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A review of non-fungicidal approaches for the control of apple scab
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Article abstract

Apple scab is the single most important disease of apple in Canada and the most costly to control. Failure to control apple scab results in severe yield losses and a reduction in market value of harvested fruits. Currently, the strategy to control apple scab relies on multiple applications of fungicides. These sprays are a significant cost to growers and the indirect environmental impact may be substantial. Reliance on fungicides can be reduced by the integration of non-fungicidal control measures that include genetic, physical, and biological approaches. Recommendations for chemical control of apple scab are often based on the highly susceptible cultivar McIntosh. This was justified because up to 60 to 70% of orchards in northeastern North America were planted with this cultivar. Today, the situation is changing as more and more growers are planting new cultivars, some of which are less susceptible to apple scab. A change in the recommendations to account for cultivar susceptibility could result in a reduction in the number of fungicide applications required. In addition, there has been long-standing research on resistant cultivars, but none of the scab-resistant cultivars have been widely accepted. However, with new molecular techniques to identify and locate the resistance genes, there is potential for progress on this front. Furthermore, fungicide applications for primary infections can be delayed if the number of ascospores is reduced after fall treatments that include leaf shredding, the application of biological control agents, or urea. These methods are preventive and can be integrated into existing management programs. However, the integration of all or some of these methods is more complex than simply the use of fungicides. Nevertheless, integrated management of apple scab may prove more sustainable on a long-term basis, mainly because it does not depend on the use of a single method. Hence the risk of the development of fungicide resistance in the pathogen population is reduced.

Review article / Article de synthèse

A review of non-fungicidal approaches for the control of apple scab

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Apple scab is the single most important disease of apple in Canada and the most costly to control. Failure to control apple scab results in severe yield losses and a reduction in market value of harvested fruits. Currently, the strategy to control apple scab relies on multiple applications of fungicides. These sprays are a significant cost to growers and the indirect environmental impact may be substantial. Reliance on fungicides can be reduced by the integration of non-fungicidal control measures that include genetic, physical, and biological approaches. Recommendations for chemical control of apple scab are often based on the highly susceptible cultivar McIntosh. This was justified because up to 60 to 70% of orchards in north-eastern North America were planted with this cultivar. Today, the situation is changing as more and more growers are planting new cultivars, some of which are less susceptible to apple scab. A change in the recommendations to account for cultivar susceptibility could result in a reduction in the number of fungicide applications required. In addition, there has been long-standing research on resistant cultivars, but none of the scab-resistant cultivars have been widely accepted. However, with new molecular techniques to identify and locate the resistance genes, there is potential for progress on this front. Furthermore, fungicide applications for primary infections can be delayed if the number of ascospores is reduced after fall treatments that include leaf shredding, the application of biological control agents, or urea. These methods are preventive and can be integrated into existing management programs. However, the integration of all or some of these methods is more complex than simply the use of fungicides. Nevertheless, integrated management of apple scab may prove more sustainable on a long-term basis, mainly because it does not depend on the use of a single method. Hence the risk of the development of fungicide resistance in the pathogen population is reduced.

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[Revue des approches non chimiques pour la lutte contre la tavelure du pommier]

Au Canada, la tavelure est la plus importante des maladies du pommier et la plus coûteuse à réprimer. Un échec dans le contrôle de la tavelure se traduira par une baisse de rendement importante et une diminution de la valeur marchande des fruits. La lutte contre la tavelure est essentiellement basée sur l'application de fongicides. Ces applications répétées de fongicides contribuent à l'augmentation des coûts de production et peuvent avoir un impact indirect sur l'environnement. La dépendance envers les fongicides pourrait être réduite par l'intégration de mesures alternatives aux fongicides, incluant des méthodes génétiques, physiques et biologiques. Les recommandations pour la lutte chimique sont souvent basées sur des études faites avec le cultivar McIntosh qui est très sensible à la tavelure. Ces recommandations étaient justifiées par la présence de 60 à 70 % de ce cultivar dans le nord-est de l'Amérique du Nord. Toutefois, cette situation change dans la mesure où les producteurs plantent de plus en plus de cultivars moins sensibles. L'adaptation de ces recommandations en tenant compte de la sensibilité moindre de certains cultivars permettrait de réduire le nombre d'applications de fongicides. Malheureusement, la recherche sur le développement de cultivars résistants n'a pas donné les résultats escomptés. Toutefois, les nouveaux outils moléculaires peuvent servir à identifier et localiser les gènes de résistance et ainsi permettre une percée importante dans le développement de cultivars résistants. De plus, les applications de fongicides faites au printemps pour réprimer les infections primaires, peuvent être retardées à la suite de traitements d'automne incluant le broyage des feuilles et l'application d'agents de lutte biologique ou d'urée. Ces mesures sont préventives et peuvent s'intégrer dans les programmes de lutte actuels. Toutefois, l'usage de ces mesures est plus complexe que de simples applications de fongicides. Par contre, la lutte intégrée contre la tavelure est plus durable dans la mesure où elle ne dépend pas d'une seule méthode de contrôle et parce qu'elle permet de réduire les risques de développement de résistance aux fongicides dans la population de l'agent pathogène.

INTRODUCTION

Ever since apples have been grown on a commercial basis, apple scab, caused by *Venturia inaequalis* (Cke.) Wint. (Sivanesan and Waller 1974), has been a major concern to producers. Since the late 1940's, the use of organic fungicides has had a major impact on the control of apple scab (Lewis 1980; MacHardy 1996). Over the years, fungicides became the sole means to control apple scab and there has been little effort to commercialize alternative strategies. Until recently, fungicidal control was perceived as the only economical control measure. This perception is changing as a result of the high costs of new molecules such as the strobilurine-based fungicides, increased fungicide resistance in populations of *V. inaequalis*, and increased appreciation of environmental costs and consumers' negative perceptions of fungicide use (Beresford and Manktelow 1994). Fungicide use entails certain environmental risks, that include disruption of pest and predator balances, such as the adverse effect on predacious mites, and health concerns for both farmers and consumers (Bower *et al.* 1995; Schneider and Dickert 1994). The pathogen has become increasingly resistant to some fungicides (e.g. dodine and benomyl) along with mounting concerns about

resistance to the DMI fungicides (sterol demethylation inhibitors) (Braun and McRae 1992; Carisse and Pelletier 1994; Hilderbrand *et al.* 1988; Jones 1981; Köller *et al.* 1991; Kunz *et al* 1997; Sholberg *et al* 1989; Smith *et al.* 1991; Thind *et al.* 1986). A case in point is the development of fungicide resistance in the pathogen population. In a survey of Ontario orchards, about 50% of the isolates of *V. inaequalis* were resistant to the eradicant fungicides currently used. For instance, resistance in *V. inaequalis* to Benlate® (benomyl) developed within only 3 yr of its release (Ontario Ministry of Agriculture and Food 1993). Furthermore, evidence of cross-resistance and partial cross-resistance was demonstrated in populations of *V. inaequalis* from the United States (Köller and Wilcox 2001). The frequencies of resistant isolates to both benomyl and DMIs were significantly higher within dodine-resistant populations than in dodine-sensitive populations. This phenomenon may result in accelerated selection of phenotypes with resistance to multiple fungicides. Because of the high costs to develop new fungicides and great public concern about environmental issues, fewer and fewer fungicides are available. Fewer fungicides will be available in the near future to control apple scab. For all these reasons, interest is increasing to develop alternative strategies to manage apple scab (Carisse *et al.* 2000a; Sutton *et al.* 2000).

As there are several review papers on apple scab, of which the most complete is by MacHardy (1996), this review paper focuses specifically on preventive management of scab based on non-fungicidal methods that include genetic resistance, physical destruction of the pathogen, and biological controls.

THE HOST

The apple has been an important fruit crop since the beginning of recorded time and continues to be important to this day. Worldwide, there were 7.3 million ha dedicated to apple culture in 1999. Apples were the second highest fruit production in the world after grapes at 60,000,000 MT (Anonymous 2000; Childers *et al.* 1995). The apple tree (*Malus* X *domestica* Borkh.) is a member of the family Rosaceae, subfamily Pomoideae (Jones and Aldwinckle 1990; Westwood 1988). The origins of the apple were likely from one or several interspecific hybridizations in Asia Minor, just south of the Caucasus Mountains. This resulted in a n = 17 haploid number of chromosomes (Juniper *et al.* 1999; Watkins 1995; Westwood 1988). However, the specifics of this process are still shrouded in mystery (Gordon 1991). One of the earliest reports of apple cultivation comes from Israel, that dates around 1000 BC and by Graeco-Roman times, the apple was common around the Old World, no doubt it followed the paths of the Greeks and Romans (Bultitude 1983; Juniper *et al.* 1999). Both the Romans and the Greeks were familiar with the modern techniques of budding, grafting, and rootstocks, although neither group is credited with their discovery. Either the Chinese or the Persians were likely to have developed these techniques (Juniper *et al.* 1999). Along with much of the technology of the Romans, these techniques were lost during the Dark Ages in northern Europe and then rediscovered or re-imported by 1000 AC. Apple cultivars became isolated in small geographic areas throughout Europe and tended to hybridize among themselves to produce new cultivars (Juniper *et al.* 1999). The europeans were responsible for transporting the cultivated apple to the new world and Australia during their explorations and colonization (Bultitude 1983; Juniper *et al.* 1999). In North America, for the two centuries after the arrival of the Europeans, very little grafting and few rootstocks were used as orchardists relied mainly on seedlings. This allowed for the emergence of many unique North American cultivars such as Golden Delicious and McIntosh. Apples are now grown in the majority of temperate countries. However, the geographical distribution of the apple is limited by a chilling requirement of 1000 to 1600 h of temperatures lower than 5°C and winter kill of trees occurs at temperatures lower than -30°C (Westwood 1988).

The terminal fruit buds of apples contain both flowers and leaves (Jones and Aldwinckle 1990). Fruit bearing branches are normally short structures called spurs that develop on the larger scaffold branches. Flower buds are initiated during the previous season. The quantity of flowers and the timing of the bloom depends on the crop load, cultivar, environment, and growth rate (Childers *et al.* 1995). An inflorescence has five to seven flowers that surround the centre flower, known as the king, which opens first (Childers *et al.* 1995; Jones and Aldwinckle 1990). After flowering, the shoot can continue vegetative growth, although on spurs this growth is minimal (Childers *et al.* 1995).

Of all the diseases that attack the apple tree, apple scab, caused by the fungus *Venturia inaequalis* (anamorph *Spilocea pomi* Fr.), is the most economically important disease in most apple growing regions of the world (Jones and Aldwinckle 1990; MacHardy 1996). One small scab lesion on an apple makes it unmarketable, even a low incidence of scab can mean significant losses to a grower. Not only can scab directly damage the crop, it can cause indirect harm by reducing the photosynthetic capacity of the foliage or outright defoliation. The distribution of apple scab is worldwide, but it is especially a problem in temperate regions with cold wet springs such as the United Kingdom, the former Soviet Union, and north-eastern North America (MacHardy 1996).

THE DISEASE

Symptoms of infection by *V. inaequalis* are found on the current year's growth except in regions with warm winter climates where the fungus can overwinter on the buds. Lesions are seen on leaves, petioles, sepals, blossoms, fruit, new shoots, and infrequently budscales (Jones and Aldwinckle 1990; MacHardy 1996). Foliar symptoms tend to be circular lesions that vary in colour from olive to brown. The lesions develop a velvety appearance caused by the abundant production of conidia. Lesions can appear either singly or in such numbers that the entire leaf surface is covered. When the lesions are young, the margins are indistinct, but margins become more distinct as lesions age if coalescence has not occurred. As the leaf grows and thickens, the infected area becomes deformed as well as necrotic because the hyphae and leaf tissue dye in the center of the lesion. Infection can occur on both upper and lower surfaces of a leaf with symptoms visible only on that surface, but some lesions kill underlying tissue, and render them visible from both sides. When many lesions are on a leaf or there is a lesion on the petiole, premature abscission is common.

On fruit, young lesions are similar to those on leaves. With age, the fungus dies in the lesion center having depleted its resources and the lesion takes on a corky appearance, often with a halo of loose cuticle. If the fruit was infected when young, it tends to become deformed as uninfected tissue around the lesion continues to grow. Often fruit cracking occurs because the callus tissue under the lesion is unable to expand. Fungal growth can continue throughout the season so that the enlarged lesion covers over half of an apple. Late-season infections develop slowly and can be very small or unnoticeable until after storage, and cause the phenomena of pin-point scab (Jones and Aldwinckle 1990; MacHardy 1996).

Apple scab is a limitation to apple production wherever apples are grown. Despite years of research on its biology and control, scab is still the most economically important disease of apple worldwide. Losses due to scab vary from one location to another and depend on disease severity and prevailing weather conditions.

EPIDEMIOLOGY

V. inaequalis overwinters in apple leaf litter. In the spring, when apple leaf litter is thoroughly wetted by rain, mature asci expand out of the pseudothecial ostioles. The asci forcibly eject their ascospores at an average distance of 3.0 mm, but many of them never

escape from the laminar air boundary layer (Aylor and Anagnostakis 1991; Jones and Aldwinckle 1990; MacHardy 1996). Once out of the laminar boundary layer, spores are dispersed by wind in the orchard, and a very few are deposited on the new growth of apple trees. The ascospores germinate if there is enough free water or relative humidity. Infection is successful as long as water is present or the relative humidity stays above 95%. Penetration of germ tube into the leaf is temperature dependent with the optimum at 20°C (Jones and Aldwinckle 1990; MacHardy 1996; Mills 1944). Once the appressoria and infection pegs are formed, the hyphae travel subcuticularly and deplete the cells around them to form a subcuticular stroma. The established *V. inaequalis* then starts to produce conidia. The disease is spread to new leaves and fruit throughout the season by splash dispersal of conidia. In autumn, when the leaves have fallen, *V. inaequalis* becomes a saprophyte. While in its saprophytic phase, two compatible mating types join to form a pseudothecium initial through fertilisation, followed by formation of the ascogonium. The fungus overwinters as pseudothecial initials in a more or less static state. Once early spring arrives and the temperatures warm, the pseudothecia mature and the whole process starts once again.

GENETIC CONTROL

Breeding for resistance has been recognized as a viable technique to control apple scab since the beginning of the twentieth century (Kellerhals 1989; Kumar and Sharma 1999; Williams and Kuc 1969). Resistance to *V. inaequalis* has been historically characterized in one of three manners: no visible symptoms from natural infection, reduced lesion number in comparison to another cultivar, and comparably smaller lesions with less severe symptoms that often includes reduced colonization of the subculticular space, reduced sporulation, and necrotic or chlorotic flecks (MacHardy 1996). There are several breeding programs for resistance to apple scab, primarily in Europe and North America. In the first half of the twentieth century much of the work to develop resistant varieties was done in Germany. Because the progeny of resistant species of *Malus* were not promising, most of the work in Germany concentrated on varieties with polygenic resistance such as Antonovka (Kellerhals 1989). Much of this work was interrupted by World War II and was not continued afterwards. One of the most prominent programs was started more than 50 years ago with the collaborative breeding effort of Purdue University, Rutgers University, and the University of Illinois. Known as the PRI program, it is responsible for the introduction of such resistant cultivars as Prima, Priscilla, and Jonafree. It was in this context that *Malus floribunda* 821, the original source of the V_f gene, was first used as a parent. Hough discovered the V_f gene in 1943 at the University of Illinois in the apple collection of Crandall (Crosby *et al.* 1992; Hough 1944). The V_f gene, while being the most frequently used over the last 50 yr, is not the only qualitative resistance gene. Qualitative genes are not dependant on environment or host conditioning and are usually dominant 'single' genes or tightly linked groups of genes (Williams and Kuc 1969). Many of the resistant species carry alleles of the V_f gene (Williams *et al.* 1966; Williams and Dayton 1968). However, there are other resistance genes such as the pit gene (V_m), from *M. micromalus* and *M. atrosanguinea*, the V_r gene that originated from *M. pumila* R12740-7A, V_{bj} from *M. baccata jackii*, V_b discovered in Hansen's *baccata* #2, and V_a from Antonovka PI 172623 (Crosby *et al.* 1992).

Good reviews on the history of the breeding programs and the origins of resistant cultivars are given by Crosby *et al.* (1992) and MacHardy (1996) so only a brief outline will be given here. As mentioned above, Hough identified *M. floribunda* 821 as a source of resistance in 1943 (Hough 1944; Crosby *et al.* 1992). Despite the promise of the resistance of *M. floribunda* 821, it was recognized early that a diverse selection of resistance genes was preferable

(Dayton *et al.* 1953; Dayton and Williams 1968; Shay *et al.* 1953). To this end, many crosses were made, but in some cases, there was little success to produce hybrid seedlings from *Malus* X *domestica* Borkh. and resistant species such as *M. honanensis* or *M. halliana* Koehne (Shay *et al.* 1953). Apples are susceptible to inbreeding depression so a modified system of backcrossing was used in breeding resistant cultivars with a different susceptible parent used in each generation (Hough *et al.* 1953). The breeding programs encountered other problems. For example, varieties were developed with 'desirable fruit characteristics' from the small fruited, often unpalatable resistant species. This is well illustrated in the early assessments of the crosses of *Malus pumila* and various commercial cultivars from the PRI program. Most progeny were rated poor for quality characteristics and some crosses resulted in fruit that were not much larger than cherries (Dayton *et al.* 1953; Hough *et al.* 1953).

Plant breeders have not been equally stringent in their definition of resistance. The main difference between programs was the classification of progeny with slight sporulation. The PRI group included slightly sporulating progeny in their resistant selections. In contrast, in two north american resistance-breeding programs of importance, the Canadian Department of Agriculture in Ottawa and the New York Agricultural Experiment Station in Geneva, the breeders excluded all progeny with sporulation (Lamb and Hamilton 1969; Spangelo *et al.* 1956). More recently, in the breeding programs in Europe, the breeders have eliminated the class of seedlings with restricted chlorotic lesions and they use only those seedlings with symptomless or hypersensitive responses (Kellerhals 1989). With their definition of resistance, the PRI group found a 1:1 segregation of resistant and susceptible progeny of V_f resistant susceptible crosses (Hough *et al.* 1953). Further intercrosses gave a dominant heterozygous gene in the resistant progeny with the classic 3:1 ratio of resistant to susceptible plants. However, when the plants with sporulating lesions are excluded, the ratios of resistant to susceptible plants were much lower (Hough *et al.* 1953; Lamb and Hamilton 1969; Spangelo *et al.* 1956). Spangelo *et al.* (1956) reported that when the results from 1950-54 were pooled, only 11% of the seedlings were considered resistant when plants with any sporulation were excluded. If the seedlings with sporulating lesions were included, then the data corresponded to the levels found by Hough *et al.* (1953). Lamb and Hamilton (1969) confirmed the findings of Spangelo *et al.* (1956). They also observed that plants differed in the numbers of spores produced per lesion. The variation in sporulation levels could be attributed to minor or quantitative genes from either the resistant or susceptible parent. Although the 1:1 resistant to susceptible ratio found by the PRI program remained consistent throughout the backcrosses, the proportion of seedlings classed with slight sporulation rose. The major resistance gene, V_f, was thought to be closely linked to a group of minor genes in *M. floribunda* 821 (Williams and Kuc 1969). However, with molecular techniques, the increase in sporulation was found to be related to the loss of resistance-modifying genes that may or may not be linked to V_f by segregation (Gardiner *et al.* 1996). There was further evidence of the effects of resistance-modifying genes. Tartarini (1996) found that the progeny of a Prima, Jerseymac cross was more resistant to scab than those from crosses between Prima and Golden Delicious or Summerred.

Despite the many yr of work on scab-resistant of apple cultivars, they have yet to gain widespread popularity, especially in North America. Cultivars resistant to scab are reputed to have low fruit quality, poor storability, low yield, and lack of market acceptance (Crosby *et al.* 1992; MacHardy 1996; Merwin *et al.* 1994). Producers are often hesitant to plant them, especially when they are relatively unknown by consumers (Crosby *et al.* 1992; Korban and Morrisey 1989; Merwin *et al.* 1994). Apples are one of the few horticultural crops that are purchased on the basis of recognition of the cultivar name. Therefore, when a cultivar is unknown to the public, sales tend to be low (Mer-

win *et al.* 1994). Although there has been little acceptance of resistant varieties in North America, acceptance has been greater in Europe, especially France. Two of the most widely planted cultivars are Priam and Judeline. However, resistant cultivars still represent a small percentage of the planted acreage (Crosby *et al.* 1992; Korban and Morrisey 1989).

As with any crop, durability of resistance is a concern. Durability is especially a concern with apples because the perennial nature of production makes it impossible to change cultivars rapidly. There is good reason for producers and breeders to be concerned. In 1956, the first three physiological races of *V. inaequalis* were described (Shay and Williams 1956). Race 1, the most commonly encountered throughout the world, did not cause disease on resistant hosts. Race 2 caused disease on *M. baccata* (L.) Borkh., some segregates of Russian seedling R12740-7A, and 'Geneva' (*M. pumila* var. *niedzwetzkyana* open pollinated). Race 2 only caused disease on 'Geneva' when the environmental conditions were optimal. However, Race 3 invariably caused disease on this cultivar but not the other species (Shay and Williams 1956). When Races 2 and 3 were evaluated for the number of genes involved to overcome the resistance in these species, there was one gene involved in the infection of *M. baccata* and Russian seedling R12740-7A, and two genes in Geneva (Shay and Williams 1956). Thereafter, races 4 and 5 were discovered (Crosby *et al.* 1992; Williams and Brown 1968). In the 1990's, race 6 of *V. inaequalis* was discovered. Race 6 was able to infect certain progeny of *M. floribunda* 821 that contained the V_f gene, but not *M. floribunda* itself (Parisi *et al.* 1993). Discovery of race 6 generated concerns among apple breeders because the majority of released resistant cultivars, 30 out of 34, contained the V_f gene. This discovery also highlighted what had already been suspected, that the resistance conferred by *M. floribunda* 821 was contributed by more than one gene (Parisi *et al.* 1993; Williams and Kuc 1969). It has since been proposed that the resistance gene in the selections susceptible to Race 6 be renamed the V_{fh} gene, the actual V_f gene having been lost early in the breeding process (Bénaouf and Parisi 2000). Recently, a race of *V. inaequalis* was found to infect *M. floribunda* 821 itself (Bénaouf and Parisi 2000; Roberts and Crute 1994). Bénaouf and Parisi (2000) have named this strain Race 7 and it is considered to infect trees with the V_f gene that includes *M. floribunda* 821. Despite the discovery of these new races, some commercial plantings of Liberty in North America are now 15 yr old, and there have been no reports of scab causing problems on these plantings.

MOLECULAR EXPLORATIONS OF RESISTANCE

Cultivars with only one resistance gene were vulnerable to *V. inaequalis* after the discovery of races that were able to overcome the major resistance genes. This problem has been apparent since the detection of races 2 and 3 (Shay and Williams 1956), but has been an even greater concern since the appearance of race 6 (Parisi *et al.* 1993). One proposed solution was to breed cultivars with more than one resistance gene (King *et al.* 1998). This is a difficult and time-consuming task with traditional breeding techniques. The actual procedure to test for resistance can be problematic also; inoculum distribution may be patchy, environmental conditions between sites are rarely the same, and growth may affect management practices and influence rates of infection (King *et al.* 1998). The release of a new cultivar can take 20 yr after the initial cross and the resulting characteristics are difficult to predict (Gardiner *et al.* 1996; Merwin *et al.* 1994; Shay *et al.* 1953). To compound the breeder's problems, apples are self-incompatible and have a relatively long juvenile period (Gianfranceschi *et al.* 1996).

Molecular techniques developed in early 1990's allowed for the identification of markers associated with the V_f gene. To date, only a small amount of work has been done with the other

resistance genes (Cheng *et al.* 1998). The benefits of marker identification are to speed and increase the accuracy of resistance screening of seedlings. Only the screening of those seedlings identified to have the markers in greenhouse or field would be necessary to confirm their resistance (Gardiner *et al.* 1996; King *et al.* 1998; Tartarini 1996). To identify seedlings with pyramided resistance genes is difficult with phenotypes, but this identification would be greatly simplified with marker-assisted selection (MAS). However, marker identification of most other genes remains to be done, despite rudimentary work on V_m and V_r (Cheng *et al.* 1998). Marker identification can also lead to the location and sequencing of the resistance genes. Once the V_f gene has been sequenced, the nature of V_f resistance will be easier to understand.

Mapping of the V_f region began in the early 1990's. Initially isozymes were investigated because of the high allozyme polymorphism of the apple. The isozyme locus *Pgm-1* was successfully identified as being linked to the V_f region at a distance of 8 cM (Manganaris *et al.* 1994) but is not currently used because of the high rate (~ 8%) of recombination between *Pgm*-1 and V_f. Also, the marker locus does not always segregate with V_f during meiosis and certain susceptible cultivars have the same allelic variant (Gianfranceschi *et al.* 1994; Hemmat *et al.* 1998; Manganaris *et al.* 1994). Other techniques, one of the first DNA markers, OPD20/600, was identified by Yang and Krüger (1994) with bulk-segregant analysis and random amplified polymorphic DNA (RAPD) techniques. This marker was later used to screen a series of resistant cultivars and selections for the V_f gene as well as susceptible cultivars (Yang and Korban 1996). Seventeen out of the 38 resistant varieties had the marker present while none of the susceptible cultivars did. This demonstrated the feasibility of this technique. Three more markers, M18, U1 (Köller *et al.* 1994), and A15 (Durham and Korban 1994) were identified soon after and the mapping process began. By 1996, it was possible to draw reasonably detailed linkage maps of the V_f gene and the markers on both sides of it (Gardiner *et al.* 1996; Gianfranceschi *et al.* 1996; Tartarini 1996). The markers on the maps were amplified from several cultivars that originated from the same resistant parent. This highlights the potential utility of the markers to screen progeny of new crosses (Gardiner *et al.* 1996; Gianfranceschi *et al.* 1996; Tartarini 1996). The RAPD markers were later transformed into sequence characterized amplified regions (SCAR) markers, which were more consistently reproduced (Gianfranceschi *et al.* 1996; Tartarini *et al.* 1999). Another benefit of marker identification has been the clarification as to whether progeny classified as 'weakly susceptible' by phenotype, such as defined by Chevalier *et al.* (1991), carry the V_f gene. These plants were found to carry the resistance gene and there was no segregation difference between those classified as 'weakly susceptible' and 'weakly resistant' (Gardiner *et al.* 1996). Resistant cultivars and advanced selections were screened to see which markers were present. All cultivars with resistance that originated from the V_f gene had a minimum of two markers although many were missing markers greater than 10 cM from V_f. In some cases, even the marker M18 was not present. This means that the entire side of the V_f introgression was missing (King *et al.* 1999). Markers greater than 10 cM are not useful for MAS because of the rates of recombination (Cheng *et al.* 1998). Marker distances in the range of 16-30 cM to the left and the right of the V_f gene as reported by Gardiner *et al.* (1996), Gianfranceschi *et al.* (1996) and Tartarini (1996) were confirmed by Hemmat *et al.* (1998) during their work to identify and map new markers. However, the location of the RAPD markers M18, AM19, and AL07 on the same side of the V_f gene (Hemmat *et al.* 1998) was contradicted by the work of King *et al.* (1998) and Tartarini *et al.* (1999), who placed these markers on opposite sides. The location of these markers is especially important because they are the closest reported to the V_f gene. Both the precise physical and genetic distances need to be established for map-based cloning projects, a tech-

nique to isolate genes where only the phenotype and genetic map position are known (Patocchi *et al.* 1999a, 1999b). There are some risks to map a gene with only one assessment technique or location as was demonstrated by King *et al.* (1998). Discrepancies were found between the experimental sites around Europe and assessment methods employed in the study when the resulting maps were compared with the consensus map of the entire data set.

With a bacterial artificial chromosome (BAC) library of the V_f carrying variety 'Florina', the feasibility to locate V_f with 'chromosome walking' was demonstrated by screening the library with a AL07 RAPD derived probe (Vinatzer *et al.* 1998). For the clarification of the marker location question, Patocchi *et al.* (1999b) and Xu and Korban (2000) saturation mapped the 20 cM that surrounded the V_f gene. With two different techniques, modified cleaved amplified polymorphic sequence (CAPS) markers for M18 (Patocchi *et al.* 1999b) and amplified fragment length polymorphism (AFLP) markers (Xu and Korban 2000) to investigate this region, the location of these three important markers was confirmed. M18 was reported to be the closest to the left of V_f and AL07, along with AM19, to the right. However the distances calculated from V_f were different; Xu and Korban (2000) measured M18 to be 0.4 cM from V_f and AL07/AM19 to be 0.2 cM whereas Patocchi *et al.* (1999b) calculated a distance of 0.2 cM from V_f for M18 and 1.1 cM for AL07. In addition Xu and Korban (2000) identified 15 new AFLP markers, seven of which were placed at the same position as V_f because no recombination events occurred between the markers and V_f in the two populations tested. Seven of the AFLP markers were clustered around the location of AL07 and AM19 with a further two markers at the position of M18. The other five RAPD markers that were mapped were found to be outside the 0.6 cM interval around V_f (Xu and Korban 2000). Eleven of the 15 AFLP markers have since been converted into more easily used SCARs (sequence–characterized amplified regions) (Xu *et al.* 2001). Patocchi *et al.* (1999a) continued work with the BAC library and chromosome walking with the map calculated in Patocchi *et al.* (1999b). Thirteen BAC clones were shown to contain the markers M18 and AM19. With 2000 plants from various segregating populations, the location of V_f was identified to be in a minimum of five BAC clones that represented approximately 350 kb. Once the approximate physical location of V_f was discovered, a closer exploration of the region between M18 and AM19 could ensue. From this region, cDNA's that hybridized to the five BAC were identified and subsequently sequenced. The cDNA clones were found to be homologues of the *Cf* (*Cladosporium fulvum* (Cooke)) resistance genes of tomato (Vinatzer *et al.* 2001). Thirty-six percent of the amino acid sequence was identical and 56% was similar to one gene, *Cf9*. When a Southern blot with one of the cDNA clones was done, multiple bands were found on three of the five previously identified BAC. This was interpreted as a gene cluster of many *Cf* homologues in the V_f region. These homologues were named the '*HcrVf* genes' (homologues to *C. fulvum* resistance genes of the V_f region) of which there was a minimum of five found to co-segregate with V_f resistance. Through analysis of the proposed amino acid sequences, it was predicted that the *HcfVf* 1-3 are membrane-bound glycoproteins as is *Cf9*. With the results of Vinatzer *et al.* (2001), currently two of the *HcfVf* genes, numbers 2 and 4, are candidates to be the V_f gene. Whether *HcfVf*1 co-segregates with V_f and its exact location is still unknown, and *HcfVf*3 has been identified as a pseudogene. However, there are two other *HcfVf* genes to be mapped and the possibility that there may be more to be discovered.

From the work on the V_f gene, the question arises: Are the V_f markers present in the species and cultivars that carry other resistance genes? Nova Easygro (reportedly V_f) and Reglindis (*VA*) had all the markers associated with the V_f gene but Murray (V_m) had none (Tartarini 1996). Gianfranceschi *et al.* (1996) went so far as to suggest that the resistance gene in Nova Easygro was an allele of the V_f gene since all of the V_f markers are linked with resistance in

Nova Easygro. This interpretation is an unlikely coincidence because of the high level of polymorphism in the apple genome, including the V_f region. Dayton and Williams (1970) originally described the two genes, V_r and V_f, to be separate. This has not been refuted to date. The fact that Gianfranceschi *et al.* (1996) used Nova Easygro in their analysis and Dayton and Williams (1970) used *M. pumila* R12740-7A, the original source of the gene could help to explain the discrepancy. *M. pumila* R12740-7A has two resistance genes, V_r and V_f, with V_r being lost during the breeding process of Nova Easygro. Some V_f-linked markers were found in unrelated accessions such as *M. hupehensis*, *M. sikkimensis*, and *M. nietzwetzkyana* but these may be the same fragments that were amplified. No evidence that these accessions are in any way related to the V_f locus has been provided (King *et al.* 1999).

Of the other resistance genes, only V_m has been extensively mapped. One marker, OBP12, was identified at the relatively long distance of 6 cM from the V_m gene. It was found only in cultivars and species closely related to *M. micromalus*. To test for resistance type, some accessions that carry V_m were inoculated and all exhibited the pit-type resistance reaction (Cheng *et al.* 1998).

Recently, a new tactic of resistance production was tried. Apple trees were transformed with genes that encoded for chitinolytic enzymes from *Trichoderma harzianum* Rifai (Bolar *et al.* 2000). The transformations of Marshall McIntosh leaf tissue were done with *Agrobacterium tumefaciens* (Smith & Townsend) Conn. as the method of gene transfer. In early tests in the greenhouse, good control of *V. inaequalis* was achieved with higher levels of enzyme expression. When compared to Liberty, a V_f carrying cultivar, the transgenic lines that exhibited relatively high levels of enzyme activity had comparable numbers of visible lesions and proportion of leaf area diseased (Bolar *et al.* 2000). The number of conidia produced was higher than on Liberty, but the overall levels were relatively low with comparable numbers of lesions. However, some transformed trees failed to thrive. In fact, at higher titers of enzyme activity, the trees were so stunted that it was difficult to distinguish between the effects of the enzyme or other factors that caused poor tree growth. When an apple tree is stressed and not rapidly growing, it is not as susceptible to infection by *V. inaequalis* because of ontogenic resistance (MacHardy 1996).

DIFFERENTIAL SUSCEPTIBILITY

Since early in the twentieth century it has been recognized, first by Aderhold in 1902, that differential or partial resistance to *V. inaequalis* exists in the apple genome (Williams and Kuc 1969). However, resistance levels of the majority of orchard varieties were too low to be of use in either breeding programs or in orchard protection strategies (Hough 1944). In breeding studies, those orchard varieties with above average resistance had a form that was controlled by multigenic factors or 'minor genes' (Hough 1944). However, the apple is difficult to breed for homozygotic gene expression, being self-sterile. In addition, because the screening process for resistance is long and results are unpredictable, efforts to use multigenic resistance was soon dropped and little research has been done in this area since the 1950's.

Polygenic resistance or partial resistance, can be expressed in many ways; reduced disease severity or incidence, increased incubation or latent periods, smaller lesions, and reduced inoculum production which combined, create different levels of susceptibility (Parlevliet 1979; Smith 1992). Partial resistance is generally found to be multigenic, and although the genotype is susceptible, the epidemic rate is moderated (Leonard and Mundt 1984; Parlevliet 1979). It is generally assumed that pathogens have greater difficulty to adapt to this broader-based resistance and is therefore considered to be more durable than single-gene resistance (Parlevliet 1979).

Until recently, little research has been done on the relative susceptibility of commercial cultivars common to the

north american industry, although some lists have been published (Aldwinckle 1974; Olivier *et al.* 1984). These reports are based on mainly anecdotal evidence from field agents and scientists. Several reasons exist as to why partial resistance in apples has not been thoroughly investigated. Fungicides have been a very effective control strategy to date and farmers who plant multiple cultivars in the same block to ensure pollination have difficulty individualizing fungicide regimes to each cultivar. Moreover, in comparison to complete resistance, partial resistance is more difficult to assess and difficult to handle in the breeding process (Hough 1944; Leonard and Mundt 1984; Parlevliet 1979). Olivier *et al.* (1984) published an anecdotal list of scab susceptibility ratings for european conditions and cultivars. Schwabe (1980) looked at several different cultivars in his article on leaf wetness periods. Differences were observed among cultivars in percent infection for both conidia and ascospores. However, there is no indication as to whether the cultivars were infected at the same time or whether they were in separate trials, therefore it is difficult to conclude from this report that there were real differences among cultivars. There was also no comment on the inoculum dose used, in particular for experiments with ascospores. Therefore, it is impossible to assess whether the differences seen between levels of infection by the ascospores and conidia were to the inoculum dose or whether one type of spore had a higher infection efficiency. Further studies on relative susceptibility were done by Jeger (1981), Jones *et al.* (1998), Olivier *et al.* (1984), Smith (1992) and Szkolnik (1978). Szkolnik (1978) looked at the relative number of lesions per leaf, spore production, and the type of lesions. He did the experiments with conidia in the greenhouse and ascospores in the field. The trees in the greenhouse were inoculated only once, whereas the trees in the field were subjected to multiple inoculations by ascospores over a six-week period. From the greenhouse work, it was concluded that there were differences amongs cultivars for all of the variables studied. Szkolnik (1978) cautioned that the number of lesions per leaf should not be considered the sole indicator of susceptibility as cultivars with comparable numbers of lesions could vary considerably in the number of spores produced. Unfortunately, the results of the field trial were neither published nor presented although it was noted that trees grown under greenhouse conditions were much more easily infected (Szkolnik 1978). Jeger (1981) took a slightly different approach to measure the comparative incidence and severity among several cultivars over time under field conditions, without separation of primary and secondary inoculum effects. The cultivars were significantly different for these disease indicators. In addition, there were interesting differences among the disease progress rates, where some cultivars were much less infected early in the season but approached similar levels of infection by the end of the season (Jeger 1981). The same author also described differences among cultivars in their susceptibility to wood scab, a problem virtually unknown in North America.

In New Hampshire, Smith (1992) conducted a large study on the relative susceptibility of apple cultivars. Several aspects of cultivar effects on the epidemiology of *V. inaequalis* were investigated. The effect of cultivar on overwintering of pseudothecia and on their rate of maturation in the following spring was examined. The cultivars had no significant effect on these processes. In addition, Smith (1992) compared cultivars upon the basis of their innate components of partial resistance. Among the components examined were disease incidence, disease severity, and the amount of available susceptible leaf and fruit tissue, the relative timing of phenological stages, incubation period, conidia production, and relative ontogenic resistance. Significant differences were observed among the cultivars for both disease incidence and severity. Although the cultivars differed phenologically and in the amount of available tissue, there was no correlation with the incidence or severity of scab. No significant differences were found among incubation periods for the cul-

tivars evaluated. The relative conidial production did differ significantly, but no effect of cultivar on the rate of production or lesion size was found. Only general conclusions could be made about ontogenic resistance from this study. Only the three youngest leaves were generally susceptible, and that did not seem to vary between cultivars.

Control recommendations in Eastern North America for *V. inaequalis* are often based on epidemiological studies using McIntosh. McIntosh is highly susceptible and, as a result, the infection models are conservative (i.e. fungicides are used more frequently than may be necessary) (Anonymous 1988; MacHardy and Gadoury 1989; Mills and Laplante 1951). However, this may not be the best strategy. For example, as the eastern Canadian apple industry changes from being McIntosh-based to a more diverse selection of cultivars, strategies to manage scab should be optimized for cultivars dependent on their susceptibility relative to McIntosh. As the industry diversifies, knowledge of the relative susceptibility of cultivars could permit management strategies such as the one proposed by Olivier *et al.* (1984) to come to the forefront. Olivier (1983, 1984) suggested a re-interpretation of the Mills' curves to include cultivar susceptibility in a decision for fungicide application. Those cultivars with high partial resistance, Olivier (1983, 1984) suggested they should be treated less frequently than what the current interpretation of the Mills' curves suggest. The majority of cultivars would be classified into lower susceptibility groups and treated according to the appropriate curve. Improved control measures using fewer fungicide applications for the less susceptible varieties would thus be available in the future.

In response to the ideas of Olivier (1983, 1984), a new study was undertaken to investigate partial resistance in susceptible cultivars by Dewdney *et al.* (2000). The authors investigated 21 cultivars common to the canadian apple industry and their relative partial resistance ratings. The components of partial resistance examined in this experiment were disease severity, incubation and latent periods, lesion surface area, and conidial production. The experiment was conducted in a greenhouse with ascospores as inoculum, the inoculum that generates primary infections under canadian conditions. Most experiments to date have used conidia as inoculum to facilitate experimentation. However, when primary infections are well-controlled, infection by conidia is rarely important (MacHardy 1996). Significant differences among cultivars were found for each component of partial resistance for both initial and final results (Dewdney 2000; Dewdney *et al.* 2000). In addition, the cultivars were grouped into susceptibility classes for each component of partial resistance by cluster analysis. A principal component analysis (PCA) was then used to determine which of the partial resistance factors were the most important in the final ranking of disease susceptibility (Fig. 1). The cultivars were grouped into a final ranking of disease susceptibility by multivariate cluster analysis (Fig. 2). The final ranking was most influenced by disease severity, incubation period, and latent period, all found on principal component one (PC-1). The effects of the partial resistance factors on individual cultivars could be gauged by where the cultivars were positioned on the axes. For example, disease severity was very important in the ranking of both McIntosh and Vista Bella. Although the other partial resistance factors were much less influential, they were still considered to be important when doing a definitive ranking. For a ranking based on partial resistance components, it was necessary to use either lesion surface area or conidial production to get a more accurate account of the cultivars (Dewdney 2000). It has been considered acceptable to use only disease severity to rank cultivars for partial resistance in breeding programs (Lateur and Populer 1994a, 1994b; Washington *et al.* 1998) mainly because of the amount of work implicated to measure other partial resistance components on hundreds of cultivars.

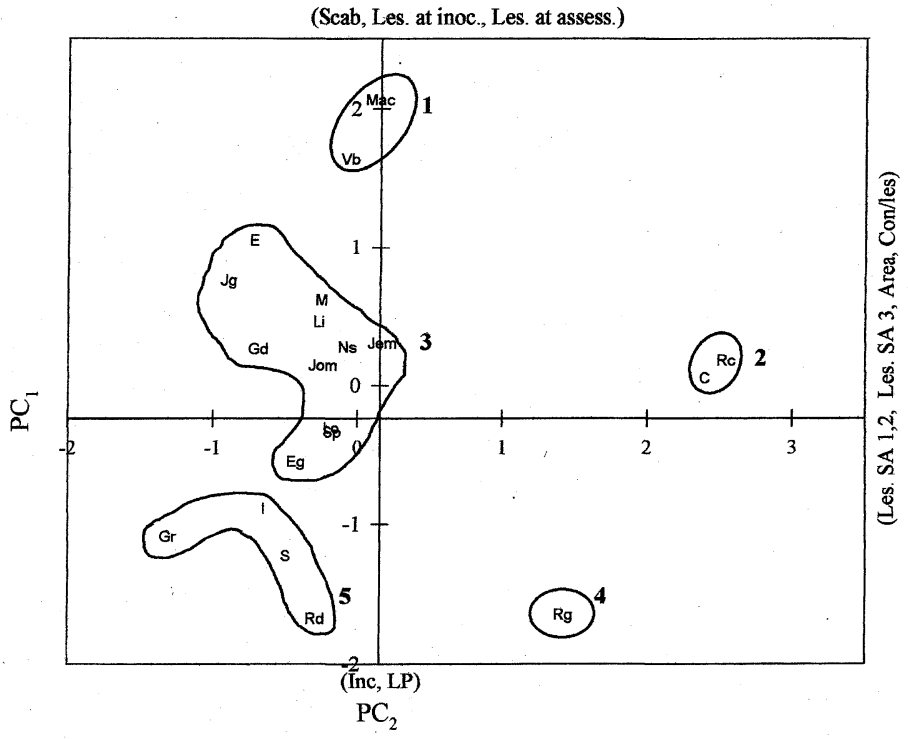

Figure 1. Susceptibility groupings of the 21 cultivars, based on the principal component analysis (PCA) as projected on the PC_1-PC_2 plane (eigenvalues 6.7898 and 1.3961 respectively). Together, PC_1 and PC_2 account for 74% of the total variation. The numbers 1 to 5 identify the clusters in order of increasing partial resistance. The legend for the cultivars are as follows C = Cortland, Eg = Early Geneva, E = Empire, Gd = Golden Delicious, Gr = Golden Russet, I = Idared, Jem = Jersey Mac, Jg = Jonagold, Jom = Jonamac, Lo = Lobo, Li = Lodi, Mac = McIntosh, M = Mutsu, Ns = Northern Spy, Rc = Red Cortland, Rd = Red Delicious, Rg = Royal Gala, Sp = Spartan, S = Sunrise, and Vb = Vista Bella. The axes abbreviations are, for the top axis Scab = relative lesions/leaf/tree, Les. at inoc = relative lesion density at time of inoculation, Les. at assess = relative lesion density at time of assessment; for the bottom axis, Inc = relative incubation, LP = relative latent period; for the right-hand axis Les. SA 1,2 = relative lesion surface area series 1 and 2, Les. SA 3 = relative lesion surface area series 3, Area = relative proportion of leaf area diseased, Con/les = relative conidia/lesion.

The relative cultivar susceptibility to apple scab is a transient phenomena. Much depends on environmental conditions the yr of observation and on the proportion of an area planted with a given cultivar. For example, Golden Delicious is considered a highly susceptible cultivar in Europe (Koch *et al.* 2000; Lateur and Populer 1994a), whereas Dewdney (2000) found that it was only moderately susceptible under conditions in eastern Canada. In Europe, Golden Delicious is one of the most widely planted varieties (Koch *et al.* 2000), but in eastern Canada, Golden Delicious has a very restricted acreage (Labrecque 1999). *Venturia inaequalis* appears to become more aggressive on a specific cultivar if the area on which that cultivar is being produced increases substantially (Bousset *et al.* 1997). In the experiment by Dewdney (2000), the inoculum came exclusively from McIntosh, the dominant cultivar of the Quebec region. Because the majority of apples produced in Quebec is McIntosh, it was considered fair to assume that the population of *V. inaequalis* was adapted

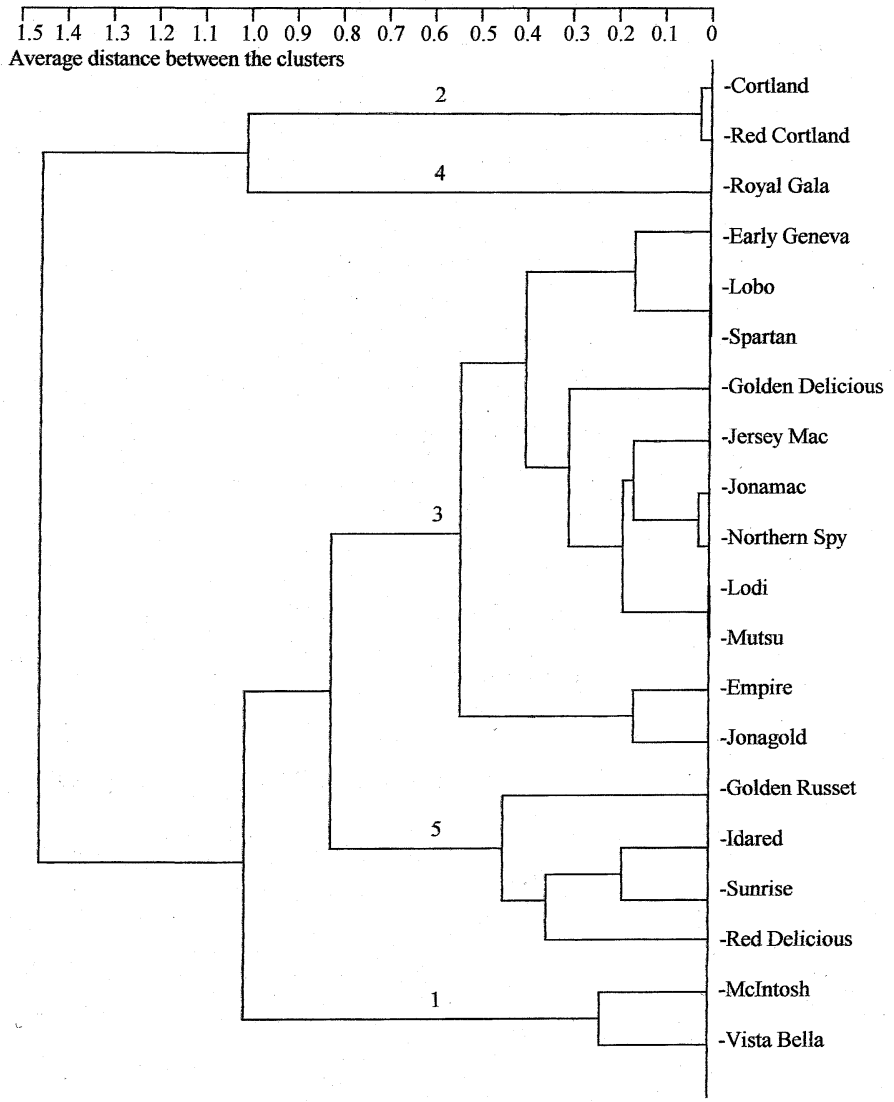

Figure 2. A multivariate hierarchical comparison of 21 apple cultivars divided into 5 susceptibility classes based on the values of principal components 1 and 2 (PC_1 and PC_2) for each cultivar. The numbers 1 to 5 identify the clusters in order of increasing partial resistance.

to that cultivar. As the proportion of McIntosh in Quebec was six times greater than any other cultivar produced, it was assumed that any other plantation would be exposed to the same population for at least the following 5 yr. It was presumed that as the proportion of other cultivars increases, the population profile of *V. inaequalis* would change and a new relative cultivar evaluation would be needed.

In the last 16 yr, since Olivier (1984) proposed his 'evolution in apple scab control', little has been done to resolve the question of relative susceptibility

of apple cultivars in comparison to one another, especially to McIntosh. McIntosh is of special importance, since this was the cultivar that Mills used for his pioneering work on infection curves (Mills 1944). Only recently, studies have begun that could lead to an analysis of the feasibility of Olivier's ideas.

MIXED CULTIVAR PLANTINGS

Apple cultivars are differentially susceptible to *V. inaequalis* (Dewdney 2000; Smith 1992; Szkolnik 1978). Isolates of *V. inaequalis* are virulent on some cultivars but either less so or not at all on others (Koch *et al.* 2000; Palmiter 1934; Sierotzki *et al.* 1994; Tenzer and Gessler 1997). It is a challenge to use differential susceptibility of cultivars and virulence of the pathogen isolates in orchard design to reduce fungicide because apple trees are perennial plantings. A computer simulation experiment was conducted to test the effects of different cultivar planting patterns on the reduction of lesions (Blaise and Gessler 1994). Combinations of no more than three cultivars were tested in solid blocks, homogenous rows, and mixed rows. The cultivars were differentially susceptible to the inoculum, primary or secondary and the 'pathotype' of the inoculum depended on the cultivar of origin. The conidia dispersion was assumed to follow a Gaussian distribution for splash dispersal with equal distribution in each direction (Blaise and Gessler 1994). Expectedly, the greatest amount of disease predicted was in a solid cultivar block. The greatest reduction, 79%, was found with three cultivar mixes within the row. When the cultivars were in alternating rows, potential scab was reduced by 65% in the simulation compared to 67% reduction when only two cultivars were in alternate rows. In this simulation, substituting one susceptible cultivar by a resistant one had no effect in the alternate-row planting scenario (Blaise and Gessler 1994). The proposed mechanisms of reduction were a greater distance for inoculum to travel because resistant trees created a barrier to the spread of inoculum and the reduction of susceptible tissue. The model was tested in an orchard planted specifically for this purpose (Bousset *et al.* 1997). There was a large reduction in the area under the disease progress curve (AUPDC) for the most heavily infected cultivar, Elstar, when it was planted within the row with less susceptible cultivars. For the less susceptible cultivar Golden Delicious, there was also a reduction of the AUPDC when within row cultivar mixes were compared with rows of a single cultivar, which supported the theory of Blaise and Gessler (1994). All possible virulence combinations of the isolates of *V. inaequalis* were found in the orchard.

Different isolates of *V. inaequalis* are not equally virulent on all susceptible cultivars. This has been known for years, being proposed first by Aderhold (1899) *in* Sierotzki *et al.* (1994). Further evidence was given in studies such as the one by Palmiter (1934). Once resistant cultivars were introduced, interest in differential virulence was substantially reduced. However some interest in this subject has been rekindled in the last 10 yr. When Sierotzki *et al.* (1994) evaluated the isolates from several susceptible cultivars, some cultivars like Boskoop and Glockenapfel, were less susceptible to inoculum that originated from other cultivars but quite susceptible to inoculum that originated from the same cultivar. Other cultivars such as Maigold and Golden Delicious were severely infected by all isolates. If a population of pathotypes were inoculated onto a cultivar in equal proportions and allowed to have three asexual cycles, then there was a shift in the frequency of pathotypes. Some isolates disappeared, some had reduced frequencies, and others substantially increased their proportions in the populations. The patterns of the isolates were dependant on the cultivar that was inoculated but generally isolates that originated from that cultivar remained. Even when isolates from a particular cultivar were inoculated back onto that cultivar, frequencies changed; one or two isolates became prominent while the other isolates either disappeared or had much reduced frequencies.

Two questions that needed to be addressed to determine whether mixed cultivar plantings would be effective in the long term were: (i) how does virulence vary among isolates and (ii) what is the distribution of the individual isolates? An attempt to quantify the virulence patterns in populations of *V. inaequalis* found in Switzerland was done by Koch *et al.* (1997, 2000). It was pointed out that when testing for virulence the interpretation of 'no disease' could be difficult (Koch *et al.* 1997, 2000). The lack of disease could be due to either an avirulent reaction between cultivar and pathogen or simply escape from infection since *V. inaequalis* has particular criteria for infection. Koch *et al.* (1997) investigated the consequences of variability in classifications of virulence or avirulence. They concluded that variability within a repetition was comparable between inoculations on cultivar of isolate origin and non-origin. Koch *et al.* (2000) looked at cultivar virulence patterns and concluded that differential resistance existed among cultivars and their corresponding virulences in the population of *V. inaequalis*. To compliment this work, the genetic diversity in populations of *V. inaequalis* was evaluated in Switzerland (Tenzer and Gessler 1997; Tenzer *et al.* 1999). Haplotype diversity was very high for the orchard but relatively low for within a single tree as would be expected for an asexually reproducing organism. The final conclusions were that the population was highly diverse but equally spread across all regions of Switzerland (Tenzer and Gessler 1997; Tenzer *et al.* 1999).

PHYSICAL CONTROL

Strategies of physical control have included: (i) pruning to increase air circulation and thereby reduce leaf wetness duration; (ii) burning of leaf litter and; (iii) the use of earthworms to increase leaf decomposition. Orchard layouts that favour wind circulation, appropriate in-row and between-row spacing, and proper pruning have been shown to reduce severity of scab (Kolbe 1983). Overall, most of these practices were developed in the context to reduce the ascospore inoculum of *V. inaequalis*. As early as 1888, it was known that overwintering scabbed leaves contributed to the initial inoculum of the following spring (Scribner 1888). However, it was not until 1924 that Curtis established a quantitative relationship between scabbed leaves in the leaf litter and initial inoculum. In 1936, Keitt reported that removal of apple leaves from the orchard, either by burning or by disc cultivation, was recommended even if the role of leaf litter in inoculum production was not fully understood. Rosenberger (1990) also reported similar effects. Sutton *et al.* (2000) demonstrated that, in northeastern United States, when apple leaf litter was destroyed by shredding the leaves in the fall (November) or in the spring (April), an 80 to 90% reduction of scab risk was observed. However, if due care was not taken and not all the leaves were shredded, scab risk was reduced by only 50 to 65%. In general, growers do not use sanitation techniques, although they can result in significant reductions in ascospore load. Reluctance to use sanitation results from the need for specialized equipment (shredder), the failure of sanitation to provide complete disease control, and lack of reliable relationships between sanitation measures and the degree to which fungicides can be reduced the following year (Biggs and Warner 1990; Sutton *et al.* 2000).

BOTANICALS AND FERTILIZERS

Gilliver (1947), tested plant extracts from 1915 different species for their effects on germination of conidia of *V. inaequalis*. Of all the plant extracts, 440 showed various levels of inhibition. In particular, extracts of watery ivy (*Hedera helix* L.) were the most effective. Later, in 1965, the ivy saponins hederacosid C and α-hederin were purified and characterized by Tschesche *et al.* The fungicidal effects of α-hederin and other saponins were demonstrated by Wolters in 1968. In 1987, Bosshard *et al.* reported that plant extracts of saponin provided a good level of scab control on

potted plants under controlled conditions, but not when tested under field conditions. Bosshard (1992) tested the effect of watery ivy extracts and reported that a 1% ivy leaf extract diluted with water to 1:8 and even as low as 1:16 completely inhibited conidial germination on glass slides. On apple seedlings, the level of scab control was high, varying from 59.0 to 99.4% dependent on whether the extracts were applied 1 or 7 d before inoculation with *V. inaequalis* (Bosshard 1992). Northover and Schneider (1993) tested several plant oils against *V. inaequalis* and reported that soybean or canola oil emulsified with Agral 90 and applied at a rate of 1% every 7 to 10 d, reduced scab severity by 66 to 81%. Some russetting was reported on Golden Delicious following the oil treatments.

It is well known that proper fertilization promotes tree health and consequently increases the host's defence against some diseases. However, there are few reports on the effect of fertiliser on scab development. Kumar and Gupta (1986) observed that a high level of potassium fertilizers increased resistance of apple tree to scab and that a similar effect was not obtained with high levels of phosphorus fertilization. Among all fertilizers, urea has been the most widely studied for the control of apple scab, with its effects on scab being known since the pioneering work of Ross (1961). From *in vitro* experiments, Ross (1961) suggested that excess nitrogen could suppress pseudothecial formation. A few yr later, Oland (1963) first tested post-harvest pre-leaf-fall urea sprays as a means to supply nitrogen to the trees. With the idea of combining nitrogen supply with scab control, Burchill *et al.* (1965) tested the effect of two post-harvest pre-leaf-fall urea sprays of 2 and 5% on the development of *V. inaequalis* in overwintering leaves. In an experiment on detached leaves dipped in 5% urea and overwintered under controlled conditions, noticeably enhanced leaf decomposition occurred with a reduction in ascospore production of 97%. Burchill (1968) further investigated the effect of urea on ascospore production and looked at different timings of urea application as well as combined autumn (5% urea) and spring (pre-budburst, 2% urea) treatments. He reported various levels of inhibition (50 to 100%) for detached leaves and field experiments. The role of urea in suppression of pseudothecial development has been investigated worldwide; in England by Ross and Burchill (1968) and Burchill and Cook (1970), in India by Gupta and Lele (1980), in United States by Moller (1981), and more recently by Sutton *et al.* 2000. From these studies, it was concluded that chemical and microbial changes, as well as the deterioration of the physical support for pseudothecia formation, were responsible for the inhibitory effect. Despite the various levels of control reported, urea treatments present several advantages that included, no known effect on auxiliary fauna, and low cost. Urea can be used alone or combined with other treatments, such as leaf shredding (Ciecierski *et al.* 1995; Gupta and Lele 1980; Sutton *et al.* 2000).

BIOLOGICAL CONTROL

This section focuses on biological control through either the introduction of a microbial control agent or the manipulation of naturally occurring populations of microorganisms. Biological control is often seen as a strategy that recently emerged from microbial biotechnology, but, in fact, research on biological control of apple scab has been conducted for over 50 yr. The nature of life cycle of *V. inaequalis* has lent itself to studies that aim to interrupt overwintering of the perfect stage or else to control infection of leaves during the spring and summer.

In 1949, Cinq-Mars pioneered biological control of apple scab as the first scientist to isolate microorganisms from apple leaves. His collection contained more than 25 different organisms, among which were fungi, bacteria, and yeasts. With these organisms, he looked at the interaction of *V. inaequalis* and sterile culture filtrates of the isolates. He showed that some of these organisms, mainly *Penicillium* species, produced antibiotics that inhibited mycelial growth of *V. inaequalis*. He also

demonstrated that some of the fungi were able to enhance leaf decomposition and consequently interfered with pseudothecial development. Ross (1953) pursued the work initiated by Cinq-Mars and extended the collection of microorganisms isolated from apple leaves in both Quebec and Nova Scotia. Ross was the first to conduct an *in vitro* experiment to show the effects of leaf decomposition on pseudothecial development. Ross was a pioneer, as he evoked the idea to used the antagonists themselves instead of the antibiotics produced by such organisms. A few yr later, inspired by the remarkable success obtained with antibiotics for the control of animal diseases, Simard *et al.* (1957) made a new collection of microorganisms with the hope to find producers of antibiotics. Of their collection, they observed that 34 antibiotics produced by fungi significantly inhibited mycelial growth of *V. inaequalis*. Unfortunately, in further tests, they observed that the effects of the substances produced by inhabitants of apple leaves were mainly fungistatic, not fungicidal.

In 1962, Hirst and Stedman reported the results and conclusions of several yr of research on the supply and liberation of ascospores of *V. inaequalis*. They suggested that low concentrations of ascospores observed in some orchards might be due to the presence of naturally occurring saprophytes. The evidence that fall applications of urea interfered with the overwintering of *V. inaequalis* and favored development of bacterial populations was shown by Crosse *et al.* (1968). They observed that the diversity of apple leaf microflora was modified after an application of urea. The bacterial population shifted from predominantly 'gram positive' to a population dominated by 'gram negative' bacteria. Fluorescent pseudomonads increased in numbers, many of which were found to suppress the development of *V. inaequalis*. Consequently, they hypothesized that a fall application of urea may help to reduce initial inoculum of *V. inaequalis* by enhanced leaf decomposition, disturbed pseudothecial development, and favored populations of microbes antagonistic to *V. inaequalis*.

Complementary work was done by Hislop and Cox (1969) when they investigated the effects of fungicides on the size and diversity of populations of microorganisms that lived on apple leaves. Hislop and Cox's work represented a turning point in the history of biological control of scab, as it was the first study to examine a possible integration of microbial and chemical control. Burchill and Cook (1970) conducted the first field study on the effects of a fall application of urea on fungal flora of apple leaves. They observed that when leaves were dipped in urea it stimulated the leaf colonization by *Cladosporium* sp., *Epicoccum* sp., *Pistillaria* sp., *Alternaria* sp., and *Fusarium* sp. When *Fusarium sporotrichioides* Sherbakoff and *F. avenaceum* (Fr.:Fr.) Sacc. were inoculated onto leaf disks, the development of pseudothecia of *V. inaequalis* was suppressed. From this study, they also concluded that bacterial populations could both stimulate and inhibit pseudothecial development.

Andrews *et al.* (1983) reinitiated research on biological control of apple scab. Their first step was to make another collection of apple leaf inhabitants and to evaluate the effects of these leaf colonizers on vegetative growth and conidial germination of *V. inaequalis*. A total of 50 microorganisms were evaluated and the most antagonistic fungi were *Aureobasidium pullulans* (de Bary) G. Arnaud, *Trichoderma viridae* Pers.:Fr., *Chaetomiun globosum* Kunze:Fr., *Microsphaeropsis olivacea* (Bonord.) Höhn., and two unidentified actinomycetes. From a series of experiments, they selected the antagonist, *C. globosum*, based on its efficacy and consistency. From their observations, they suggested that the antagonistic activity was due to both nutrient competition and antibiosis.

Cullen *et al.* (1984) evaluated the potential of *Chaetomiun globosum* as a biofungicide against apple scab. A spore suspension of *C. globosum* applied every 1 to 2 wk to apple trees in an orchard reduced scab severity by 20% as compared to untreated control. In further trials, Boudreau and Andrews (1987) demonstrated that *C. globosum*

did not colonize apple leaves even when biological control activity was observed. Instead, they observed that dead cells of *C. globosum* were as effective as living cells to prevent leaf infection by *V. inaequalis*. Unfortunately, they also noted that the antibiotics produced by *C. globosum* were short-lived and lost their activity when exposed to common environmental conditions such as prolonged light.

Recently, Burr *et al.* (1996) screened 931 strains of bacteria and yeasts isolated from apple leaves. They looked at the effects of these microorganisms on mycelial growth, conidial germination, and scab development on seedlings. Of the 931 strains, 92 isolates significantly inhibited mycelial growth of *V. inaequalis*, 32 inhibited conidial germination, 104 significantly reduced scab severity on apple seedlings. Unfortunately, no correlation was observed between *in vitro* and *in vivo* tests and promising bacteria were not tested under field conditions. Ouimet *et al.* (1997a, 1997b) examined at the effects of fungal antagonists on *in vitro* inhibition of vegetative growth of *V. inaequalis*. An isolate of *Ophiostoma* sp. completely prevented mycelial growth independent of the temperature, pH, or light conditions. However, in the experiments conducted by Philion *et al.* (1997), it reduced ascospore production by 88.7% in one trial, but only by 8.2% in another. When tested under orchard conditions, this isolate failed to inhibit production of ascospores with an average ascospore inhibition of 8%.

In their study, Burr *et al.* (1996) and Ouimet *et al.* (1997a, 1997b) aimed to develop a microbial control agent for use against leaf infection. This approach was fraught with difficulty because it is much harder to attack *V. inaequalis* in its active phase (parasitic phase) than in its saprophytic phase. Furthermore, it will be difficult for a biofungicide to compete with available chemical fungicides on the basis of cost and efficiency and perhaps safety. Several researchers (Burchill and Hutton 1965; Keitt 1936; Keitt and Palmiter 1937; Keitt *et al.*, 1941; Palmiter 1946) demonstrated that a reduction in the ascospore inoculum results in lower disease severity the following spring and consequently makes scab management more secure, possibly with fewer fungicides sprays. MacHardy *et al.* (1993) demonstrated the possibility to delay the first fungicide applications up to the pink stage, when the inoculum potential is very low.

Before the information to delay an application of fungicide in orchard with low ascospore inoculum was published, Heye (1982) and Heye and Andrews (1983) examined the possibility to screen fungal antagonists on the basis of their ability to inhibit pseudothecial development rather than vegetative growth of the fungus. From their fungal collection, 57 apple leaf saprophytes were screened, and *Athelia bombacina* Pers. was selected because of its ability to inhibit completely pseudothecial development of *V. inaequalis*. *Athelia bombacina* was also tested under field conditions for its efficacy to reduce ascosporal inoculum. In a first field trial, Young and Andrews (1990) showed, with immunocytochemical detection, that *A. bombacina*, when applied to naturally infected apple leaves, inhibits both the growth of hyphae and the initiation of pseudothecia by *V. inaequalis*. From these studies, the fungus *A. bombacina* was identified as a potential biological control agent. However, complete pseudothecial inhibition was obtained in field trials only when very high doses of the antagonist inoculum were used (Heye 1982; Heye and Andrews 1983). When a lower rate was used, pseudothecial inhibition was reduced to only 60 to 70% (Miedtke and Kennel 1990). More recently, *A. bombacina* was used as a positive control in a field evaluation of potential biocontrol agents (Carisse *et al.* 2000b). In this experiment, with relatively low amounts of inoculum, the ascospore inhibition by *A. bombacia* was 84.2%, but *Trichoderma* sp. and *Microsphaeropsis* sp. were as good as *A. bombacina* in the reduction of the production of ascospores.

As the research on the development of biofungicides evolves, the required characteristics of the microbial agents are becoming more clearly defined. To be commercialized, a microbial control agent must be effective at an economic

dose, which is defined by the mode of industrial production, it must be easily implemented by growers, and it must provide observable benefits for the growers. In this context, *A. bombacina* will be difficult to commercialize because of the amount of inoculum required and because large-scale production of Basidiomycetes is costly.

Bernier *et al.* (1996) collected dead apple leaves in the early spring and late fall of 1993 in six abandoned orchards located in the different apple growing regions of Quebec. A total of 189 fungal isolates were recovered from the leaves collected in the spring and 156 from those collected in fall. Most of the isolates (75%) were Deuteromycetes and 15 had never been recorded previously as colonizers of apple leaves in North America (Bernier *et al.* 1996). From this collection of fungal inhabitants of apple leaves, Philion *et al.* (1997) screened isolates for their ability to inhibit the production of ascospores in *in vitro* tests. The candidates were also selected on the basis of their ability to colonize apple leaves under the relatively cold conditions generally encountered in the fall, their potential for industrial production, and multiple modes of action against *V. inaequalis.* From this evaluation, five fungal isolates (*Microsphaeropsis* sp., *M. arundinis* (Ahmad), *Ophiostoma* sp., *Diplodia* sp., and *Trichoderma* sp.) were selected based on their ability to inhibit ascosporal production. These potential biocontrol agents were further tested under orchard conditions. The most consistent reduction in the production of ascospores was obtained with the isolate *Microsphaeropsis* sp., strain P130A (Carisse *et al.* 2000b). This isolate was identified as a new species of *Microsphaeropsis* and named *M. ochracea* (Carisse and Bernier 2001). Benyagoub *et al.* (1998) investigated the interaction between *M. ochracea* and *V. inaequalis* with light and electron microscopy. They proposed that *M. ochracea* acted as a mycoparasite based on observation of penetration and active growth of *M. ochracea* in *V. inaequalis* cells (Figs. 3 and 4). They also observed important alteration of cell walls of *V. inaequalis*, an indication of the possible role of hydrolytic enzymes produced by *M. ochracea* (Fig. 4).

Microsphaeropsis ochracea was further tested in orchard plots at the Agriculture and Agri-Food Canada Experimental Farm, in Frelighsburg, Quebec, Canada, in 0.41 ha of mature orchard planted with the cultivars McIntosh and Lobo. *M. ochracea* was applied at a rate of 10^{11} spores ha^{-1}, as a post-harvest, pre-leaf-fall treatment in mid October. The effect of *M. ochracea* on ascosporal production was evaluated the next spring by measuring the concentration of ascospores of *V. inaequalis* in the air during each rain event during the primary infection period from the end of April until late June. The application of *M. ochracea* resulted in a reduction of 70.7% in 1997 and 79.8% in 1998, in the total amount of airborne ascospores trapped as compared to the control plots (Carisse *et al.* 2000b). In other similar trials, conducted in 1998-99, the biological control agent reduced ascosporal production by 70% to 85% depending on the inoculum potential in the orchards (Carisse *et al.* 2000b). This reduction of inoculum allowed nearly 40% reduction of fungicide sprays. Further reduction of inoculum was obtained when the biological control agent was mixed with 5% urea (46% N). Trials were conducted in orchards with different levels of inoculum. In orchards with low inoculum, the application of the biofungicide alone or mixed with urea resulted in a substantial reduction in the number of fungicide sprays required the next yr (five as compared to nine in the untreated plot). However, in orchards with a high inoculum potential, the fall application of the biofungicide alone or mixed with urea resulted in a small reduction in the number of fungicides required (five as compared to six in the untreated plot). The incidence of scab was substantially reduced from 12% in the unsprayed plot to 2.21% and 1.18% in the sprayed plots.

Despite the tremendous amount of research and number of publications on biological control of plant pathogens, there are only a few biofungicides registered in the world, most of them be-

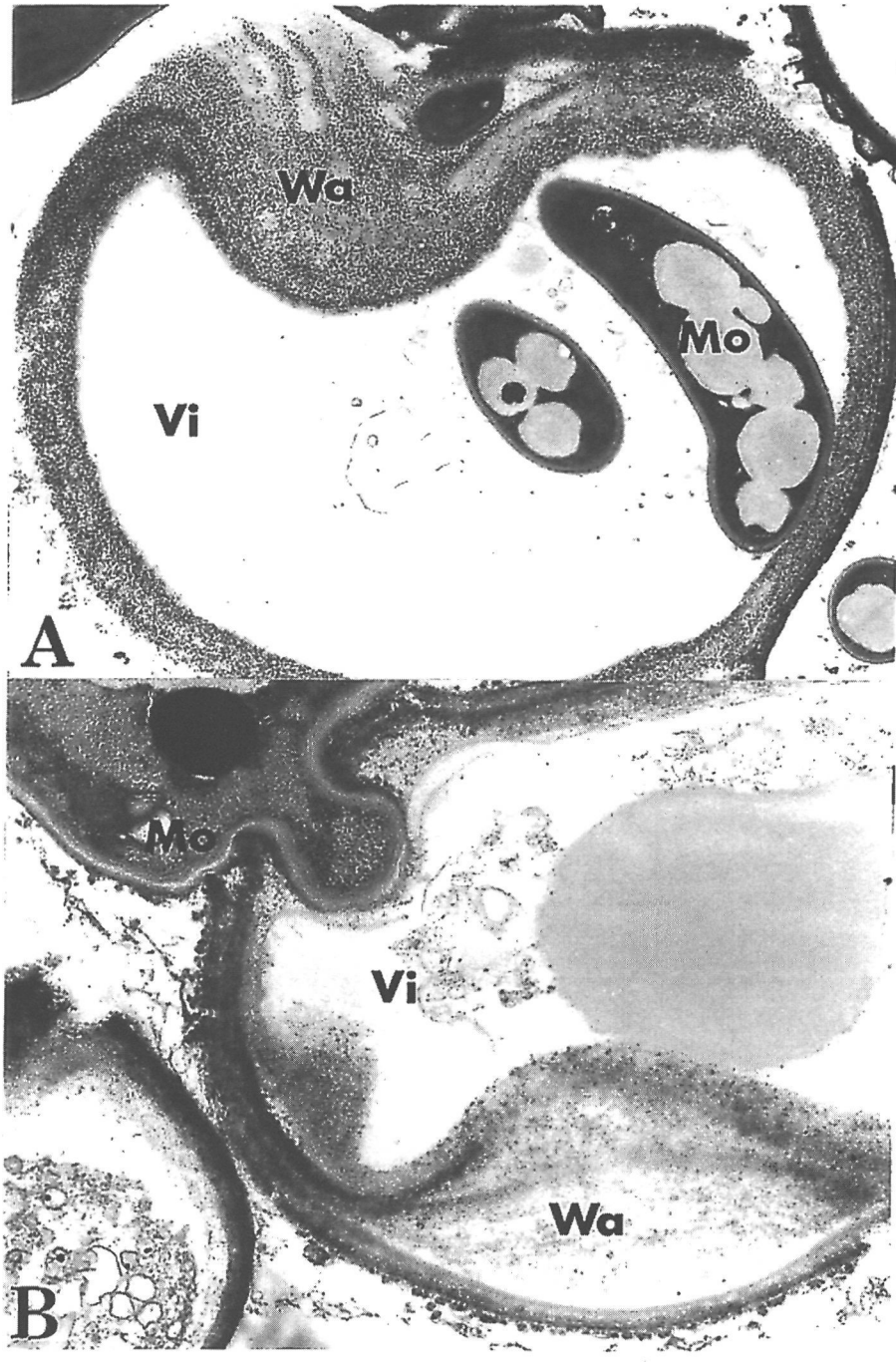

Figure 3. Transmission electron micrographs of the interaction between *Microsphaeropsis ochracea* (Mo) and *Venturia inaequalis* (Vi) cells. In A and B, *V. inaequalis* hyphae colonized by *M. ochracea* with wall apposition (Wa) developed in response to the invasion by *M. ochracea*, (A: 1.00 cm = 1μm X 10,000, and B: 0.45 cm = 1μm X 20,000).

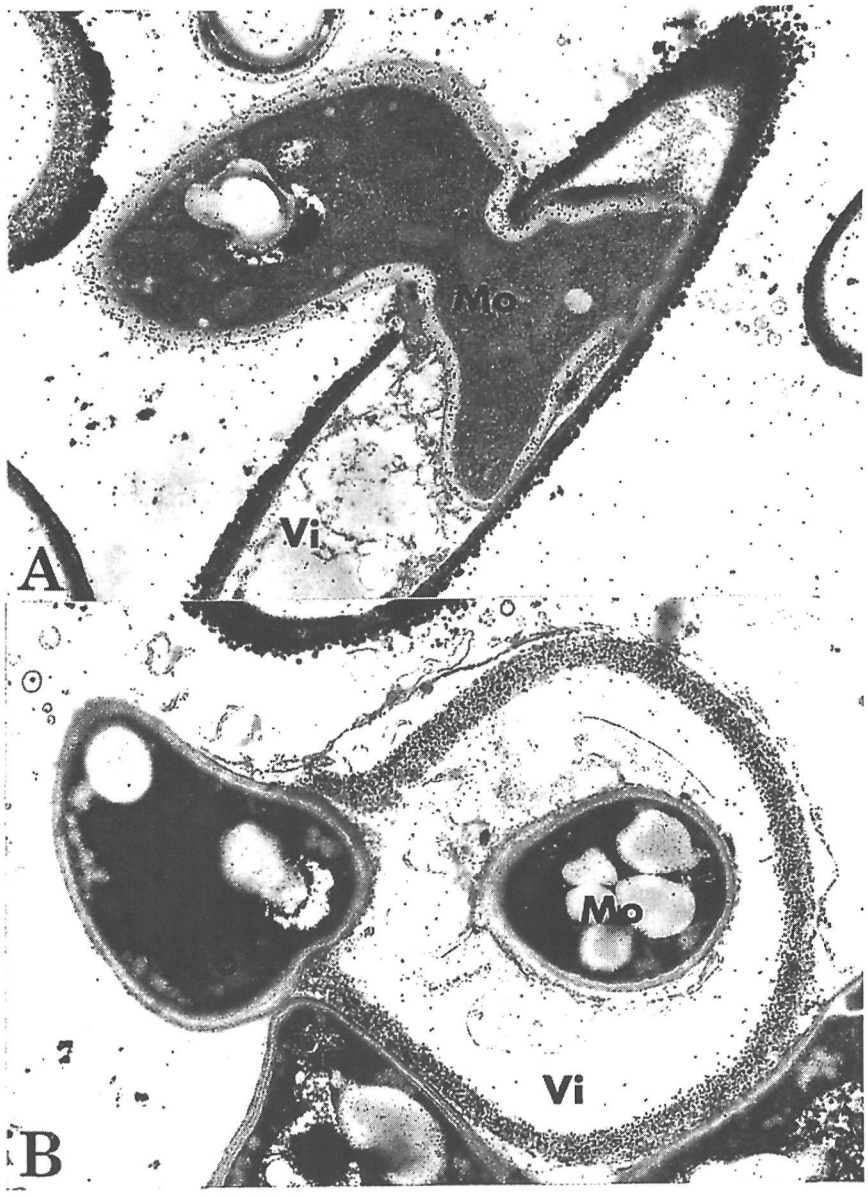

Figure 4. Enzymatic degradation of *V. inaequalis* (Vi) membrane by *M. ochracea* (Mo). In A, labelling of chitin with wheat germ agglutinin/ovomucoid-gold complex is less abundant in zones of penetration by *M. ochracea* (0.71 cm = 1μm X 16,000; in B, labeling of cellulose with exoglucanase-gold complex (0.55 cm = 1μm X 20,000).

ing commercialized for specific niches, such as high value crops for which there is a demand for pesticide-free products. To be commercialized, biofungicides must be effective (at least as compared to available chemical fungicides), consistent in their effectiveness, adaptable to IPM and therefore compatible with chemicals and other biological treatments, compatible with common agricultural practices, environmentally safe, and not more expensive than available chemicals. Furthermore, biofungicides are often made of a single strain of an antagonist that was selected on the basis of its specificity. In practice, this limits the use of the biofungicide against other diseases. In the near future, research on biological control will probably focus on enhancing the expression of active genes in biocontrol agents, such as degrading enzymes. It is likely that biofungicides will have to be used in conjunction with other products in an integrated control program. As mentioned previously, this implies that the biocontrol agent is compatible with chemical fungicides and agronomic practices commonly used.

Despite improvements in fungicide efficacy, timing, and application, apple scab is still a limiting factor in apple production. This may be at least in part a consequence of management programs based exclusively on chemical control. Chemical control can be seen as a curative approach as fungicides are applied in response to an existing or anticipated pathogen's attack. An integrated preventative approach will result in a more complex management program that includes major changes in grower practices. These changes include designing orchards so that cultivars with differential susceptibility can be treated with fungicides based on different schedules and using post harvest treatments, such as leaf shredding or application of biological control agents. New knowledge of the resistance mechanisms in *Malus* may also present new control options. Despite the increased complexity of integrated scab management, it may prove more sustainable because it does not depend on the use of only one method and because it will reduce the risk of development of resistance to fungicides in the pathogen population. Ultimately, sustainability will depend on the cost effectiveness of integrated approaches as compared to total dependence on fungicides to control apple scab.

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