-897.

Lakra, B.S., G.S. Saharan, and **P.R. Verma.** 1988. Factors affecting sporangial germination of *Albugo candida* causing white rust of mustard. Indian Phytopathol. 41:287. (Abstract).

Liu, Q., and **S.R. Rimmer.** 1991. Inheritance of resistance in *Brassica napus* to an Ethiopian isolate of *Albugo candida* from *Brassica carinata*. Can. J. Plant Pathol. 13:197-201.

Liu, Q., S.R. Rimmer, and **R. Scarth.** 1989. Histopathology of compatibility and incompatibility between oilseed rape and *Albugo candida*. Plant Pathol. 38:176-182.

Long, M., and **N.T. Keen.** 1977. Genetic evidence of diploidy in *Phytophthora megasperma* var. *sojae*. Phytopathology 67:675-677.

Melhus, I.E. 1911. Experiments on spore germination and infection in certain species of Oomycetes. Wisconsin Agric. Exp. Stat. Bull. 15:25-91.

Michelmore, R.W., and **S.H. Hulbert.** 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Ann. Rev. Phytopathol. 25:383-404.

Michelmore, R.W., T.W. Ilott, S.H. Hulbert, and **B.F. Farrara.** 1988. The downy mildews. Pages 53-79 *in*: G. Sidhus, D.S. Ingram, and P.H. Williams eds., Advances in Plant Pathology, Vol. 6. Academic Press, London.

Michelmore, R.W., and **D.S. Ingram.** 1980. Heterothallism in *Bremia lactucae*. Trans Br. Mycol. Soc. 75:47-56.

Michelmore, R.W., and **D.S. Ingram.** 1981. Recovery of progeny following sexual reproduction of *Bremia lactucae*. Trans. Br. Mycol. Soc. 77:131-137.

Michelmore, R.W., and **D.S. Ingram.** 1982. Secondary homothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 78:1-9.

Michelmore, R.W., J.M. Norwood, D.S. Ingram, I.R. Crute, and **P. Nicholson.** 1984. The inheritance of virulence in *Bremia lactucae* to match resistance factors 3, 4, 5, 6, 8, 9, 10, and 11 in

lettuce (*Lactuca sativa*). Plant Pathol. 33:301-315.

Michelmore, R.W., and **E.R. Sansome.** 1982. Cytological studies of heterothallism and secondary homothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 79:291-297.

Mortimer, A.M., D.S. Shaw, and **E.R. Sansome.** 1977. Genetical study of secondary homothallism in *Phytophthora drechsleri*. Arch. Microbiol. 111:255-259.

Napper, M.E. 1933. Observation on spore germination and specialization of parasitism in *Cystopus candidus*. J. Pomol. Hortic. Sci. 11:81-100.

Newton, A.C. 1987. Markers in pathogen populations. Pages 187-194 *In:* P.R. Day and G.J. Jellis eds., Genetics and Plant Pathogenesis. Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne.

Newton, A.C. and **I.R. Crute.** 1989. A consideration of the genetic control of species specificity in fungal plant pathogens and its relevance to comprehension of the underlying mechanisms. Biol. Rev. 64:35-50.

Norwood, J.M., and **I.R. Crute.** 1984. The genetic control and expression of specificity in *Bremia lactucae* (lettuce downy mildew). Plant Pathol. 33:385-400.

Norwood, J.M., R.W. Michelmore, I.R. Crute, and **D.S. Ingram.** 1983. The inheritance of virulence in *Bremia lactucae* to match resistance factors 1, 2, 4, 6, and 11 in *Lactuca sativa* (lettuce). Plant Pathol. 32:177-186.

Person, C. 1959. Gene-for-gene relationships in host:parasite systems. Can. J. Bot. 37:1101-1130.

Petrie, G.A. 1973. Diseases of *Brassica* species in Saskatchewan, 1970-1972. I. Staghead and aster yellows. Can Plant Dis. Surv. 53:19-25.

Petrie, G.A. 1975. Prevalence of oospores of *Albugo cruciferarum* in *Brassica* seed samples from western Canada, 1967-73. Can. Plant Dis. Surv. 55:19-24.

Petrie, G.A. 1988. Races of *Albugo candida* (white rust and staghead) on cultivated Cruciferae in Saskatchewan. Can. J. Plant Pathol. 10:142-150.

Petrie, G.A., and **P.R. Verma.** 1974. A simple method for germinating oospores of *Albugo candida*. Can. J. Plant Sci. 54:595-596.

Pidskalny, R.S. 1984. Studies of *Albugo candida* on rapeseed: yield

effects, development on cultivar mixtures, and infection dynamics. M.Sc. Thesis. Dept. of Plant Science, University of Manitoba, Canada.

Pidskalny, R.S., and **S.R. Rimmer**. 1985. Virulence of *Albugo candida* from turnip rape (*Brassica campestris*) and mustard (*Brassica juncea*) on various crucifers. Can. J. Plant Pathol. 7:283-286.

Pound, G.S., and **P.H. Williams**. 1963. Biological races of *Albugo candida*. Phytopathology 53:1146-1149.

Ribeiro, O.K. 1983. Physiology of asexual sporulation and spore germination in *Phytophthora*. Pages 55-70 *in*: D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao, eds., Phytophthora: Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, MN.

Ruben, D.M., **Z.R. Frank**, and **I. Chet**. 1980. Factors affecting behavior and developmental synchrony of germinating oospores of *Pythium aphanidermatum*. Phytopathology 70:54-59.

Salvatore, M.A., **F.A. Gray**, and **R.B. Hine**. 1973. Enzymatically induced germination of oospores of *Phytophthora megasperma*. Phytopathology 63:1083-1084.

Sansome, E. 1980. Reciprocal translocation heterozygosity in heterothallic species of *Phytophthora* and its significance. Trans. Br. Mycol. Soc. 74:175-185.

Sansome, E., and **F.W. Sansome**. 1974. Cytology and life-history of *Peronospora parasitica* on *Capsella bursa-pastoris* and of *Albugo candida* on *C. bursa-pastoris* and on *Lunaria annua*. Trans. Br. Mycol. Soc. 62:323-332.

SAS Institute Inc. 1985. SAS user's guide: Statistics, Version 5 edn., Cary, NC: SAS Institute Inc., 956pp.

Savage, E.J., **C.W. Clayton**, **J.H. Hunter**, **J.A. Brenneman**, **C. Laviola**, and **M.E. Gallely**. 1968. Homothallism, heterothallism, and interspecific hybridization in the genus *Phytophthora*. Phytopathology 58:1004-1021.

Shattock, R.C. 1988. Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. Plant Pathol. 37:4-11.

Shattock, R.C., **P.W. Tooley**, and **W.E. Fry** 1986. Genetics of *Phytophthora infestans:* Determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology 76:410-413.

Shaw, D.S. 1967. A method of obtaining single-oospore cultures of *Phytophthora cactorum* using live water snails. Phytopathology 57:454.

Shaw, D.S. 1983a. The Peronosporales: A Fungal Geneticist's Nightmare. Pages 85-121 *in*: S.T. Buczacki ed., Zoosporic Plant Pathogens: A Modern Perspective. Academic press, London.

Shaw, D.S. 1983b. The cytogenetics and genetics of *Phytophthora*. Pages 81-94 *in*: D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao, eds., Phytophthora: Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, MN.

Shaw, D.S. 1988. The *Phytophthora* species. Pages 27-51 *in*: G. Sidhus, D.S. Ingram, and P.H. Williams eds., Advances in Plant Pathology, Vol. 6. Academic Press, London.

Shaw, D.S., and **C.G. Elliott.** 1968. Streptomycin resistance and morphological variation in *Phytophthora cactorum*. J. Gen. Microbiol. 51:75-84.

Shen, C.Y., L.A. Bower, D.C. Erwin, and **P.H. Tsao.** 1983. Formation of sex organs in the A1 mating type of *Phytophthora infestans* induced chemically by A2 isolates of other species of Phytophthora. Can. J. Bot. 61:1462-1466.

Sherriff, C., and **J.A. Lucas.** 1989a. Cytogenetic study of heterothallic and homothallic isolates of *Peronospora parasitica*. Mycol. Res. 92:302-310.

Sherriff, C., and **J.A. Lucas.** 1989b. Heterothallism and homothallism in *Peronospora parasitica*. Mycol. Res. 92:311-316.

Sherriff, C., and **J.A. Lucas.** 1990. The host range of isolates of downy mildew, *Peronospora parasitica*, from *Brassica* crop species. Plant Pathol. 39:77-91.

Shew, H.D. 1984. In vitro growth response of *Phytophthora parasitica* var. *nicotianae* isolates to metalaxyl. Plant Dis. 68:764-766.

Skidmore, D.I., R.C. Shattock, and **D.S. Shaw.** 1984. Oospores in cultures of *Phytophthora infestans* resulting from selfing by the presence of *P. drechsleri* isolated from blighted potato foliage. Plant Pathol. 33:173-183.

Spielman, L.J., B.J. McMaster, and **W.E. Fry.** 1989. Dominance and recessiveness at loci for virulence against potato and tomato in *Phytophthora infestans*. Theor. Appl. Genet. 77:832-838.

Spielman, L.J., J.A. Sweigard, R.C. Shattock, and **W.E. Fry.** 1990. The genetics of *Phytophthora infestans:* Segregation of allozyme markers in F_2 and backcross progeny and the inheritance of virulence against potato resistance genes *R2* and *R4* in F_1 progeny. Exp. Mycol. 14:57-59.

Stack, J.P., and **R.L. Millar.** 1985. Isolation and characterization of a

metalaxyl-insensitive isolate of *Phytophthora megasperma* f. sp. *medicaginis*. Phytopathology 75:1387-1392.

Stanghellini, M.E., and J.D. Russell. 1973. Germination in vitro of *Pythium aphanidermatum* oospores. Phytopathology 63:133-137.

Stone, J.R., P.R. Verma, J. Dueck, and **D.T. Spurr.** 1987. Control of *Albugo candida* race 7 in *Brassica campestris* cv. Torch by foliar, seed, and soil applications of metalaxyl. Can. J. Plant Pathol. 9:137-145.

Tiwari, A.S., G.A. Petrie, and R.K. Downey. 1988. Inheritance of resistance to *Albugo candida* race 2 in mustard (*Brassica juncea* (L.) Czern.). Can. J. Plant Sci. 68:297-300.

Togashi, K., and K. Shibasaki. 1934. Biometrical and biological studies of *Albugo candida* (Pers.) O. Kuntze in connection with its specialization. Imperial College Agric. and Forestry (Marioba, Japan) Bull. 18, 88pp.

Tosa, Y. 1989. Evidence on wheat for gene-for-gene relationship between *formae speciales* of *Erysiphe graminis* and genera of gramineous plants. Genome 32:918-924.

U, N. 1935. Genome-analysis in *Brassica* with special reference to experimental formation of *B. napus* and peculiar mode of fertilization. Japan. J. Bot. 7:389-452.

Valdes, E., and **L.V. Edgington.** 1983. Development of resistance in *Albugo candida* to metalaxyl when used alone and in combination with mancozeb on radish (*Raphanus sativus)*. Can. J. Plant Pathol. 5:212. (Abstract).

Valent, B., L. Farrall, and **F.G. Chumley.** 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. Genetics 127:87-101.

Vanterpool, T.C. 1959. Oospore germination in *Albugo candida*. Can J. Bot. 37:169-172.

Verma, P.R., H. Harding, G.A. Petrie, and **P.H. William.** 1975. Infection and temporal development of mycelium of *Albugo candida* in cotyledons of four *Brassica* species. Can. J. Bot. 1016-1020.

Verma, P.R., and **G.A. Petrie.** 1975. Germination of oospores of *Albugo candida*. Can J. Bot. 53:836-842.

Verma, P.R., and **G.A. Petrie.** 1980. Effect of seed infestation and flower bud inoculation on systemic infection of turnip rape by *Albugo candida*. Can. J. Plant Sci. 60:267-271.

Verma, P.R., D.T. Spurr, and **G.A. Petrie.** 1983. Influence of age, and

time of detachment on development of white rust in detached *Brassica campestris* leaves at different temperatures. Can. J. Plant Pathol. 5:154-157.

Verma, U., and **P.T. Bhowmik**. 1989. Inheritance of resistance to *Brassica juncea* pathotype of *Albugo candida* in *B. napus*. Can. J. Plant Pathol. 11:443-444.

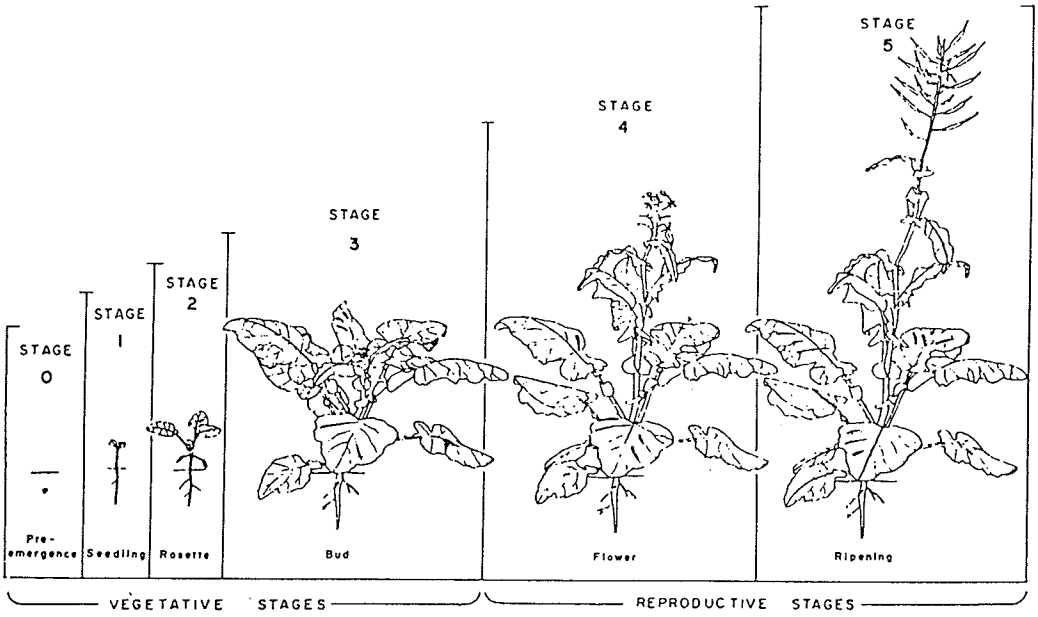
Webster, J. 1980. Introduction to Fungi, 2nd edn., Cambridge University Press, Cambridge, London, New York, Rochelle Melbourne, Sydney. pp. 186-190.

Williams, P.H. 1985. Crucifer Genetics Cooperative (CrGC) Resource Book. Department of Plant Pathology, University of Wisconsin, Madison, WI.

Williams, P.H., and **C.B. Hill**. 1986. Rapid-cycling populations of *Brassica*. Science 232:1385-1389.

Williams, P.H., and **G.S. Pound**. 1963. Nature and inheritance of resistance to *Albugo candida* in radish. Phytopathology 53:1150-1154.

APPENDIX 1



Key to the growth stages in *Brassica* crops (Harper & Berkenkamp 1975). Descriptions are based on the main stem.

Stage 0 - Preemergence

1 - Seedling

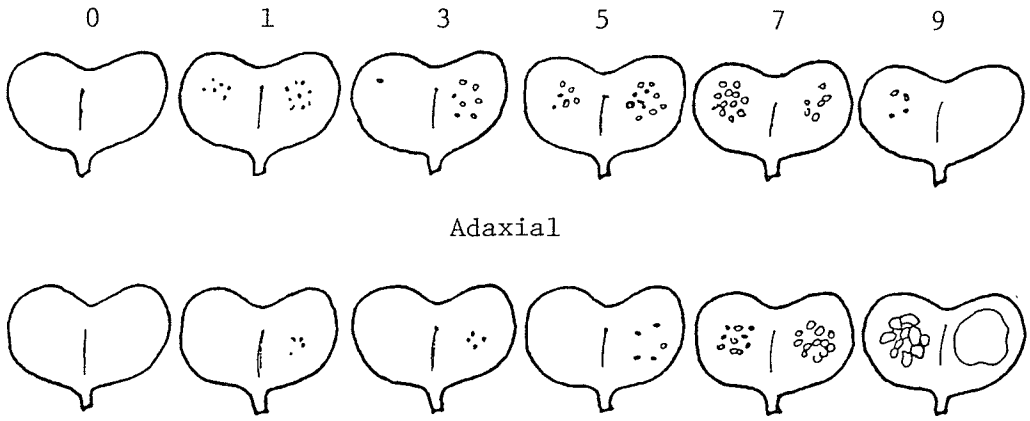
2 - Rosette
- 2.1 First true leaf expanded
- 2.2 Second true leaf expanded (add 0.1 for each additional leaf)

3 - Bud
- 3.1 Inflorescence visible at centre of rosette
- 3.2 Inflorescence raised above level of rosette
- 3.3 Lower buds yellowing

4 - Flower
- 4.1 First flower open
- 4.2 Many flowers open, lower pods elongating
- 4.3 Lower pods starting to fill
- 4.4 Flowering complete, seeds enlarging in lower pods

5 - Ripening
- 5.1 Seeds in lower pods full size, transparent
- 5.2 Seeds in lower pods green
- 5.3 Seeds in lower pods green-brown mottled
- 5.4 Seeds in all pods brown, plant senescent

APPENDIX 2



White rust rating scale. Symptoms and signs for the interaction phenotype ratings on the adaxial/abaxial cotyledon surfaces are: 0 = no symptoms on either cotyledon surface, 1 = necrotic flecks/none to few necrotic flecks, 3 = few, minute pustules/none to very few pustules, 5 = few to many small pustules/few small pustules, 7 = many to few small pustules/many large pustules, 9 = very few to no pustules/large coalescing pustules (Williams 1985).