

# Non- Chemical Management of Apple Scab- A Global Perspective

Misba Majeed<sup>1\*</sup>, Nazir A. Bhat, Zaffar A. Badri, Vaseem Yousuf, Tanveer A. Wani, Mudasir Hassan, Md. Saleem, Stanzin Dorje, Shazia Paswal<sup>\*\*</sup>

<sup>\*</sup>Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India, <sup>\*\*</sup>Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, J&K, India.

**Abstract**— Apple scab, caused by the fungus *Venturia inaequalis* (Cooke) G. Wint. is the most widespread disease in apple orchards worldwide. In order to manage apple scab and produce a marketable crop, growers across the globe have relied on 10 to 18 applications of synthetic chemicals at an annual cost of US\$202 to \$506 per hectare. Until recently, fungicidal control was perceived as the only economical control measure but this perception is changing because 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. For all these reasons cited above, interest is increasing to develop alternative strategies to manage apple scab. These changes include re-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 management options. Despite the increased complexity of integrated scab management, it can prove more sustainable as it involves the use of more than one method and reduces 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.

**Keywords**—Apple scab, *Malus*, Strobilurine-based fungicide, Sustainability, *Venturia inaequalis*.

## I. INTRODUCTION

Apple scab, which is caused by the ascomycete *Venturia inaequalis* (Cke.) Wint. is the most important disease in all apple-growing districts with high spring and summer rainfall (Belfantiet al., 2004). In some circumstances, the losses from apple scab can be 70% or more of the total fruit

value (Agrios, 2005). Floral buds are first exposed to ascosporous infection (Falk et al., 1995; Godec, 2004; Percival, 2008). Under dry weather conditions, the number of primary infections may be low and growers can use extended spray intervals (Rosenberger and Cox, 2010). Conidiophores, on which conidia (the asexual spores) are produced and cause secondary infections on all succulent plant parts throughout the growing season (Stensvand et al., 1998). These lesions produce infective conidial spores, and they may overwinter and not undergo a sexual reproduction cycle in warmer areas (Schwabe et al., 1984). Over the years, fungicides has become the sole means to control apple scab and there has been little effort to commercialize alternative strategies. Even in Integrated Pest Management systems, scab is currently controlled by up to 15–20 applications of protective and curative fungicides during the growing season, regardless of the presence of ascospores in the orchards (Demeyere and De Turck, 2002). Like other apple growing regions worldwide, scab too is currently being managed by fungicidal sprays from pink bud to harvest in Jammu and Kashmir state of India. (Padder et al., 2013). Until recently, it 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; Smith et al.,

1991). In a survey of Ontario orchards, about 50% of the isolates of *V. inaequalis* were resistant to the eradicator fungicides currently used (Ontario Ministry of Agriculture and Food, 1993).

For all these reasons, interest is increasing to develop alternative strategies to manage apple scab based on non-fungicidal methods that include genetic resistance, physical destruction of the pathogen, and biological controls (Carisse *et al.*, 2000; Sutton *et al.*, 2000).

## II. 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).

### 2.1. Pruning

To discourage scab, it is advisable to keep the leaves as dry as possible, in other words, to avoid planting too close together, to ventilate the canopy by pruning and to avoid planting in wet, low-lying areas (Corroyer and Petit, 2002). Kolbe (1983) showed that orchards which promote circulation of air through the rows and between the rows by means of appropriate pruning have lower levels of scab in the long term. Holb (2005) compared three pruning models (intense, moderate and none) on two very susceptible cultivars (cv. Jonagold and cv. Mutsu), two susceptible cultivars (cv. Elstar and cv. Idared) and two resistant cultivars (cv. Liberty and cv. Prima) in an organic orchard. He concluded, notably, that intense pruning of susceptible cultivars results in significantly less scab on the leaves and fruit than in the other two models. Simon *et al.* (2006) showed the favourable effects of centrifugal training compared with conventional solaxetraining on scab control, interpreting these results as being due to better ventilation within the tree and, therefore, a microclimate which is unfavourable to scab.

### 2.2. Inoculum Reduction

Scab overwinters mainly on dead leaves that have fallen on the ground and these are therefore the main source of the primary inoculum that causes contamination the following spring (MacHardy *et al.*, 2001). The two main ways of reducing the primary inoculum are (i) to reduce the mass of scabbed leaf litter and (ii) to prevent *V. inaequalis* developing in the litter that remains (MacHardy *et al.*, 2001).

Several studies have shown the effects of sanitary practices such as burning or burying leaves in the soil (Gomez *et al.*, 2004), leaf shredding (Vincent *et al.*, 2004; Holbet *et al.*, 2006) and a combination of shredding and using urea (Sutton *et al.*, 2000) on reducing scab inoculum. These studies showed an ascospore inoculum reduction of between 40 and 95% and a correlated scab reduction of 45 to 85%. Collecting leaves from the ground in the inter-rows in autumn along with burying the leaves left along the row has a positive effect in reducing primary contamination (Gomez *et al.*, 2004). Gomez *et al.* (2004) showed that for two consecutive years the practice of 'raking and ridging' reduced the severity of scab on the fruit by 68 to 74%, depending on the year. Burchill *et al.* (1965) first showed that application of 5% urea to English orchards in the autumn completely suppressed ascospore production the following spring. Burchill (1968) treated Bramley's Seedling trees at two sites in Kent with a postharvest, pre-leaf fall application of 5% urea; scab lesions on blossom-spur leaves were reduced by 59% and 46%, respectively, the following spring compared to the untreated control. Mitre *et al.* (2012) studied the effect of applications of urea 5% after harvest but before leaf-fall, as foliar application, in order to restrict perithecial production by *Venturia inaequalis* in a commercial super intensive apple orchard situated near Cluj-Napoca, Romania. The results (Table 1) showed large reductions in spore production, often as high as 70 to 80%, following application of 5% urea. Spraying the surface of the leaves on the ground with urea 5% reduced primary infection by about 60%.

Table.1: Attack degree(%) of apple scab on 'Golden Delicious' cultivar in the experience regarding effect of urea application in Romania (Mitre *et al.*, 2012)

Variant	Attack degree (%)	Relative attack degree (%)
V <sub>1</sub> (Untreated)	81.67	100.00
V <sub>2</sub> (sprayed in autumn with 5% urea )	21.33	26.12
V <sub>3</sub> (sprayed in autumn with 5% urea, followed by a second -pre-bud burst application at 2%)	11.00	13.47

### 2.3. Fertilization

Professional fruit growing requires regular supplement of minerals to warrant fruit set and quality. Heavy nitrogen fertilization supports tree and fruit growth and therefore is a prominent controlling tool for yield. An enhanced vegetative growth of apple trees, however, is often correlated with an increasing susceptibility to pathogens such as *V. inaequalis* (Leser and Treutter, 2005). This may be due to the concomitant decrease of phenolic compounds by high nitrogen uptake (Leser and Treutter, 2005) indicating that environmental conditions favouring plant growth reduce investment of carbon for defence. Kumar and Gupta (1986) observed that a high level of potassium fertilizers increased resistance of apple tree to scab but a similar effect was not obtained with high levels of phosphorus fertilization.

### 2.4. Alternative Protectant Products

#### 2.4.1. Botanicals

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. 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 days 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 days, reduced scab severity by 66 to 81%. Some russetting was reported on Golden Delicious following the oil treatments.

#### 2.4.2. Bicarbonate salts

The fungicidal properties of bicarbonates have long been known (Clayton *et al.*, 1943) but have never been significantly exploited and used in agriculture. However, bicarbonate salts have experienced a revival of attention in recent years as alternatives for plant disease control (Tamm *et al.*, 2006). Bicarbonate of sodium, potassium and ammonium, in particular, are known to have fungicidal properties. A small body of research is currently available highlighting the effectiveness of bicarbonate salts in apple scab control (Schulze and Schonherr, 2003; Tamm *et al.*, 2006). Ihlanet *al.*, (2006) show the scab-reducing effect of

1% sodium bicarbonate treatments in orchards during the primary infection season. A new commercial formulation of potassium bicarbonate, called Armicarb, has recently been developed in the USA, especially for foliar applications (McGovern *et al.*, 2003).

### Reluctance For Sanitation Practices

Sutton *et al.* (2000) observed that in general, growers do not use sanitation techniques, although they can result in significant reductions in ascospore load. Reluctance to use sanitation practices is through:

- 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.

## III. GENETIC CONTROL

Breeding for resistance has been recognized as a viable technique to control apple scab since the beginning of the 20<sup>th</sup> century (Kellerhals 1989; Kumar and Sharma 1999). 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 sub-cuticular space, reduced sporulation, and necrotic or chlorotic flecks (MacHardy 1996). Several breeding programme started across Europe and North America, but the work during first half of 20<sup>th</sup> century remained affected due to World War II. 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. The PRI apple breeding program began in 1926 when crosses made from the crab apple, *Malus floribunda* 821, were found to show some resistance to apple scab. The  $V_f$  gene, while being the most frequently used over the last 50 years, is not the only qualitative resistance gene. 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. baccatajackii*,  $V_b$  discovered in Hansen's baccata #2, and  $V_a$  from Antonovka PI 172623 (Crosby *et al.*, 1992). Until now, mostly the  $Rvi6$  gene previously known as  $V_f$  gene has been incorporated into

commercially available cultivars for resistance. Table 2 depicts the list of R-genes imparting scab resistance.

Despite the many years of work on scab resistant of apple cultivars, they have yet to gain widespread popularity. 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). Producers are often hesitant to plant them, especially when they are relatively unknown by consumers (Crosby *et al.*, 1992). 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 (Merwinet *et al.*, 1994). Hence, efforts to produce durable scab resistant cultivars with market acceptability should be given priority in breeding programmes. In order to devise such programmes with success, apple genotypes have to be screened for scab resistance under *in vitro* condition with fungus races present in particular region. Molecular techniques developed in early 1990's allowed for the identification of markers associated with the Vf gene. 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 (Tartarini, 1996). Mapping of the Vf region began in the

early 1990's. Initially isozymes were investigated because of the high allozyme polymorphism of the apple. With a bacterial artificial chromosome (BAC) library of the Vf carrying variety 'Florina', the feasibility to locate Vf with 'chromosome walking' was demonstrated by screening the library with a AL07 RAPD derived probe (Vinatzeret *et al.*, 1998). Of the other resistance genes, only Vm has been extensively mapped. One marker, OBP12, was identified at the relatively long distance of 6 cM from the Vm gene. It was found only in cultivars and species closely related to *M. micromalus*. To test for resistance type, some accessions that carry Vm were inoculated and all exhibited the pit-type resistance reaction (Cheng *et al.*, 1998). The isolates of *V. inaequalis* are hypervariable and exhibit differential pathogenicity on apple cultivars (known as differential hosts). Based upon such differences, the pathogen has been categorized into eight physiological races (Bus *et al.*, 2005; MacHardy, 1996). This is one of the good reason for producers and breeders to be concerned. The salient features of these races are summarized in Table 3. In India, particularly Kashmir valley, there is little information on the susceptibility of apple cultivars to scab races, including the cultivars such as Lal Ambri, Gulshan, Shreen, Firdous, Akbar, Shalimar 1 and Shalimar 2 which have been bred in the J&K state

Table.2: List of apple R-genes imparting scab resistance

S.No	R-Gene		Source /host	Linkage group	Reference
	Old name	New name			
1	V <sub>a</sub>	Rvi10	Antonovka Type PI 172623 Differential host: h10	LG-1	Gessler <i>et al.</i> , 2006
2	V <sub>b</sub>	Rvi12	Hansen's baccata #2 Differential host: h12	LG-12 (Distal end)	Gessler <i>et al.</i> , 2006
3	V <sub>bj</sub>	Rvi11	<i>Malus baccata jackii</i> Differential host: h11	LG-2 (Distal end)	Gessler <i>et al.</i> , 2006
4	V <sub>d</sub>	Rvi13	Durello di Forli Differential host: h13	LG-10 (Proximal end)	Gessler <i>et al.</i> , 2006
5	V <sub>d3</sub>		1980-015-025	LG1	Soriano <i>et al.</i> , 2009
6	V <sub>dg</sub>	Rvi9	J34; Differential host: h9	—	Patocchiet <i>et al.</i> , 2009
7	—	Rvi14	Dülmener Rosenapfel Differential host: h14	LG-6 (Proximal end)	Soufflet-Freslonet <i>et al.</i> , 2008
8	V <sub>f</sub>	Rvi6	"Priscilla" Differential host: h6	LG-1 (Distal end)	Gessler <i>et al.</i> , 2006
9	V <sub>fh</sub>	Rvi7	<i>Malus floribunda</i> 821 Differential host: h7	LG-8	Durel, 2006
10	V <sub>g</sub>	Rvi1	Golden Delicious Differential host: h1	LG-12 (Distal end)	Gessler <i>et al.</i> , 2006
11	V <sub>h2</sub>	Rvi2	<i>Malus pumila</i> R12740-7A (TSR34T15) Differential host: h2	LG-2 (Distal end)	Gessler <i>et al.</i> , 2006
12	V <sub>h3.1</sub>	Rvi3	Q71; Differential host: h3	—	Patocchiet <i>et al.</i> , 2009
13	V <sub>h4/Vr1</sub>	Rvi4	<i>Malus pumila</i> R12740-7A (TSR33T239)	LG-2 (Distal end)	Gessler <i>et al.</i> , 2006



			Differential host: 4		
14	V <sub>h8</sub>	Rvi8	<i>Malus sieversii</i> W193B Differential host: 8	LG-2 (Distal end)	Gessler <i>et al.</i> , 2006
15	V <sub>m</sub>	Rvi5	<i>Malus micromalus</i> 245-38, <i>Malus atrosanguinea</i> 840 Differential host: h5	LG-17 (Distal end)	Cheng <i>et al.</i> , 1998
16	V <sub>r2</sub>	Rvi15	GMAL 2473 Differential host: h15	LG-2 (Proximal end)	Gessler <i>et al.</i> , 2006

Table.3: Physiological races of *V. inaequalis* (Bus *et al.*, 2005; MacHardy, 1996)

Races	Pathological characteristics on apple cultivars
Race1	Non sporulating lesion on Dolgo, R 12740-7A (a Russian cultivar) and Geneva
Race 2	Sporulating lesions on Dolgo, Geneva and some progenies of R 12740-7A
Race 3	Sporulating lesions on Geneva, and non sporulating lesion on Dolgo, R 12740-7A
Race 4	Non sporulating lesion on Dolgo, Geneva and sporulating lesion on those progenies of R12740-7A on which race 2 isolates cannot sporulate
Race 5	Sporulating lesions on Vm R gene containing cultivars
Race 6	Sporulating lesions on Vf hybrids but cannot infect <i>Malus floribunda</i> 821 containing Vf <sub>h</sub> R gene
Race 7	Can infect cultivars having Vf and Vf <sub>h</sub> R gene but cannot infect Golden delicious which contains Vg gene
Race 8	Can infect Golden delicious, Royal gala, and cultivars containing Vh8 R gene

#### IV. MIXED PLANTING CULTIVARS

The monoculture of a genetically uniform crop is a major feature of modern agricultural systems. However, this genetic uniformity favours the rapid development of plant diseases and renders these agricultural systems dependent on high pesticide input. One strategy proposed for increasing the spatial diversification of host resistance is the use of multiline cultivars or cultivar mixtures (Mundt, 2002). Apple cultivars are differentially susceptible to *V. inaequalis* (Dewdney, 2000). 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. Expectedly, the greatest amount of disease predicted was in a solid cultivar block.

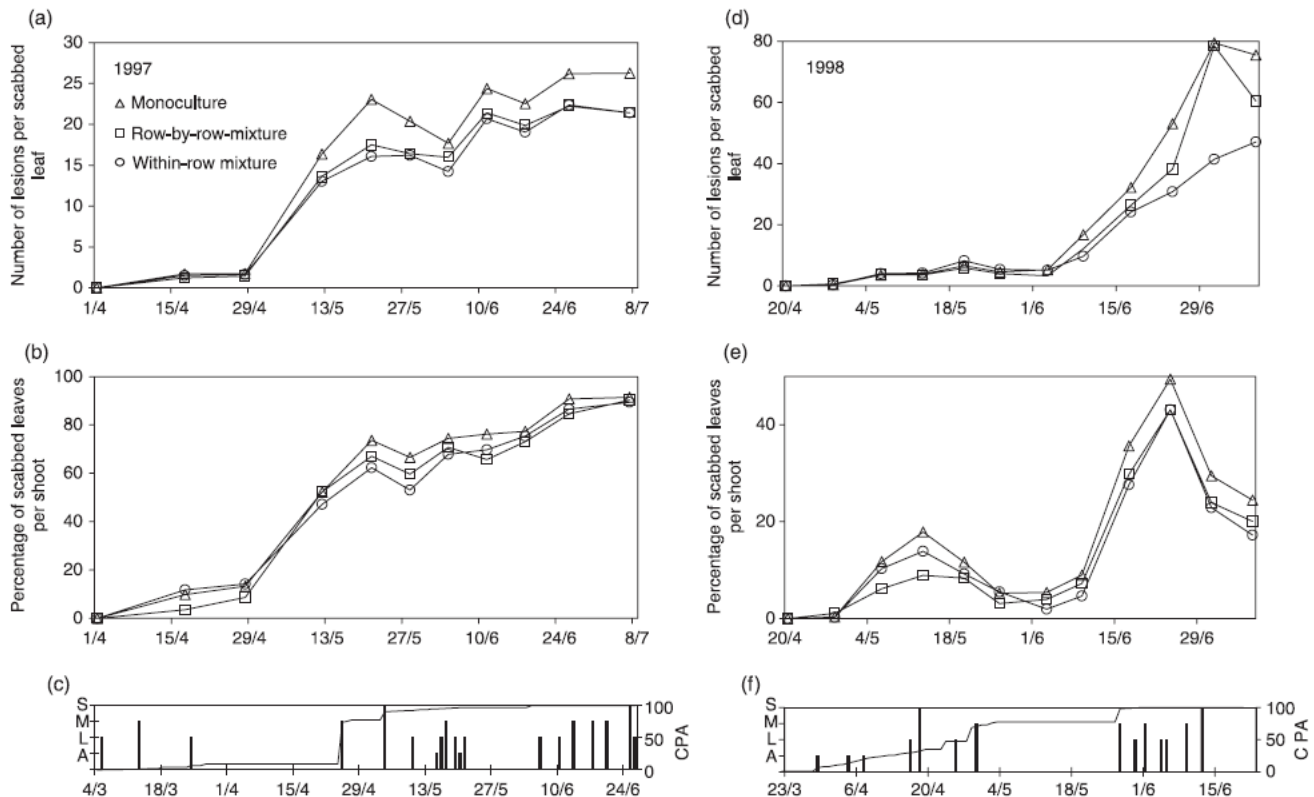
The greatest reduction, 79%, was found with three cultivar mixes within the row. When the cultivars were in alternating rows, potential scab was reduced by 65% in the simulation compared to 67% reduction when only two cultivars were in alternate rows. In this simulation, substituting one susceptible cultivar by a resistant one had no effect in the alternate-row planting scenario.

Didelot *et al.* (2007) studied the effects of two mixtures of resistant and susceptible apple cultivars on the development of scab caused by *Venturiainaequalis* in an experimental orchard over four years, initially for two years without fungicides against scab, and subsequently for two years with a moderate fungicide schedule. The row-by-row and within-row mixtures included a susceptible cultivar and a resistant cultivar in equal proportions. Without fungicides, the results showed a significant reduction of disease incidence over both years (7.3 to 21.3%), and severity in the second year (35.4%) in the within-row mixtures, compared to the monoculture of the susceptible cultivar. The best results were obtained when the within-row mixture was associated with moderate fungicide treatments; in this case the reduction in disease incidence reached 75.1% on leaves and 69.7% on fruits during the growth phase. The characteristics of the *Venturia inaequalis*/*Malus*×

domestic pathosystem and the results obtained in this experiment suggest a moderate but not negligible ability of cultivar mixtures for reducing epidemics of the disease.

**Figure 1** depicts the apple scab severity over the years of

trial. In the future, this kind of planting system, combined with other methods such as sanitation or biological control, could represent an interesting alternative to chemical control.



**Fig.1:** Evolution of (a, d) scab severity and (b, e) incidence on apple cv. Smoothie in monoculture plots and in mixed cultivar plots, and (c, f) infection periods (dark bars) and cumulative percentage of ejected ascospores (CPA, line without symbols), in (a–c) 1997 and (d–f) 1998. A, L, M and S are the different levels of the Mills and Olivier infection periods: Angers, Light, Moderate and Severe, respectively.

## V. BIOLOGICAL CONTROL

Biological control can be adopted through either (i) the introduction of a microbial control agent or (ii) the manipulation of naturally occurring populations of microorganisms. 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. Cinq-Mars (1949) pioneered biological control of apple scab as the first scientist to isolate microorganisms from apple leaves with more than 25 different microorganisms, including fungi, bacteria, and yeasts. He showed that some of these organisms, mainly *Penicillium* species, produced antibiotics that inhibited mycelial growth of *V. inaequalis*. Hislop and Cox's (1969) 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 by

investigating the effects of fungicides on the size and diversity of populations of microorganisms that lived on apple leaves. Cullen *et al.* (1984) evaluated the potential of *Chaetomiunglobosum* as a biofungicide against apple scab. A spore suspension of *C. globosum* applied every 1 to 2 week to apple trees in an orchard reduced scab severity by 20% as compared to untreated control. *Microsphaeropsisochracea*, a coelomycete isolated from dead apple leaves, recently has been identified as a biological control agent of apple scab (Carisse and Bernier, 2001). When *M. ochracea* was applied onto naturally infected leaves that were overwintered on the orchard floor, ascospore production the following spring was reduced by 76 to 84% and fall applications on the tree canopy (at 10% leaf fall) and on the ground (at 90% leaf fall) resulted in 75.5 and 62% fewer ascospores than control. (Carisse *et al.*, 2000). Foliar sprays of plant extracts, derived from

*Artemisia absinthium*, *Urticadioica* and *Equisetum arvensae*, were combined with two antagonistic microorganisms, *Trichoderma asperellum* and *Pythium oligandrum*, and tested in organic apple orchards. The spray with only the microorganism *T. asperellum* showed the most efficacy during primary scab infection period and the

level of scab was significantly different from the water control. During the secondary scab infection period, *T. asperellum* alone plus *T. asperellum* with each of the extracts and *P. oligandrum* alone showed significantly less apple scab when compared to the water control as shown in Table 4 (Kowalska et al., 2010).

Table.4: The incidence of apple scab on leaves in spring and autumn time (2009-2010) (Kowalska et al., 2010)

No	Treatment	Mean % affected leaves during primary infection period (spring time)	Mean % affected leaves during secondary infection period (autumn time)
1	<i>T. asperellum</i>	1,47*	3,88
2	<i>T. asperellum</i> + <i>Urticadioica</i>	3,17	3,50
3	<i>T. asperellum</i> + <i>Artemisia absinthium</i>	4,75	14,88*
4	<i>T. asperellum</i> + <i>Equisetum arvensae</i>	3,17	17,38*
5	<i>P. oligandrum</i>	8,75	19,63*
6	<i>P. oligandrum</i> + <i>U. dioica</i>	4,20	47,63
7	<i>P. oligandrum</i> + <i>A. absinthium</i>	4,15	49,00
8	<i>P. oligandrum</i> + <i>A. arvensae</i>	4,42	72,25
9	Untreated (water)	2,24	31,65

\* - statistically different from untreated trees

The potential of the antagonistic isolate *Cladosporium cladosporioides* H39, originating from a sporulating colony of *V. inaequalis*, to control apple scab development was tested by Kohl et al. (2015) in eight trials during 2 years in orchards in Eperjeske (Hungary), Dabrowice (Poland), and Bavendorf (Germany) planted with different cultivars. Treatments were conducted as calendar sprays or after infection periods. Additional trials in an orchard in Randwijk (The Netherlands) focused on the effect of timing of antagonist application before or after infection periods. The overall results of the field trials consistently showed for the first time that stand-alone applications of the antagonist *C. cladosporioides* H39 can reduce apple scab in leaves and fruit. This was demonstrated in an organic growing system as well as in conventional orchards and the same control levels could be reached as with common fungicide schedules. Efficacies reached 42 to 98% on leaf scab incidence and 41 to 94% on fruit scab.

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 being commercialized for specific niches, such as high value crops for which there is a demand for pesticide-free products. Second, biofungicides are often made of a single strain of an antagonist and thus limits the use of the biofungicide against other diseases.

## VI. CONCLUSION

- Apple scab is of major economic importance and if not managed, the disease can cause extensive losses following humid and cool weather conditions during the spring months. Direct losses result from fruit infections and indirect losses from defoliation, which can reduce tree vigor, winter hardiness, and subsequent yield
- Fungicidal control is generally considered the sole economically feasible control measure against apple scab. However, this may change due to the high costs of new fungicides, increased fungicide resistance in populations of *V. inaequalis*, and increasing concerns of environmental costs and consumers negative perceptions of fungicide use
- Repeated use of fungicides can interfere with pest and predator balance and have adverse effect on predacious mites and increasing health concerns for both farmers and consumers.
- For all of these reasons, interest in the development of alternative strategies such as genetic, physical, and biological approaches to manage apple scab and to reduce reliance on 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. Ultimately, sustainability will depend on the cost effectiveness of integrated approaches as compared to total dependence on fungicides to control apple scab

## REFERENCES

- [1] Agrios, G.N. (2005). Plant Pathology. 5<sup>th</sup> edition. Academic Press Ltd, San Diego, California, USA.
- [2] Belfanti, E., Silfverberg-Dilworth, E., Tartarini, S., Patocchi, A. et al., (2004). The HcrVf2 gene from a wild apple confers scab resistance to a transgenic cultivated variety. *Proc Natl AcadSci U S A* **101**: 886-890.
- [3] Beresford, R.M., and Manktelow, D.W.L. (1994). Economics of reducing fungicide use by weather-based disease forecasts for control of *Venturia inaequalis* in apples. *N. Z. J. Crop Hortic. Sci.* **22**: 113- 120.
- [4] Blaise, P.H. and Gessler, C. (1994). Cultivar mixes in apple orchards as a means to control apple scab? *Norweg. J. Agric. Sci.* **17**: 105-112.
- [5] Bosshard, E. (1992). Effect of ivy (*Hedera helix*) leaf extract against apple scab and powdery mildew. *ActaPhytopatholEntomol Hung.* **27**: 135-140.
- [6] Bower, K.N., Berkett, L.P. and Costante, J.F. (1995). Non-target effect of a fungicide sprayprogram on phytophagous and predaciousmite populations in a scab resistantapple orchard. *Environ Entomol* **24**: 423-430.
- [7] Braun, P.G. and McRae, K.B. (1992). Composition of a population of *Venturia inaequalis* resistant to myclobutanil. *Can J Plant Pathol* **14**: 215-220.
- [8] Burchill, R.T. (1968). Field and laboratory studies of the effect of urea on ascospore production of *Venturia inaequalis* (Cke.) Wint. *Ann Appl Biol.* **62**: 297-307.
- [9] Burchill, R.T., Hutton, K.E., Crosse, J.E. and Garrett, C.M. (1965). The inhibition of the perfect stage of *Venturia inaequalis* (Cooke) Wint. by urea. *Nature* **205**: 520
- [10] Bus, V. G. M., Laurens, F. N. D., van de Weg, W. E. et al. (2005). The Vh8 locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the Vh2locus in *Malus pumila* R12740-7A. *New Phytol* **166**: 1035-1049.
- [11] Carisse, O. and Bernier, J. (2001). *Microsphaeropsisochracea* sp. nov. associated with dead apple leaves. *Mycologia* **94**: 297-301.
- [12] Carisse, O. and Pelletier, J.R. (1994). Sensitivity distribution of *Venturia inaequalis* to fenarimol in Québec apple orchards. *Phytoprotection* **75**: 35-43.
- [13] Carisse, O., Svircev, A. and Smith. R. (2000). Integrated biological control of apple scab. IOBC/WPRS Bulletin **23**: 23-28.
- [14] Cheng, F. S., Weeden, N. F., Brown, S. K. et al. (1998). Development of a DNA marker for Vm, a gene conferring resistance to apple scab. *Genome* **41**: 208-214.
- [15] Cinq-Mars, L. (1949). Interactions between *Venturia inaequalis* (Cke) Wint. And saprophytic fungi and bacteria inhabiting apple leaves. M.Sc. Thesis, McGill University, Montreal, Quebec, Canada. 114 pp.
- [16] Clayton, E.E., Smith, T.E., Shaw, K.J., Gaines, J.G., Graham, T.W. and Yeager, C.C. (1943). Fungicidal tests on blues mold (*Peronosporatabacina*) of tobacco. *J Agric Res* **66**: 261-276.
- [17] Corroyer, N. and Petit, J.L. (2002). Le pommier. pp. 230-261. In Produire des fruits enAgriculture Biologique. ITAB. 253 p.
- [18] Crosby, J.A., Janick, J., Pecknold, P.C., Korban, S.S. O'Connor, P.A., Ries, S.M., Goffreda, J. and Voordeckers, A. (1992). Breeding apples for scab resistance: 1945-1990. *Fruit Varietal Journal* **46**: 145-166.
- [19] Cullen, D., Barbee, F.M. and Andrews, J.H. (1984). *Chaetomiunglobosum* antagonizes the apple scab pathogen, *Venturia inaequalis*, under field conditions. *Can J Bot* **62**: 1814-1818.
- [20] Demeyere, A. and De Turck. (2002): Utilisation des produitsphytopharmaceutiquesdans les principales cultures enBelgique durant la décennie 1991-2000. Ministère des Classes Moyenneset de l'Agriculture, Bruxelles, Belgium.
- [21] Dewdney, M. (2000). Susceptibility of apple cultivars to *Venturia inaequalis*. M.Sc. Thesis, McGill University, Montreal, Quebec, Canada. 95 pp.
- [22] Didelot, F., Brun, L. and Parisi, L. (2007). Effects of cultivar mixtures on scab control in apple orchards *Plant Pathol. (Oxf.)* **56**: 1014-1022.



- [23] Falk, S.P., Gadoury, D.M. and Seem, R.C. (1995). Analysis of risk of primary apple scab infection. *Phytopathology* **85**: 1556.
- [24] Godec, B. (2004). New scab resistant apple cultivars recommended in Slovenia. *Journal of Fruit and Ornamental Plant Research* **12**: 225–231.
- [25] Gomez, C., Brun, L., Chauffour, D., De Le Valee, D. and Dumont, E. (2004). Effet de la reduction de l'inoculum d'automne sur le développement des épidémies de tavelure en verger de pommiers biologiques. Dans : *Journées techniques fruits et légumes biologiques*. Saint- Pierre-des-Corps, les 30 nov. & 1er déc. 2004: pp 45-52, ITAB.
- [26] Gilliver, K. (1947). The effect of plant extracts on the germination of the conidia of *Venturia inaequalis*. *Ann Appl Biol.* **34**: 136-143.
- [27] Hislop, E.C. and Cox, T.W. (1969). Effects of captan on the non-parasitic microflora of apple leaves. *Trans Br Mycol Soc* **52**: 223-235.
- [28] Holb, I.J. (2005). Effect of pruning on apple scab in organic apple production. *Plant Dis* **89**: 611-618.
- [29] Holb, I.J., Heijne, B. and Jeder, M. (2006). Effects of integrated control measures on earthworms, leaf litter and *Venturia inaequalis* infection in two European apple orchards. *Agric. Ecosyst. Environ.* **114**: 287-295.
- [30] Ihlan, K., Arslan, U. and Karabulut, O. A. (2006). The effect of sodium bicarbonate alone or in combination with a reduced dose of tebuconazole on the control of apple scab. *Crop Prot* **25**: 963-967.
- [31] Kellerhals, M. (1989). Breeding disease resistant apple cultivars in Switzerland. *IOBC/WPRS Bulletin* **2**: 130-136.
- [32] Kohl, J., Scheer, C., Holb, I., Masny, S. and Molhoek, W. (2015). Toward an Integrated Use of biological control by *Cladosporium cladosporioides* H39 in Apple Scab (*Venturia inaequalis*) management *The American Phytopathological Society* **99**: 535-543.
- [33] Kolbe, W. (1983). Effects of different pruning systems and chemical retardants compared with no pruning on apple trees on yield fruit quality and disease incidence in the long term trial at Höfchen (1959-1982). *Erwerbsobstbau* **25**: 246-255.
- [34] Kowalska, J., Remlein-Starosta, D. and Drozdzyrski, D. (2010). Efficacy of bioagents against apple scab in organic orchards, preliminary results.
- [35] Kumar, J. and Gupta, G.K. (1986). Influence of host response and climatic factors on the development of conidial stage of apple scab fungus (*Venturia inaequalis*). *Indian J Mycol Plant Pathol* **16**: 123-135.
- [36] Kumar, K. and Sharma, S.D. (1999). Breeding scab resistant apples: new directions. *Journal of Genetics and Plant Breeding* **53**: 155-164.
- [37] Leser, C. and Treutter, D. (2005). Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. *Physiologia Plantarum* **123**: 49-56.
- [38] MacHardy, W.E. (1996). Apple Scab: Biology, Epidemiology, and Management. *The American Phytopathological Society*, St. Paul, MN.
- [39] MacHardy, W.E., Gadoury, D.M. and Gessler, C. (2001). Parasitic and biological fitness of *Venturia inaequalis*: Relationship to disease management strategies. *Plant Dis* **85**: 1036–51.
- [40] McGovern, R.J., Winston, A.E., Rouse, R.E. and Welch, A.W. (2003). Reduction of defoliation in citrus caused by *Mycosphaerella citri* with a novel biocompatible fungicide. *Plant Dis* **87**: 134-138.
- [41] Merwin, I.A., Brown, S.K., Rosenberger, D.A., Cooley, D.R. and Berkett, L.P. (1994). Scab resistant apples for the northeastern United States - new prospects and old problems. *Plant Dis* **78**: 4-10.
- [42] Mitre, V., Mitre, L., Adriana, F., Petrisor, C. and Sestras, R. (2012). Reducing primary inoculum of apple scab using foliar application of urea in autumn. *Bulletin of the University of Agricultural Sciences & Veterinary* **69**: 415-420.
- [43] Mundt, C.C. (2002). Use of multiline cultivars and cultivar mixtures for disease management. *Annu. Rev. Phytopathol.* **40**: 381–410.
- [44] Northover, J. and Schneider, K.E. (1993). Activity of plant oils on diseases caused by *Podosphaera leucotricha*, *Venturia inaequalis*, and *Albugo occidentalis*. *Plant Dis* **77**: 152-157.
- [45] Ontario Ministry of Agriculture and Food. 1993. 1994-1995 Fruit production recommendations. Publication 360. 111 pp.
- [46] Padder, B.A., Sofi, T. A., Ahmad, M., Shah, M. D., Hamid, A., Saleem, S. and Ahanger, F. A. (2013). Virulence and molecular diversity of *Venturia inaequalis* in commercial apple growing regions in Kashmir. *Phytopathology* **161**: 271-279.

- [47] Percival, G.C. (2008). Paclobutrazol soil drenches provide partial reductions in symptoms of apple scab of ornamental trees and Guignardia leaf blotch of horse chestnut. *J. Environ. Hortic.* **26**: 87–92.
- [48] Rosenberger, D.A. and Cox, K.D. (2010). Apple scab management options for high-inoculum orchards. *New York Fruit Quarterly* **18**: 3–8.
- [49] Schneider, E.F. and Dickert, K.J. (1994). Health costs and benefits of fungicides used in agriculture: a literature review. *Journal of Agromedicine* **1**: 19–37.
- [50] Schulze, K. and Schonherr J. (2003). Calcium hydroxide, potassium carbonate and alkyl polyglycosides prevent spore germination and kill germ tubes of apple scab (*Venturia inaequalis*). *J. Plant Dis Protect* **110**: 36–45.
- [51] Schwabe, W.F.S., Jones, A.L. and Jonker, J.P. (1984). Changes in the susceptibility of developing apple fruit to *Venturia inaequalis*. *Phytopathology* **74**: 118–121.
- [52] Simon, S., Lauri, P. E., Brun, L., Defrance, H. and Sauphanor, B. (2006). Does manipulation of fruit tree architecture affect the development of pests and pathogens? A case study in an organic apple orchard. *J. Hortic. Sci. Biotechnol.* **81**: 765–773.
- [53] Smith, F.D., Parker, D.M. and Koller, W. (1991). Sensitivity distribution of *Venturia inaequalis* to sterol demethylation inhibitor flusilazole: baseline sensitivity and implications for resistance monitoring. *Phytopathology* **81**: 392–396.
- [54] Stensvand, A., Amundsen, T., Semb, L., Gadoury, D.M. and Seem, R.C. (1998). Discharge and dissemination of ascospores by *Venturia inaequalis* during dew. *Plant Dis.* **82**: 761–764.
- [55] Sutton, D.K., MacHardy, W.E. and Lord, W.G. (2000). Effect of shredding or treating apple leaf litter with urea on ascospores dose of *Venturia inaequalis* and disease buildup. *Plant Dis.* **84**: 1319–26.
- [56] Tamm, L., Amsler, T., Scharer, H. and Refardt, M., (2006). Efficacy of Armicarb (potassium bicarbonate) against scab and sooty blotch on apples. pp 87–92. In "Proceedings 12<sup>th</sup> International Conference on Cultivation Technique and Phytopathological. Problems in Organic Fruit Growing", edn Fördergemeinschaft Ökologischer Obstbaue.V.: Weinsberg, Germany.
- [57] Tartarini, S. (1996). RAPD markers linked to the Vf gene for scab resistance in apple. *Theor. Appl. Genet.* **92**: 803–810.
- [58] Vinatzer, B.A., Zhang, H.B. and Sansavini, S. (1998). Construction and characterization of a bacterial artificial chromosome library of apple. *Theor. Appl. Genet.* **97**: 1183–1190.
- [59] Vincent, C., Rancourt, B. and Carisse, O. (2004). Apple leaf shredding as a non-chemical tool to manage apple scab and spotted tentiform leaf miner. *Agric. Ecosyst. Environ.* **104**: 595–604.