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Innovative strategies for the control of apple scab (*Venturia inaequalis* [Cke.] Wint.) in organic apple production

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Summary / Résumé

JAMAR Laurent (2011) – Innovative strategies for the control of apple scab (*Venturia inaequalis* [Cke.] Wint.) in organic apple production (Thèse de doctorat). University of Liege – Gembloux Agro-Bio Tech, Belgium. 188 pages, 21 tables, 34 figures.

The aim of this thesis, conducted over six growing seasons (2003−2008), is to define new measures for reducing the use of fungicides and in particular, copper fungicides, for the control of apple scab (*V. inaequalis*) in organic apple production. Special emphasis is put on primary scab infection control during spring season. An original approach is proposed for defining a specific spray timing involving spraying during the infection processes, especially before fungal penetration, determined by the RIMpro software warning system. This 'during-infection' spray strategy allows reducing from 30 to 50% the amount of fungicide usually used for effective apple scab control, on high scab-susceptible cultivars. The field study shows that six alternative products (e.g., potassium bicarbonate and lime sulphur), among 60 products tested, have the potential to reduce copper use. However, copper use, even with low doses, seems to be still necessary in presence of scab-susceptible cultivars. The results obtained in these experiments could not be attributed to the specific technical performances of the tunnel sprayer used, which however, offer valuable environmental benefits. On the basis of the present study, the organic management system seems a good farming approach for maintaining soil quality with regard to biological indicators. This work shows and draws attention to the fact that (i) planting cultivars with polygenic scab-resistance traits, (ii) increasing emphasis on sanitation practices aimed at reducing initial inoculum in autumn, and (iii) applying an accurate spray strategy in spring, as defined in the present study, are the three most promising approaches for substantial further reductions in protection products fully compliant with international organic crop production standards.

JAMAR Laurent (2011) – Stratégies innovantes de protection vis-à-vis de la tavelure du pommier (*Venturia inaequalis* [Cke.] Wint.) en production biologique. (Thèse de doctorat). Université de Liège – Gembloux Agro-Bio Tech, Belgique. 188 pages, 21 tableaux, 34 figures.

L'objectif de cette thèse, conduite durant six saisons de croissance (2003-2008), est de définir des nouvelles mesures de réduction d'usage de fongicides, en particulier du cuivre, pour se protéger de la tavelure du pommier (*V. inaequalis*) en production biologique. L'accent est mis sur la protection des arbres lors des infections primaires au printemps. Une approche originale est proposée pour définir les moments d'application des traitements, spécifiquement durant les processus d'infection, avant pénétration du champignon, déterminés à l'aide du logiciel d'avertissement RIMpro. Cette stratégie dite «durant-infection », permet de réduire de 30 à 50% la quantité de fongicide habituellement utilisée pour assurer une protection efficace, sur variétés sensibles. L'étude en verger montre que six produits alternatifs (par ex., bicarbonate de potassium et bouillie sulfocalcique), parmi 60 produits expérimentés, ont le potentiel de réduire l'usage du cuivre. Cependant, le cuivre, même à faibles doses, semble être encore nécessaire en présence de variétés sensibles à la tavelure. Les résultats obtenus dans ces expérimentations ne peuvent pas être attribués aux performances techniques du pulvérisateur tunnel utilisé, qui cependant, offre de précieux avantages sur le plan environnemental. Sur base de cette étude, le système de production biologique semble une bonne approche pour maintenir la qualité du sol du point de vue de ses indicateurs biologiques. Ce travail montre et porte l'attention sur le fait que (i) planter des variétés à composante polygénique de résistance, dites tolérantes à la tavelure (ii) accroitre l'accent sur les pratiques sanitaires visant à réduire l'inoculum initial, et (iii) appliquer une stratégie de traitement adéquate au printemps, telle que celle décrite dans cette étude, sont les trois approches les plus prometteuses pour réduire l'usage futur de fongicides à un niveau plus cohérent avec le règlement international de production biologique.

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General introduction

Although the development of the concept of sustainable agriculture may lead to greater interest in environmentally friendly methods of crop disease and pest control, agriculture is currently still a big consumer of pesticides. Some 320,000 tonnes of active substances are consumed each year in the European Union (the 15 pre-enlargement states), making up one-quarter of the world plant protection product market in 2002. Fungicides accounted for 43% of the volume by weight (Anon, 2002).

In Belgium, an analysis of the data published in the report on the agricultural use of plant protection products in the 10-year period 1991-2000 carried out by the Ministry of Agriculture (Demeyere and de Turck, 2002) shows that fungicide consumption in agriculture remains at a high level. Overall, fungicides represent 46% of all products used, or a minimum of 1,660 tonnes of active fungicidal ingredients consumed in each growing season. Of that quantity, commercial pome fruit growing uses about 30%. Consequently, in Belgium, conventional pome fruit production system still accounts for about 30% of the fungicides used in agriculture, on only 1% of the useful agricultural area. In France, in 2005, 2006 and 2007, under reasoned chemical protection, which is used on the largest part of the French fruit growing area, the total annual TFI (Treatment Freqency Index) with phytosanitary products varies from 35 to 43 in apple orchards depending on production region (Sauphanor *et al.,* 2009). Apple growing sector is, therefore, one of the most intensive users of plant protection products per hectare.

Among the means of protecting plants against bio-aggressors, chemical control using synthetic active substances remains the most effective solution. It is relatively easy to use and appears to be the most cost-effective in the medium term. However, it is associated with various environmental issues, risks of residues and risks of resistant pathogens developing (Deguine and Ferron, 2004).

Several recent studies have reported the negative impact of pesticides on the environment (Cenci and Sena, 2006; Brussaard *et al.*, 2007; Lewis *et al.*, 2007; Hutchings *et al.*, 2008; Lewis *et al.*, 2009) and on human health (Andersen, 2008; Benachour and Seralini, 2008; Bjørling-Poulsen *et al.*, 2009; Costello *et al.*, 2009; Orsi *et al.*, 2009; Thompson *et al.*, 2008; Veillerette, 2010; Wigle *et al.*, 2009; Winchester *et al.*, 2009).

Frequent use of plant protection products acting on specific pathogen sites encourages the development of pathogenic populations that are resistant to these products (Köller *et al.*, 2004; Köller *et al.*, 2005).

Alternative and complementary solutions are therefore needed. This is leading to research into new global strategies that aim to integrate all the agro-ecological components of cropping systems. The UN conferences in Stockholm (1982), Rio de Janeiro (1992) and Johannesburg (2002) have brought environmental issues increasingly to the forefront of international concern, in particular through the Convention on Biological Diversity and the 'World Plan of Action' (Agenda 21) which recommend more sustainable agricultural practices (Lévèque and Mounoulou, 2001).

At European level, the sixth version of the Environmental Action Programme proposes reducing the effects of pesticides on human health and the environment (Decision No 1600/2002/EC). As a consequence of this community-wide commitment, in 2006 a thematic strategy on the sustainable use of pesticides was drawn up. The European Union has harmonised the conditions and procedures for authorising plant protection products and drawn up a list of authorised substances as well as a phased programme for evaluating substances already on the market. This is the so-called REACH programme, the European Community Regulation on chemicals and their safe use (EC 1907/2006). It deals with the Registration, Evaluation, Authorisation and Restriction of Chemical substances. The law entered into force on 1 June 2007. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. In concrete terms, this draft Regulation has led to the banning of more than 60% of active substances, with all pesticide families being affected. This Directive is to be repealed with effect from 14 June 2011 by Regulation (EC) No 1107/2009 on the placing on the market of plant protection products (EC, 2009).

This strategy gave rise, among things, to the draft Framework Directive on the sustainable use of pesticides in each Member States. Each Member States have to draw up an action plan for reducing the use of pesticides (National Action Plan - NAP). The action plan will contain target figures. For example, since 2007, the French Minister of Agriculture and Fisheries put forward various plans defining the possible ways to reinventing an agriculture that reconciles economic performance with ecological effectiveness. This firm belief has produced the '*Objective Lands 2020*', plan for a new model for agriculture in France, the '*Plan Ecophyto 2018*' designing a plan for a 50 % reduction in the use of pesticdes over a period of ten years and the '*Agriculture biologique: horizon 2012*', which is a plan aimed at providing better structure for the whole organic sector and assist the development of organic farming. This plan, which was included in the Grenelle environment conference in France, has as a core target a threefold increase by 2012 in the land area farmed using organic methods (Anon., 2009). In Belgium, since 2005, a new Program for Reduction of Pesticides and Biocides (PRPB) was drawn up with the overriding objective to reduce the environmental impact of pesticides for agricultural use and in other sectors on which approved pesticides and permitted biocides have an impact.

A steps have already been taken in integrated fruit production to produce high-quality fruit with the emphasis on ecologically sounder methods that reduce undesirable secondary effects and reduce the use of agrochemicals, in order to improve environmental protection and human health (Bosshard *et al.* 1987; Giraud *et al.*, 1996; Anon., 2004; Boller *et al.*, 2004).

However, for the past 6 years the annual European report on pesticide residues in food (Anon., 2003) has stated that about 40% of food sold on the European market contains detectable residues, with about 4% exceeding the maximum permitted limit. According to the report on pesticide residue monitoring in food of plant origin in Belgium (AFSCA, 2007), 6.9% of fruits and vegetables in the Belgian market exceeded the maximum residue levels set in the legislation. The percentage of samples with detectable residues increases each year and exceeded 55% in 2007. According to an important report commissioned by the Environment Directorate General of the European Commission, the EU do not assess and manage the risks from exposure to chemical mixtures, better known as the "cocktail effect" (Kortenkamp and Faust, 2009).

Organic farming goes further by rejecting the use of all synthetic pesticides and mineral fertilisers that are too readily soluble in surface water and underground water tables. In March 2008, the Internationnal Federation of Organic Agriculture Movements (IFOAM) has approved the following definition for organic agriculture: "*Organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved*" (IFOAM, 2011).

Organic agriculture is commercially practised in 120 countries, representing 31 million hectares and a market of US$40 billion in 2006, as noted by FAO in the paper 'Organic Agriculture and Food Security' presented at an International Conference on Organic Agriculture and Food Security (FAO, 2007).

In the European context of structural overproduction of apples grown by conventional techniques, demand for certified organically farmed apples is rising sharply. A report published by EUROSTAT in March 2010 shows that the significance of organic farming in the European Union (EU) is increasing all the time. The organic area increased by 21% in the EU between 2005 and 2008 (Wullt and Rohner-Thielen, 2010). More than 4.1% of the useful agricultural area (UAA) in the 27 EU member states was cultivated in accordance with organic farming specifications in 2007. However, the situation varies greatly between EU countries, which fall into three groups: countries that have converted more than 6% of their UAA (Austria, Italy, Sweden, Finland and Denmark); countries that have converted between 3-5% of their UAA to this method of production (UK, Germany, Norway, Portugal and Spain); and countries with less than 3% of their UAA under organic farming, including the Benelux countries, France, Greece and Ireland. Belgium is therefore distinctly lagging behind others in terms of its self-imposed target of converting 10% of its UAA to organic farming by 2010.

Therefore, over the past decade, public concern about pesticide residues on fruit and in the environment coming from intensive fruit production has generated much interest in organic apple production. But to satisfy current consumer requirements the organic products marketed need to be first-rate in terms of taste and appearance, as well as being reasonably priced (Sylvander and François, 2005). According to a relatively recent socioeconomic study (Thonon and Cnudde, 2001), consumers choose organically farmed products principally for the sake of their health, then on grounds of taste and quality, with environmental protection reasons coming last. Now, organic farming is the first production method to have acquired legislation and a European specification and to have its activities controlled by independent supervisory bodies.

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is the main disease targeted by an average of 70% of plant protection product treatments in conventional fruit growing (MacHardy, 1996). The disease can affect 100% of the yield if no control measures are applied. In France, in 2005, 2006 and 2007, under reasoned chemical protection, the annual TFI (Treatment Freqency Index) varies from 13 to 20 for the scab control, depending on production region, that is to say an increase of 25% in comparison with 1997 (Codron *et al.*, 2003; Sauphanor *et al.,* 2009).

Converting this perennial crop to organic farming methods is difficult, chiefly due to the use of commercial varieties that are generally extremely susceptible to disease (Lateur *et al.*, 2001; Anon. 2008; Lateur *et al.,* 2009; Warlop *et al.*, 2009), the large number of diseases and pests occurring in our high density orchards and, lastly, the very small number of active substances authorised in Belgium and listed in the Annex to the European Regulation on Organic Production, EC No. 884/2007 and 889/2008 (EC, 2008).

Sulphur and copper are among the few active substances authorised in organic farming in Belgium for controlling fungal diseases. These are two contact fungicides whose action is mainly preventive (Holb *et al.*, 2003). A new European Regulation (EC No. 1107/2009), which will replace Council Directives 79/117/EEC and 91/414/EEC, is due to come into force on 14 June 2011. This will limit the use of copper to a cumulative annual total of 4 kg/ha instead of the present 6 kg/ha, whereas there were no limits before 2002. In some countries, such as Denmark and the Netherlands, the use of copper as fungicides in arboriculture is totally prohibited. These constraints drive growers to use large quantities of sulphur-based fungicides, which may have other drawbacks. All these factors force organic farmers to make fundamental changes to their practices.

The foregoing shows the great need for research into new strategies for controlling apple scab in organic apple production. Is there an alternative to using copper for scab control? What strategies will enable this disease to be tackled in the context of organic farming without resorting to large-scale use of sulphur- or copper-based products?

This thesis aims to define new strategies for controlling apple scab disease that, on the one hand, will limit the use of copper and, on the other, be appropriate for use in organic fruit growing.

Very few scientific studies have been undertaken into scab control strategies in organic apple growing in our pedoclimatic conditions. Research is needed in order to (i) identify and create commercial varieties with lasting resistance characteristics, (ii) improve the effectiveness of phytosanitary practices aimed at reducing the inoculum and develop knowledge of the potential of such practices in order to reduce demand for fungicides, (iii) find alternative fungicides to sulphur and copper, (iv) refine strategies and protection schemes, (v) optimise treatment timing, (vi) improve plant protection product application techniques and (vii) develop specific cultural practices that are less favourable to the development of the disease.

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Chapter I

Literature review

Foreword

This literature review considers the issue of apple scab disease control in organic fruit production as a whole. It covers all aspects which this production method may draw on to deal with the problems of this disease. All the means of controlling this fungus are generally based on full knowledge of its life cycle.

I Chapter I : Literature review

I.1. Apple scab (*Venturia inaequalis*)

I.1.1. Biology

I.1.1.1 The disease

Apple scab caused by the fungus *Venturia inaequalis* (Cke.) Winter (anamorph : *Spilocaea pomi* Fr ou *Fusicladium dentricum* (Wallr.) Fck.) is the most important disease of apple worldwide, and it occurs very likely in every country where the cultivated apple (*Malus* x *domestica* Borkh.) is grown. The disease is restricted to the genus *Malus* (MacHardy, 1996). Apple scab is especially severe in temperate regions that have cool, wet weather in spring. It is not known when scab first appeared in orchards. The first report on scab was published by Fries in Sweden in 1819 (Fries, 1819), but the oldest clue to the existence of scab dates from 1600, in a painting by Michelangelo Caravaggio ('The Supper at Emmaus'), held at the National Gallery, London (MacHardy et al., 2001).

The fungus is virulent on all green herbaceous organs of the apple tree. The fungus may infect and colonise the sepals, leaves, fruits, petioles, blossoms and even twigs of the tree, with symptoms commonly observed on the leaves and fruits. Olive green spots, or lesions, first appear on the leaves soon after bud break. The lesions begin on the underside of the leaf, but are more conspicuous on the upper surface. As the fungus progresses the entire leaf appears a 'dirty olive' and falls to the ground. Scab lesions affecting the fruit begin on the sepals (green portions) of the flower bud. Once present on the fruit itself, the lesions are round, velvety and olive green turning darker, scabby and often cracked. Later in the spring, these primary infections produce secondary spores which infect other leaves and fruits. These secondary infections may continue throughout the growing season during wet periods. The Apple Scab fungus itself does not kill the tree, but apple scab infection results in leaf and fruit loss and many susceptible trees are severely defoliated by mid summer. Scab symptoms on woods are rare on apple trees but frequent on pear trees. However, scab on pear is caused by another fungus species named *Venturia pirina*.

The major economic loss to the grower, caused by scab, is the reduction in fruit quantity and fruit quality. The disease affects the crop but also the tree in many ways. Severe infection may prevent the setting of fruit. Infections on flowers, pedicels, petioles and young fruits during or immediately following the blossoming period may cause young apples to drop (MacHardy, 1996). Severe attacks of scab had a negative effect on tree viability the following year, due to lower leaf photosynthetic activity, fewer fruit buds and leaves being formed, more leaves being droped and that scab also had a debilitating effect on normal wood growth needed for high yields. Important losses occur also due to the development of scab in storage (Tomerlin et Jones, 1983).

I.1.1.2 The pathogen

Apple scab is caused by a pathogen fungus including two different states: *Venturia inaequalis* (Cke) Winter, the perfect (sexual) or saprophytic state and *Spilocaea pomi* Fr., the imperfect (asexual) or parasitic state (Bonne, 1971).

A taxonomic arrangement of the fungi includes *Venturia inaequalis* (Cke) Winter in the subdivision Ascomycota, class Loculoascomycètes, order Pléosporales and family Venturiaceae. *Spilocaea pomi* Fr. is placed in the subdivision Deuteromycota, class Hyphomycètes, order Moniliales (Lepoivre, 2003). *V. inaequalis* is basically a non pathogen to all non *Malus* plants. However, pathogens responsible for scab on *Malus* sp. and *Pyracantha* sp. are considered as two *formae speciales* belonging to *V. inaequalis* (Le Cam et al., 2002). Yet, while the genus *Malus* is the main host of *V. inaequalis*, not all Malus genotypes are susceptible. *Malus* is a complex genus consisting of a broad range of species, its taxonomy still debated. Since almost all its species are intra-fertile, large collections of single or multiple genotypes from the various species and districts worldwide have been established. While scab resistance within these repositories has proved quite common, all commercially important cultivars are still reputed to be susceptible to varying extents. It has also been found that the extent of resistance in some commercial genotypes changes over time because of the adaptation of the fungus to a specific plant host (Gessler, 1989) and that even the immunity of some wild *Malus* species or genotypes can be broken by the development of new scab races (Williams and Brown, 1968; Roberts and Crute, 1994; Benaouf and Parisi, 2000). A good example is 'Golden Delicious,' which was thought to be moderately resistant to scab in the early 1900s but today is one of the most susceptible (Gessler *et al.*, 2006).

I.1.1.3 The infection cycle

***Winter*:** The apple scab fungus survives during winter primarily in fallen leaves. Microscopic flask-shaped black fruiting bodies, called pseudothecia, will develop in overwintering infected leaves (see § I.1.2.1). Occasionally, in certain regions and on certain cultivars, the fungus can survive in infected bud scales and on twigs, as mycelium or conidia (Holb *et al.,* 2005b). (Figure I.1).

***Early spring – primary infections*:** The ascospores (sexual spores) contained in the pseudothecia reach maturity by March. When leaves on the orchard floor become wet from rain, the ascospores are forcibly ejected into the air in daylight. This discharge continues after rain events until middle June in Belgium, by which time all the ascospores have been released. Most are discharged between the pink bud to petal fall stages (see § I.1.2.2). At this time of maximum growth, young plant tissues are highly susceptible to infection. Air currents carry the ascospores into the trees and onto developing flowers, leaves and fruitlets where they adhere quickly on contact and resist removal by more rain. Stronger wind can transport them throughout the orchard as well as to neighbouring or more distant orchards. If the leaves and fruitlets remain wet long enough, the ascospores germinate and grow into the tissue. The time needed for germination and infection to take place varies with the temperature and is called the infection period. Depending on the temperature, olive-green,

velvety scab lesions will appear after 9 to 17 days. These spots are the result of primary infections and bear masses of dark conidia or summer spores (MacHardy, 1996).

Spring to summer – secondary infections: Rain or irrigation water washes or splashes the conidia (asexual spores) onto other leaves and fruit within the tree. Unlike the ascospores, conidia may be released at any time of the day or night and can be in the air in both dry and wet weather. Wind can carry them throughout the orchard. They can be spread by clothing, picking bags, insects and birds. Once the conidia are deposited on leaves or fruits, they require free moisture and relative humidity above 95% to germinate (see § I.1.2.4). A few days later, new velvety scab lesions will appear, bearing more conidia. This cycle of secondary infections can continue during summer, under the right weather conditions. Cooler, wetter periods in spring, early summer and autumn are ideal for the development of secondary infections. Fruit is always susceptible but needs longer periods of surface moisture near harvest to become infected (Schwabe *et al.,* 1984)

Autumn : Pseudothecia are formed on fallen infected leaves by the union of mycelium from compatible mating types. They will mature during winter, building up an inoculum for the start of a new cycle in spring.
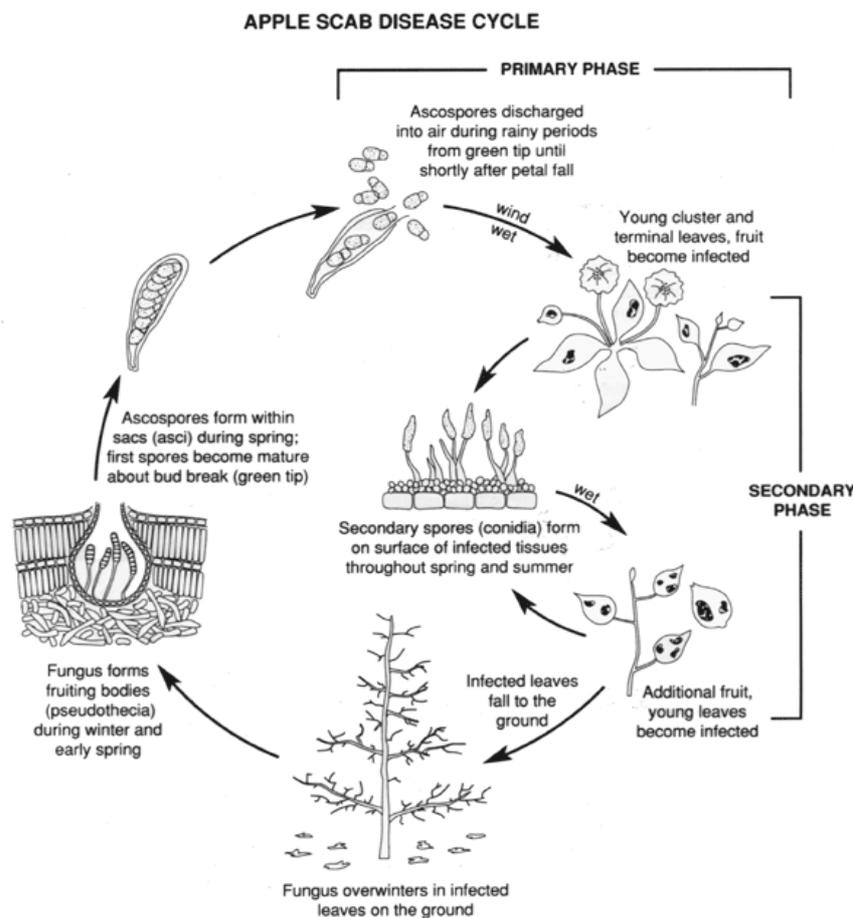
Figure I.1 − Apple scab disease cycle (Cornell University, NYSAES, Geneva, NY.).

I.1.1.4   Apple scab fertility

Infection starts in spring, with the formation of a haploid mycelium from the ascospores. During the parasitic stage, the cycle is asexual. During the saprophytic phase, in autumn, this mycelium produces antheridia, on the one hand, and ascogonia, on the other. Through plasmogamy (fusion of cytoplasms from two haploid gametes) followed by caryogamy (fusion of the nuclei), the pathogen enters the diploid phase. Meiosis quickly leads to the formation of haploid ascospores in the perithecia. During this sexual phase of the cycle the fungus undergoes genetic variations.

In October, a few days after abscission of the infected leaf, a hypha develops a helical lump at the apex which swells to form an ascogonium inside the foliar tissue (female gamete), surrounded by the walls of the future pseudothecia. Another hypha differentiates into an antheridium (MacHardy, 1996). Plasmogamy will then take place.

According to some authors (Semal, 1989), *V. inaequalis* is homothallic, that is, the cells of one and the same thallus comprise male gametes (antheridia) and female gametes (ascogonia) which can fuse. Keitt and Palmiter (1938), MacHardy (1996) as well as most of the authors takes the opposite view that this is a heterothallic species, i.e., mating can take place only between thalli of different polarities. However, MacHardy *et al.* (2001) postulate that, in some cases, mating between incompatible thalli may occur, as has been observed in *Unciluna necator*.

I.1.1.5   Apple scab physiological races

The concept of race as a fixed genetic unit is not valid for an obligatory sexually reproducing organism. The terminology 'race' used for *V. inaequalis* designates an isolate capable of infecting and finally sporulating on a particular host resistant to other isolates; as such it should be called physiological race as in the early literature. In other words, in the case of an obligatorily sexually reproducing pathogen like *V. inaequalis*, the word 'race' indicates nothing more than the presence or lack of virulence traits with respect to specific hosts on which the isolate is tested.

Eight physiological races of scab are currently defined according to their virulence on 'specific host' varieties (Table I.1). The first three of these were identified by Shay and Williams (1956). Race 1 is taken as a well sporulating isolate on popular domestic cultivars and eliciting flecks or necrotic lesions without sporulation on *Malus* clones Dolgo, R12740-7A and Geneva (Shay and Williams, 1956). Race 2 can sporulate on 'Dolgo,' 'Geneva' and certain offspring of 'R12740-7A.' Race 3 is characterized as being able to sporulate on 'Geneva,' otherwise being the same as race 1, and race 4 differs from 1 by sporulating on those offspring of 'R12740-7A' that race 2 is not able to sporulate on. Race 5 has the ability to sporulate on carriers of the *Vm* resistance and can thus circumvent the resistance of *Malus micromalus*. Race 6 first appeared at Ahrensburg, in Germany, in the nineteen-eighties (Parisi et al., 1993). It is virulent on most varieties containing the *Vf* gene, but not on clone

821 of *Malus floribunda*, the origin of this resistance. Race 7 was discovered more recently at East Malling in the UK. This is virulent on *Malus floribunda* 821 (Roberts and Crute, 1994), but avirulent on 'Golden Delicious' and on certain varieties that carry the *Vf* gene, such as 'Prima'. Bénaouf and Parisi (2000) thus identified the *Vg* gene which confers resistance on 'Golden Delicious' and some of its progeny. Recently, Bus et al. (2005) have described a further race, 8, capable of breaching a heretofore unknown ephemeral resistance denominated *Vh*8, whence race 8 (Gessler et al., 2006). Race 7 appears to spread faster than race 6 (Parisi et al., 2000). It occurs not only in Britain but also in France, in Belgium (Lateur et al., 2001) and in other countries such as the Netherlands, Italy, Switzerland and Denmark (Parisi et al., 2000).

Table I.1 − Pathogenicity of different *Venturia inaequalis* races on the set of differential host cultivars, updated after MacHardy, 1996 (Data are from Parisi and Lespinasse, 1996; Bénaouf and Parisi, 1997, 2000; Bus et al., 2005)

Differential cultivars	R gene	*Venturia inaequalis* races[b]							
		1	2	3	4	5	6	7	8
Royal Gala (h1)	None	S	S	S	S	S	S	S	S
Dolgo (h2)	None	R	S	R	R	R	R	R	R
TSR34T132 (h2)[a]	*Vh2*	R	S	S	R	R	R	R	R
Geneva (h3)	Not named	R	S	S	R	R	R	R	R
TSR33T239 (h4)	*Vh4*	R	R	R	S	R	R	R	R
9-AR2T196 (h5)	*Vm*	R	R	R	R	S	R	R	R
Florina (h6)a	*Vf* and *Vg*	R	R	R	R	R	S	R	n.a.
M. floribunda 821 (h7a)	*Vf*	R	R	R	R	R	R	S	R
Golden Delicious (h7b)	*Vg*	S	S	S	S	S	S	R	S
M. sieversii W193B (h8)	*Vh8*	R	S	n.a.	n.a.	n.a.	n.a.	n.a.	S

[a] The differential hosts for race 2 and 6 in Bus et al. (2005) were TSR34T15 and Prima

[b] n.a. = data non available

Bus *et al.* (2009) presented recently a new nomenclature of *V. inaequalis* races that suites better to the increasing complexities of comninations of genes involved in both race-specific and race-nonspecific recognition by the host, while at the same time updates the name of scab resistance loci (Rvik) and QTL loci (Qvik) to international standards. Fourteen specific hosts are therefore newly defined that corresponds to thirthen resistance and avirulence new loci.

I.1.2. Epidemiology

I.1.2.1 Pseudothecial production and ascospore maturations

The two main parameters influencing perithecia development stages are temperature and moisture. According to James and Sutton (1982), no perithecia appeared on leaves placed in dry air. If moisture is not a limiting factor, temperature has a major influence on perithecia development phases (Gadoury and MacHardy, 1982a). Perithecia formation starts as soon as the temperature exceeds 4°C.

To estimate ascospore maturity, Gadoury and MacHardy (1982b) proposed a mathematical model based on accumulated degree-days (base 0°C) calculated from the first appearance of a mature ascospore. When the temperature is high or if prolonged dry periods occur, ascospore ripening slows down and the model has to be modified (MacHardy, 1996).

Szkolnik (1969) defined five stages in ascus development, the observations being based on crushed perithecia: (i) asci formed but contents undifferentiated, (ii) asci containing spores in the process of formation, (iii) spores are all formed, usually septate but uncoloured, (iv) asci containing spores that are formed, septate and olivaceous in colour, indicating that the spores are mature, (v) empty asci.

I.1.2.2   Ascospore discharge, dispersal and deposition

The release of mature ascospores from the perithecia is triggered by water. A fine film of water covering the perithecia is necessary for ascospore release (Gadoury and MacHardy, 1986). When the leaf bearing the perithecia is wet, the water penetrates the ascocarp either via the ostiole or via the permeable wall, and then enters the ascus by osmosis (Aylor and Anagnostakis, 1991). The water causes the ascus to swell and extend to the neck opening. The asci then burst open, shooting their contents up into the air. The supply of water must be accompanied by an impact, such as that of a drop of water. The ascospores can then be dispersed by the wind.

On average, the spores are propelled 3 mm from the leaf (Aylor and Anagnostaki, 1991). They are carried upwards by the turbulence caused by wind and rain and they move through the canopy until they fall back to the ground, leave the orchard or are deposited on host vegetative parts (Gadoury and MacHardy, 1986). According to Holb *et al*. (2004) the effects of an inoculum source would become insignificant beyond 18 and 33 m in Boskoop and Jonagold orchards, respectively. Despite the apparent lack of traits that confer competence for longer-range dispersal, recently developed models indicate that some *V. inaequalis* ascospores would be transported 2 to 5 km from point and area sources of inoculum (Aylor, 1998), and presumably at such distances desiccation and irradiation are not significant mortality factors, given that the dispersal may occur in a matter of minutes at the range, and often during a rain period. The exact number of such ascospores that survive, are deposited on host tissue, and eventually cause disease at such distances remains unknown. Presently, scab is generally managed based upon assessments of potential ascospore dose within an orchard (MacHardy *et al.*, 2001).

The rainfall must be greater than or equal to 0.1 mm for the ascospores to be released into the air (MacHardy, 1996; Rossi *et al.,* 2000). Rossi *et al* (2000) described cases of rain not causing ascospore dispersal. They noted in particular that (i) daytime rain may not cause release if it occurs less than 5 hours after a previous release, (ii) in general, night-time rain does not cause immediate releases, in contrast to daytime rain (MacHardy and Gadoury, 1986; MacHardy *et al.*, 2001) and, in this case, release can occur in the first two hours after sunrise, (iii) in the absence of light, in particular light in the 710 to 730 nm range, the ascospores are not released, unless 80% of the primary inoculum has already been released or one-third of the season's total inoculum is mature and is inside perithecia, ready for release.

In the specific case of cv. 'McIntosh', Szkolnick's observations (1969) show the relationship between ascospore release and phenological stages. Spores are generally not released before the phenological stage corresponding to flower bud opening (stage C3 on the Fleckinger scale). Thereafter, the number of ascospores released increases substantially, week by week, reaching a peak which normally occurs between full flowering and petal fall (MacHardy and Gadoury, 1986). After that, the quantity of ascospores released decreases until the inoculum reserve is exhausted and the perithecia contain no more viable ascospores. Exhaustion of the primary inoculum generally happens after about thirty rainy spells over a total period of 50 to 80 days after emergence of the first mature ascospores. Often, though, 85% of the ascospores may be released after three or four spells of heavy rainfall (MacHardy, 1996). There is thus a remarkable synchronisation between the main ascospore releases and the most sensitive stages in the phenological development of the host plant (Figure I.2).

The few studies that exist concerning ascospore survival times on orchard leaves are contradictory (MacHardy, 1996). It has been shown that ascospores present on leaves may well germinate even after exposure to six days' sunshine, whereas other studies show that mortality occurs in about 80% of ascospores after only 2 to 8 h of sunshine in the orchard. Conidial viability, on the other hand, can persist for several days under different temperature and weather conditions.
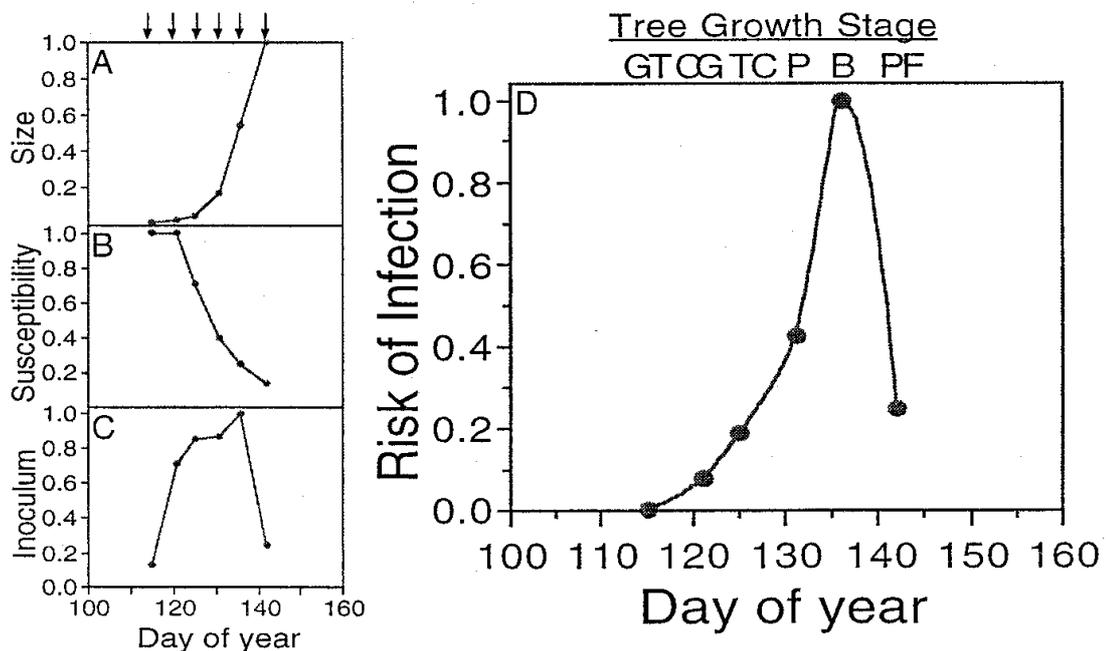
Figure I.2 – Changes in leaf cluster size (A); susceptibility to scab (B); and amount of airborne inoculum of Venturia inaequalis (C) with growth stage of McIntosh trees. The product of A*B*C is the relative risk of infection (D). GT = green tip, CG = 12.5 cm green, TC = tight cluster, P = pink, B = bloom, and PF = petal fall (From MacHardy et al., 2001).

I.1.2.3   Ascospore germination

The ascospores adhere to the epicuticular surface by producing a mucilaginous substance (Gessler and Stumm, 1984). One of the two cells composing the spore develops a protuberance. A germ tube grows out at the end, generally forming an appressorium. Alternatively, a branched germ tube may penetrate the cuticle directly without forming an appressorium. An infectious hypha travels through an appressorium wall pore and penetrates under the cuticle, assisted by the pressure exerted by the appressorium and cutin-esterase. It would take about 320 degree-hours for the ascospores to penetrate under the cuticle (Nusbaum and Keit, 1938; Smereka et al., 1987).

The germination and penetration stage can only take place if free water is present on the epicuticular surface for a sufficient time (MacHardy *et al.*, 2001). The wetting time needed for germination varies with temperature. Mills (1944) and Mills and LaPlante (1951) proposed curves (called Mills curves) that predict three levels of ascospore infection risk (light, moderate and heavy) as a function of temperature and duration of leaf wetting. According to these authors the time is one-third shorter for conidial infection. MacHardy and Gadoury (1989) adjusted Mills and Laplante's forecasts in that, according to them, ascospore infection would take three hours less than Mills' forecasts indicate (Figure I.3) and what the conidia need is not two-thirds of the time required for ascospores, but two and a half hours longer. Germination and elongation of the germ tube take place in a wide temperature range, from 0.5°C to 32°C, with an optimum at 17°C (Stensvand *et al.*, 1997).
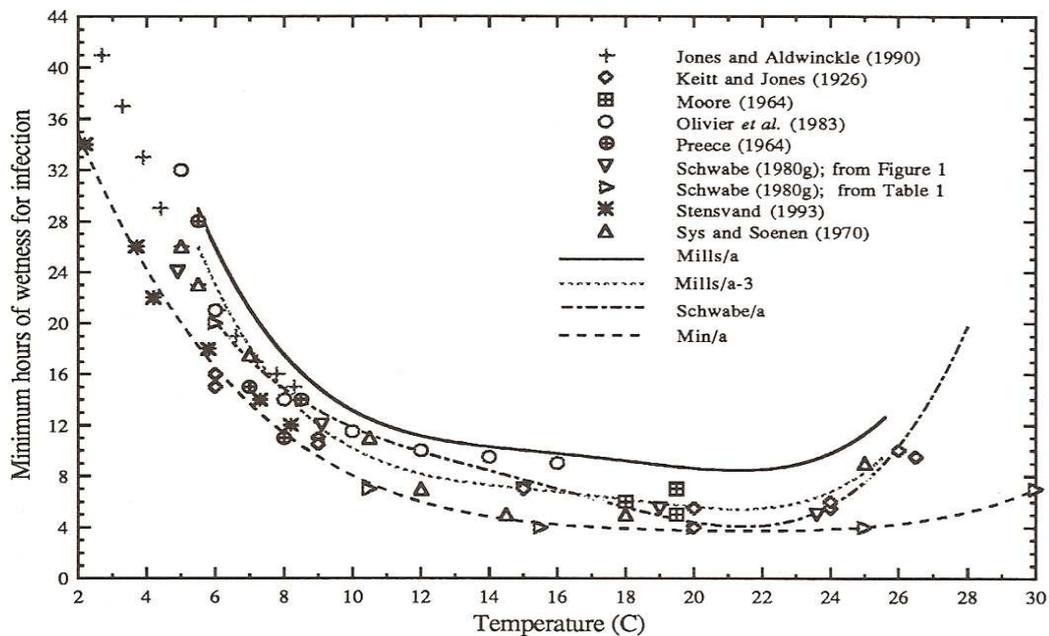
Figure I.3 − The relationship between hours of leaf wetness, temperature, and infection by ascospores of *V. inaequalis* reported in several orchard and laboratory studies. The 'Mills/a' infection curve was derived from Mills' table for light infection (Mills and LaPlante, 1951). The 'min/a' curve identifies the minimum hours of wetness in which infection occurred after a highly susceptible cultivar was inoculated with a high inoculum dose (from MacHardy, 1996).

Schwabe (1980) linked the incidence of infection expressed in degree-hours (DH) with scab development on the leaves. The number of degree-hours is equivalent to the number of hours of wetting multiplied by the average temperature during the wetting period. He found that from a high inoculum pressure, 'slight', 'moderate' or 'heavy' infection can occur on sensitive leaves after 125, 170 and >225 DH of leaf wetting, respectively.

After an incubation period of approximately two weeks, the first symptoms appear. This latent period could differ according to cultivars susceptibility. From these lesions the conidia will be produced and will form the secondary inoculum (Szkolnik, 1969).

I.1.2.4   Secondary infection by conidia, the parasitic phase

The second part of the scab infection cycle, corresponding to the parasitic phase, is due to *Spilocaea pomi* Fries. This form, classed as an imperfect fungus, is characterised by asexual reproduction. The conidia produced correspond to the secondary inoculum source. The conidial and mycelial cells are generally uninucleate. This parasite takes the nutrients required for its growth and development from the host tissues (Nusbaum and Keitt, 1938), and it can use epidermal cell walls as a food basis (Semal, 1989).

The conidia are released in a film of water covering the conidiophore. If this film is formed by dew, the conidium remains on the same leaf, thus aggravating the severity of the disease on that leaf (Sutton et al., 1976). When rain falls, splashes carry the conidium to other leaves, generally lower down in the canopy (MacHardy *et al*., 2001). Rainfall is probably one of the main factors in conidia release (Hirst and Stedman, 1962). Spore release may also be mechanical, and promoted by wind bringing leaves into contact with one another. Conidia survived radiation doses equivalent to 12h in full sunlight (Aylor and Sanogo, 1997).

The pathogen responsible for apple scab overwinters mainly as perithecia on dead leaf debris on the ground. However, the pathogen may also be found in parasitic form (conidia) between bud scales or on stem lesions (Becker *et al*., 1992, Holb *et al*, 2004; Holb *et al*, 2005b), but this form of infection is rarer. Comparing 18 orchards in the Netherlands managed either conventionally or according to organic practices, Holb *et al* (2005b) observed that the quantity of viable conidia present inside buds is generally greater (2 to 11%) in the organic orchards than in the conventional ones (0 to 6%). They also stated that the risk of early infections caused by conidia is greater in organic orchards and that protective measures need to be taken in early spring in this type of orchard. The relationship between autumn scab incidence and numbers of overwinteredconidia with shoots or buds was exponential (Holb *et al*., 2005b).

I. 2. Scab control strategies

I.1.3.   Resistance to scab in Malus

The average life of a current commercial apple orchard is quite long, between 12-15 years. The choice of the right varieties is therefore very important. Besides considerations

such as productivity, fruit quality, ease of tree management and commercial criteria, it is also essential in integrated and organic growing to use varieties that are more resistant to diseases and pests.

I.1.3.1   Vertical and horizontal resistance

Vanderplank (1984) defined ***vertical resistance*** as a resistance specific to certain pathogen races. ***Horizontal resistance***, on the other hand, confers the same level of resistance irrespective of the race of pathogen.

Resistance may be monogenic (that is, controlled by a single gene), oligogenic (controlled by a small number of genes) or polygenic (controlled by many genes).

As a general rule, vertical resistance is mono- or oligogenic and horizontal resistance is polygenic. However, different concepts are involved. Vanderplank (1984) offers some exceptions to this rule. Likewise, the concepts of total resistance or immunity and monogenic resistance are often confused, forgetting that 'total' resistance, like 'vertical' resistance, refers to the phenotype, whereas 'monogenic' is a genotype characteristic.

Total resistance or *immunity,* totally prevents infection. Host infection by the pathogen ceases before sporulation occurs, and therefore halts secondary inoculum production and prevents an epidemic from breaking out (Geesler *et al.,* 2006). This type of resistance is generally monogenic or oligogenic. It is qualitative (William and Kuć, 1969), being 'all or nothing'. This kind of resistance is insensitive to environmental effects, but it is conditioned by a specific host-pathogen relationship.

Compatibility between the host and the pathogen does not mean that the host is completely susceptible. The host may develop reactions that prevent invasion by the pathogen. This is termed ***partial resistance*** and it reduces the infection rate. This type of resistance is described as quantitative (Gessler *et al*., 2006). The term 'partial' is used according to Gessler *et al*., 2006, in the sense of 'incomplete'. In theory, this system cannot be circumvented and it generally occurs in older varieties (Chevalier *et al*., 1991). Heredity of partial resistance is generally polygenic, that is, controlled by many genes (Gessler *et al*., 2006).

Partial resistance is expressed in all conditions, but its intensity may vary according to the interaction between the infection pressure and environmental factors (William and Kuć, 1969). In this case, symptoms appear but the progress of the cycle of infection and the epidemic is very much slower and application of plant protection products on a smaller scale can maintain yield.

Berger (1977) divides partial resistance into various components: (i) smaller number of organs affected (or decreased incidence of the disease), (ii) smaller size and fewer lesions (or decreased severity of the disease), (iii) less lesion sporulation, (iv) longer latency period (period from the time of infection to the start of sporulation), (v) shorter infectious period.

I.1.3.2 Ontogenic resistance

The dynamics of the infection process are controlled partly by variety resistance and also by leaf age related resistance (Gessler and Stumm, 1984). It appears that only actively growing leaves that are expanding and increasing their area at the time of inoculation are susceptible to *V. inaequalis*, whereas the fully expanded leaves become resistant. This applies to the whole *Malus* genus. It is termed 'ontogenic resistance' (Figure I.4). Ontogenic resistance corresponds to a gradual increase, linked to leaf age, in the incubation time needed for infection until, finally, no symptoms appear (Schwabe, 1979).

Gessler and Stumm (1984), Valsangiacomo and Gessler (1988) showed that the pathogen passes through the leaf cuticle, irrespective of leaf age or cultivar. The cuticular membrane does not, therefore, constitute a preformed physical barrier. Resistance is induced after fungus penetration. According to MacHardy (1996), ontogenic resistance is conditioned by the pH level in the intercellular space or, more generally, is due to the pathogen entering an unfavourable environment after penetrating the cuticle. Leaf ageing is in fact associated with a drop in leaf sap pH, but also with changes in a series of other environmental factors. Ontogenic resistance cannot, therefore, be associated with individual gene expression. Physiologically speaking, ontogenic resistance is truly durable, as it is found in all cultivars and throughout development, and has never been circumvented by the pathogen (Gessler *et al.,* 2006).

Moreover, Kollar (1996) observed a loss of ontogenic resistance in senescent leaves of certain varieties. This change in resistance could be attributable to environmental factors possibly altering the physiology of the plant.

The scab control strategy must take account of this particular susceptibility of young leaves and plant organs that are mainly present in phases of intensive growth, for instance in spring around flowering time, and also during some summer periods.
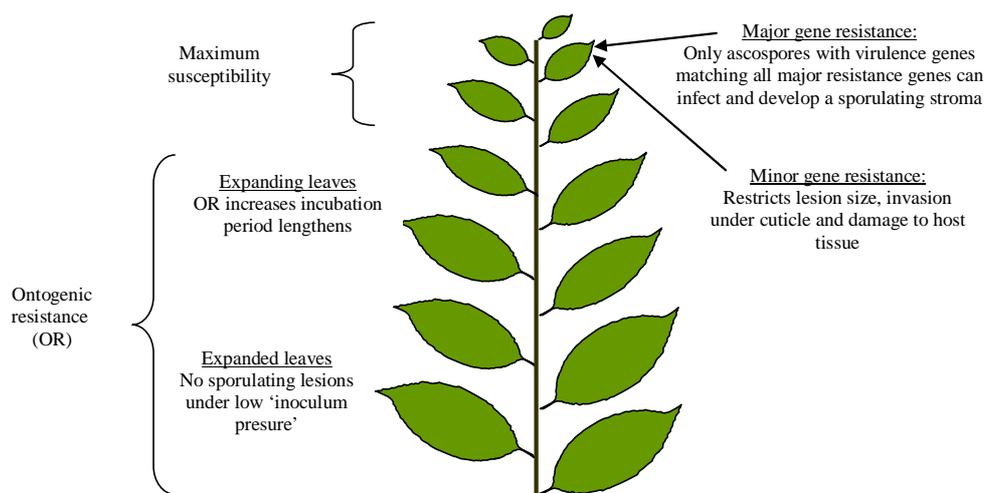
Figure I.4 − Diagrammatic representation of ontogenic, major gene (vertical) and minor gene (horizontal) host resistances to *V. inaequalis* in an apple extension shoot with respect to incubation period and expression of symptoms (redrawn and adapted from MacHardy et al., 2001).

I.1.3.3   Resistance durability

Durable resistance is defined by Johnson (1984) as resistance that remains effective for a 'long period' when the varieties that have it are widely grown in an environment favourable to the pathogen. The duration of this 'long period' obviously depends on the crop concerned. Account must be taken of the time needed for a variety to become established and the expected commercial life of that variety.

It is generally observed that partial, polygenic resistances are durable, whereas total, monogenic resistances are short-lived. This observation is based on the fact that there is less selection pressure in the former case. In the case of monogenic resistance, indeed, a single mutation or new genetic combination at the pathogen's sexual reproduction stage is sufficient to circumvent resistance. However, cases of durable total resistance do exist (Burdon, 1993).

It is very hard to assess the durability of resistance of a given variety *a priori*. Two kinds of test are available for the purpose. The first involves testing resistance at a number of points. However, the total area of these tests cannot be compared with that occupied by a widely grown variety. The second method tests resistance with different existing races of pathogen. This test does not, therefore, take any account of recently emerged races or races that could have evolved in the wake of resistance (Burdon, 1993).

It has been shown that the extent of resistance of some commercial apple genotypes changes over time, because of the scab strains' ability to adapt to a specific host plant (Gessler *et al.,* 2006). So, the immunity of some species of wild *Malus* or certain cultivated genotypes can break down if new scab races appear (Williams and Brown, 1968; Roberts and Crute, 1994; Bénaouf and Parisi, 2000). One good example is 'Golden Delicious', which was regarded as relatively scab resistant at the beginning of last century and has now become extremely susceptible (Gessler et al., 2006).

I.1.3.4   Scab resistance sources for breeders

Fruit cultivars are clones which are mainly propagated by grafting, sometimes by cuttings. By these multiplication methods, it is possible to preserve and to trace the parent phenotype, given that sexual reproduction generating dissociation traits is avoided. The apple tree is a cross-pollinated or allogametic plant and has gametophytic self-incompatibility (Lespinasse, 1992).

The literature review undertaken by Gessler *et al*. (2006) covers most of the scab resistance sources currently available to apple tree breeders. These different species or cultivars are bred with the aim of producing either oligogenic resistance (one major gene along with a few minor genes) or polygenic resistance (governed by a set of minor genes). There are thus several resistances regarded as monogenic that can be used in breeding programmes: *Vf, Vr, Va, Vb, Vbj* and *Vm* (Lespinasse, 1989). In reality, the resistances

originating from *Malus floribunda* and *pumila* are believed to be oligogenic. *Vm* resistance, which produces hypersensitivity symptoms, was very quickly circumvented.

The resistance originating from *Malus floribunda*, conferred by *Vf* gene, is by far the most commonly used. Of 53 scab resistant apple tree varieties already in commercial use, 40 have *Vf* resistance and three have combined *Vf* and *Va* resistance. Only two varieties have *Va* resistance, two have *Vm* resistance and six have *Vr* resistance (Geesler *et al.*, 2006).

The advantage of using monogenic resistance is that it provides often total resistance in natural conditions and, furthermore, a very high percentage (40 to 50%) of progenies expressing the resistance can be obtained. However, the parents are botanical *Malus* that bear small-sized fruit, with poor organoleptic qualities and few attractive agricultural characteristics, and this type of resistance is readily circumvented by new scab races (Lateur *et al.,* 1999).

Lespinasse (1989) mentions a series of *Malus* sp. containing polygenic resistance. He states that this type of resistance occurs in some old European varieties. Several teams of scientists have in fact assessed the possibilities offered by these old varieties (Laurens, 1999; Fisher and Dunemann, 2000).

The crossing strategies proposed by various authors (Lespinasse, 1989; Laurens 1999; Lateur *et al.,* 1999; Fisher and Dunemann, 2000) for durable resistance all follow the same approach: diversification of resistance sources, association of various resistance sources and tolerance to a low-level attack below the economic threshold. As to associating various resistance sources, one can (i) associate monogenic resistance sources, (ii) associate polygenic resistance sources, or (iii) associate monogenic with polygenic resistance, thereby integrating effectiveness and durability of genetic protection into one and the same plant (Laurens, 1999).

I.1.3.5   Plant symptoms

In the case of a compatible interaction, the scab symptoms are obvious to most phytopathologists, horticulturalists and arboriculturalists. The brown lesions on the upper surface of the leaves and the blackish spots and deformed fruit are symptoms that result in commercial losses. However, the scab symptoms that can appear on leaves vary greatly, indicating the complexity of the possible interactions between *V. inaequalis* and *Malus.* The varying intensity of the symptoms or types of symptoms can be attributed to ontogenic resistance, the particular cultivar genotype, interaction by a given variety with the particular genotype of the pathogen, and a revival of autumn susceptibility in all *Malus* genotypes (Gessler *et al.*, 2006).

The two main kinds of interaction are a compatible relationship, where the pathogen actively multiplies, and an incompatible relationship, in which fungal growth and, thus, host colonisation ends prematurely. In between these two situations, the different types of interaction between *V. inaequalis* and the plant produce different classes of symptoms which have been described notably by Gessler (1989), Chevalier *et al.* (1991) and Gessler *et al.* (2006) (Figure I.5).

Host-pathogen incompatibility causes a hypersensitivity response characterised by 'pinpoint' pits 0.1 to 1 mm in diameter. This pitting is due to rapid breakdown of the epidermal cells and appears four or five days after inoculation, but does not develop further thereafter (Chevalier *et al.*, 1991). *Malus micromalus* carries the *Vm* gene responsible for this type of reaction, although the hypersensitivity response has more recently been shown to be associated with various other forms of resistance (Gessler *et al.*, 2006).

Keitt *et al.* (1948) defined resistance expression classes from observations under controlled conditions. These classes are as follows: 0 = no symptoms, 1 = 'pinpoint pits', no sporulation – this is attributed to a hypersensitivity response, 2 = irregular chlorotic or necrotic lesions, no sporulation, 3 = restricted lesions, with moderate sporulation, 4 = extensive lesions with abundant sporulation. In 1991, Chevalier *et al.* (1991) revised the classification system and replaced class 3 by class 3a (necrotic and chlorotic lesions with occasional very slight sporulation) and 3b (clearly sporulating chlorotic and necrotic lesions) (Figure I.5).
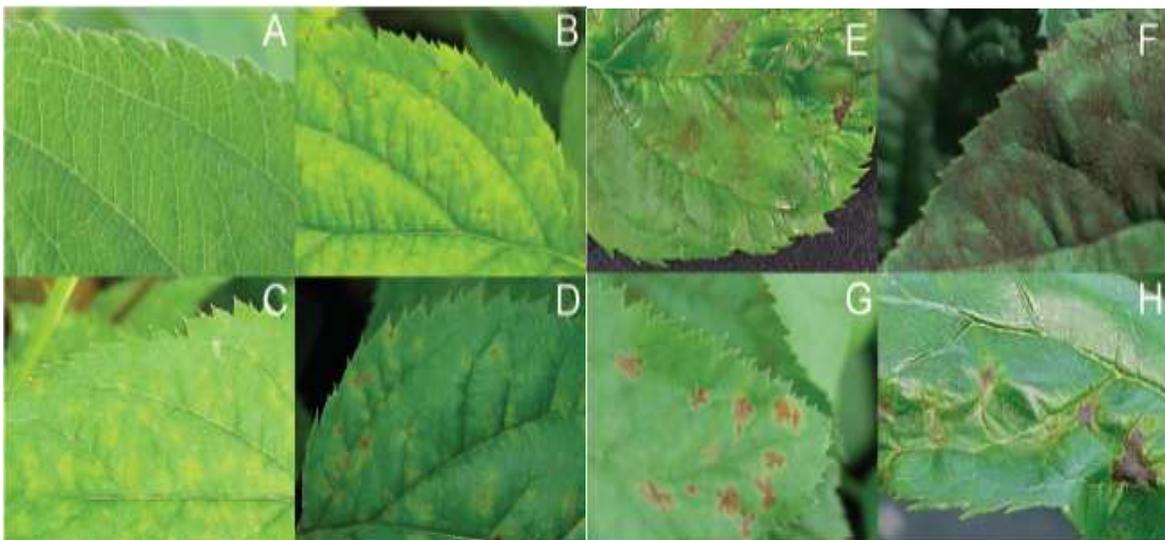
Figure I.5 ‒ Pictures of some scab reaction classes after *V. inaequalis* inoculation (about 21 days after the inoculum): A. class 0 or no symptoms; B. class 1 or 'pinpoint pits' (e.g. *Vm and Vh4*); C. class 2; D. class 3a; E. class 3b; F. class 4 or complete susceptibility; G. stellate necrotic (*Vh2* and*Vh8*) and H. *Vg*-necrosis. (From: Gessler *et al.,* 2006).

Table I.2 gives the macroscopic symptoms induced by specific different races of *V. inaequalis* on some domestic apple cultivars. It clearly shows that functionally different resistances are present in commercial apple varieties and that the corresponding virulences or avirulences are present in the pathogen (Gessler *et al.,* 2006).

Table I.2 − Macroscopic symptoms induced by selected differential isolates of *Venturia inaequalis* on domestic apple cultivars

Origin of isolate	Test cultivar					
	Ananas Reinette	Boskoop	Glockenapfel	Golden Delicious	Jonathan	Gravenstein
Ananas reinette	3 [ab]	1	0	2	0	0
Boskoop	0	2	0	0	1	0
Glockenapfel	2	1	4	3	1	0
Golden Delicious	1	0	1	4	0	0
Jonathan	0	0	0	0	3	- [c]
Gravenstein	0	0	0	0	0	4

[a] Symptoms : 0 = no visible symptoms; 1 = chlorotic and/or necrotic spots; 2 = chlorotic and/or necrotic spots with sparse sporulation; 3 = lesions with moderate sporulation ; 4 = lesions with abundant sporulation.
[b] The exemplary isolates were selected based on the host-of-origin and ability to differentiate as many differentials as possible. Isolate-cultivar combinations were considered compatible if they induced symptoms scored with 2, 3 and 4, and incompatible with score of 0 or 1 (adapted from MacHardy et al., 2001).
[c] Not tested

I.1.3.6   Apple cultivars adapted for organic agriculture

Cultivars should be chosen according to the production site, the level of intensity of the orchard and the marketing method. Preference should be given to resistant varieties that are also hardy overall (Corroyer and Petit, 2002). Ellis *et al.* (1998) showed a reduction in fungicide treatments from 9 to 5 and from 13 to 7, along with a financial saving of 57 and 73%, in orchards containing resistant varieties (Liberty) compared with orchards comprising susceptible varieties (McIntosh) in the case of conventional and organic orchards, respectively.

The principal selection criteria are: (i) the ripening date, according to the grower's constraints and the marketing method, (ii) suitability for the soil and the climate: problems of flesh consistency and lack of colour are common in the south, whereas the size potential is lower in the north and some very late varieties cannot be grown there, (iii) the agronomic properties of the variety: vigour, productivity and taste, post-harvest firmness, etc. (iv) the overall hardiness of the variety: susceptibility to certain health problems, (v) susceptibility to alternate bearing: alternate bearing varieties are difficult to regulate in organic farming, and (vi) susceptibility to early fall.

Corroyer and Petit (2002) established three new scab resistant cultivar classes for France: (i) to be maintained, (ii) to be confirmed and (iii) less attractive. The varieties to be maintained include 'Initial', 'Harmonie Delorina', Topaz', Florina Querina and Goldrush. The varieties to be confirmed are 'Santana', 'Dalinbel', 'Ecolette', 'X3263' (Inra hybrid), 'Juliet' and

'Dalinred'. The varieties ranked less attractive include 'Saturn', 'Prima', 'Sirprize', 'Priscillia', 'Liberty' and 'Baujade'. They also describe 28 classic varieties that are generally susceptible to scab but which may be attractive for organic farming. Unfortunately, some varieties like 'Initial' and 'Goldrush' have already to a great extent been circumvented by new scab races (Lateur et al., 2009). In a recent study conducted in South France, the old French cultivar 'Reine des Reinettes' appeared to express a low susceptibility, needing reduced number of treatment for scab control (Brun *et al.,* 2008).

In addition, there are the so-called 'old' varieties that have been grown in some parts for a very long time and which may be hardy or have specific scab resistance (generally polygenic, and thus durable). The problem of reintroducing these varieties into organically farmed production orchards is often one of profitability, even for growers who sell directly. This is due to a number of factors: some old varieties are highly susceptible to certain diseases, there are problems of biennial bearing, early fall, less commercial attractiveness and being harder to sell (Corroyer and Petit, 2002).

In Northern France, Stievenard and Lebrun (1996) described 78 old apple varieties some of which have good, durable scab resistance, for instance 'Calville des Prairies', 'Amère Nouvelle', 'Belle Fleur Double', 'Baguette d'Hiver', 'Court Pendu Rouge', 'Cabarette', 'Reinette des Capucins', 'Gueule de Mouton', 'Lanscailler', 'Rambour Blanc', 'Reinette de Flandre', 'Reinette de France', 'Reinette Etoilée', etc.

Among the old varieties of Belgian origin selected by the fruit tree genetic resources laboratory at CRA-W, Gembloux, some present low susceptibility to scab. By order of ripening these are 'Grenadier', 'Reinette Evagil', 'Président Roulin', 'La Paix', 'Cwastresse Double', 'Radoux', 'Joseph Musch', 'Godivert', 'Gris Braibant', 'Reinette Hernaut' (Lateur *et al*., 2001). New accessions bred by CRA-W Gembloux that have partial disease resistance have good potential for adapting to low-tech, low-input cultural methods (Lateur *et al*., 2009).

I.1.4. Cultural practices and prophylactic measures affecting scab epidemics

I.1.4.1 Orchard agro-ecosystem and management

The orchard structure

Why was scab apparently not a serious problem to growers until the 1800s? One hypothesis is that scab was present for centuries but remained obscure because apple trees with high resistance to scab were grown as seedlings in small plantings (MacHardy et al., 2001). The diversity of resistant genotypes in the seedlings is a very likely explanation of how a low infection potential was maintained. After 1800 and until the early 1900s, apple was also grown in monasteries and other religious havens and on farms (mainly for family use) in meadow orchards planted with trees (mostly crabapple seedlings grafted with sweet varieties) widely spaced and interspersed with pear and other fruit trees (Fig. 3). Single apple trees at the borders of fields and apple trees along roads were also common. A high number of different varieties was probably present, so we can assume that each tree was mostly surrounded by trees of a different genotype. The distance for ascospores (the primary inoculum) to travel from the ground to different trees, the diversity of genotypes in

seedlings, and practices such as cultivation and grazing by sheep and cows that very likely removed infected leaf litter, all mitigated the severity of scab and kept the disease from rendering the fruit unusable. All that changed in the 1800s when controlled pollination followed by vegetative propagation resulted in the production of genetically uniform trees with improved characteristics and increased consumer demand for fresh apples and for specific apples required changes in marketing and in apple production (MacHardy et al., 2001).

Therefore, with the aim to meet that demand, farmers started growing apples for the market, and clearly defined varieties were planted in rows in meadow orchards (Figure I.6). These orchards were the precursors of present-day orchards and marked the start of modern apple production. The new production systems were less efficient in reducing the leaf litter. As intensive single variety orchards became the norm, the conditions were then in place for scab to become a major problem. Scab epidemics had to be controlled to protect the nascent apple industry in the 1890s (MacHardy et al., 2001).

The role of the rootstock in scab development is mainly evident in the fact that the more dwarfing the rootstock, the more readily the ascospores from the dead leaves on the ground (primary inoculum) will come into contact with the tree's canopy and the canopies of other trees (MacHardy et al., 2001). The distances to reach the leaves by ascospores became considerably shorter. Ascospores concentration decreased rapidly with height above the ground, values at 3.0 m height were only about 6% of the values measured at 0.15 m (Aylor, 1998). However, some ascospores are easely spread over several dozen meters; this factor would play also a role for ascospore dispersal but under quite limited extend (see § I.1.2.2.).

Figure I.6 − The progression of orchards from a meadow orchard (upper left) of mostly crabapple seedlings grafted with sweet varieties and interspersed with pear and other fruit trees, no row structure, and trees often widely spaced (upper middle) to modern orchards with genetically uniform trees planted in rows, first of large standard trees (upper right), then of trees grafted onto semidwarfing (lower right and middle), and finally onto dwarfing (lower right) rootstock that resulted in much smaller trees and much greater planting densities. (from: MacHardy *et al.,* 2001).

Site

In temperate regions the choice of site is a vital and fundamental factor that will determine the entire life and profitability of the orchard. Generally speaking, well ventilated locations on a south-facing slope greatly facilitate control of fungal diseases. A sound knowledge of the microclimate history is necessary for an optimum choice of site (Corroyer and Petit, 2002).

Tree shape

As far as tree training is concerned, 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). When fully grown the orchard should be ventilated both between trees and within trees, throughout the season. 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. In another study Simon *et al*. (2006) showed the favourable effects of centrifugal training compared with conventional solaxe training on scab control, interpreting these results as being due to better ventilation within the tree and, therefore, a microclimate which is unfavourable to scab.

Cultivar mixing

Apple cultivars have differing degrees of susceptibility to *V. inaequalis* (Lateur *et al.,* 1999; Gessler *et al*., 2006). *V. inaequalis* strains are virulent for some cultivars, and either less virulent or not at all virulent for other cultivars (Tenzer and Gessler, 1997; Gessler *et al*., 2006).

In various experiments cultivar mixes have proved efficacious in reducing scab epidemics. It has been shown that combining resistant cultivars with susceptible cultivars will check the spread of scab on susceptible cultivars (Carisse and Dewdney, 2002). Overall, mixing within the row produces better results than mixing by alternate rows.

In a computer-simulated virtual experiment Blaise and Gessler (1994) showed the advantages of mixing three cultivars with different degrees of susceptibility in a row (79% scab reduction) compared with cultivar mixing in alternate rows (65% scab reduction). These results were later validated in field conditions (Bousset *et al*., 1997; Didelot *et al*., 2010).

This strategy nevertheless raises the following practical question: how can different treatments be applied according to cultivar susceptibility?

In a literature review Carisse and Dewdney (2002) raised two other questions that have not yet been answered with regard to establishing whether cultivar mixes will be effective in

the long term: (i) how does strain virulence develop? and (ii) how does the distribution of individual strains develop?

In the case of cultivar mixes using cultivars with monogenic resistance, Carisse and Dewdney (2002) advised taking a few precautions to avoid the risks of the resistance being circumvented by new scab races.

Orchard managements

Other factors affecting scab epidemics mentioned in the literatures are:

Ground cover canopy: The ground cover canopy is another aspect that may influence pest and disease developments. Herbicide-treated strips on the rows of fruit trees and regularly mowing and cutting the grass in the inter-rows, could give newly discharged ascospores a clear path towards the tree canopy (Aylor, 1998). Field experiments and modelling efforts are currently under way to determine the magnitude of this effect.

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. Heavy nitrogen fertilization (often correlated with pursuit of very high yields) may be an aggravating factor with regard to scab also because to the extent of vegetation, the prolonged presence of susceptible organs, the late leaf falls (accentuated by milder autumn weather) (MacHardy *et al*., 2001).

Hail netting : In areas with a high incidence of hail, it is necessary to use protective netting which has the side-effects of raising humidity. Hail netting extends the duration of leaf wetting and may increase the risks of scab contamination (Weibel and Häseli, 2003).

I.1.4.2 The potential ascospore dose

The 'noxiousness threshold' or 'harvest scab threshold' has been defined by MacHardy (1996) as 'the lowest pathogen population density (e.g., cumulative dose of discharged ascospores) or injury level (e.g., incidence of foliar scab on extension shoots) at which >1.0% incidence of scabbed fruit at harvest is projected if control action is not taken'.

The 'action threshold' for scab was defined by MacHardy (1996) as the 'ascospore dose threshold' at which action should be taken to prevent the noxiousness threshold at harvest being exceeded. According to MacHardy *et al.* (1993) and MacHardy (1996), the lower the potential ascospore dose (PAD), the more the spring application of fungicidal treatments can be delayed.

The method for calculating the potential ascospore dose (PAD) to quantify the extent of the primary inoculum was proposed by Gadoury and MacHardy (1986). The PAD is the

product of the number of scab spots per square metre of foliar tissue of the terminal shoots at leaf fall and the number of perithecia per lesion, asci per perithecium, ascospores per ascus and the ratio of orchard floor covered by dead leaves at bud break. The PAD is expressed as the number of ascospores per m^2 per year and may be formulated as follows:

$$PAD = LD \cdot PD \cdot AD \cdot LLD \cdot n$$

where LD = number of scab spots per square metre of foliar tissue of the leading shoots at leaf fall, PD = perithecia density (number of mature ascocarps per visible lesion at bud break), AD = ascus density (number of asci per perithecium at bud break), LLD = dead leaf density (proportion of ground covered by dead leaves at bud break), and n = number of ascospores per ascus at bud break.

Gadoury and MacHardy (1986) linked the incidence of the disease at leaf fall with the percentage of lesions producing mature perithecia of *V. inaequalis*. The model developed by Gadoury and MacHardy (1986) is straightforward to use. There are only two measurements to be taken, requiring no more than two hours per orchard: the incidence and severity of the disease should be assessed just before leaf fall and the dead leaf density on the ground should be assessed at bud break. If the inoculum is low and focally distributed, this method is more sensitive and gives less random results than the method involving spore capture in spring.

MacHardy (1996) indicated that when the ascospores are the only source of primary inoculum and the ascosporic inoculum is low (PAD ≤ 600), then there is no need to apply fungicide before the pink bud stage unless three periods of infection have occurred before then. Scab could thus be controlled with two to five fewer treatments compared with the number of treatments applied in a conventional programme.

Holb *et al*. (2005a) established that if the incidence of leaf scab is less than 4% at the beginning of August, then the scab level at harvest will not exceed 1%.

I.1.4.3   Reduction of the ascosporic inoculum using physical practices

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), though in some cases a small part of the primary inoculum can also come from conidia that have overwintered in the buds (Becker *et al*., 1992).

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 (Curtis, 1924, Louw, 1948; Gomez *et al*., 2004), leaf shredding (Sutton *et al*., 2000; Vincent *et al*., 2004; Holb *et al*., 2006) and a combination of shredding and using urea (Sutton *et al*., 2000; Vincent *et al*., 2004; Holb *et al*., 2006) on reducing scab inoculum.

These studies showed an ascosporic inoculum reduction of between 40 and 95% and a correlated scab reduction of 45 to 85%.

Sutton *et al.* (2000) showed that shredding in conjunction with autumn and spring urea application reduced the ascosporic inoculum by at least 50%. More recently, leaf shredding combined with the use of antagonistic fungi (*Microsphaeropsis ochracea*) and leaf shredding in combination with urea have also been studied on a small scale (Vincent *et al.*, 2004). Such treatments reduce *V. inaequalis* ascospore production by 93.9 and 90.5%, respectively. On a bigger scale, in an ICM orchard, a combination of leaf shredding with urea treatments and with a contact fungicide (Captan) on dead leaf litter in autumn was studied for three years in succession in the Netherlands and in Hungary (Holb *et al.*, 2006, Holb, 2008). These studies showed that this kind of autumn treatment significantly reduced both the springtime leaf litter density and the scab damage on the leaves and fruit, with a less pronounced effect in a continental climate.

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.

Arboriculturists generally have a variety of tools at their disposal for reducing the inoculum: sweepers, shredders, mowers, mechanical weeders. Shredding seems to be the easiest method, given that many arboriculturists use a shredder for cutting prunning wood or for weed control. Even though shredders only roughly chop the leaves, they do help to speed up decomposition. Several manufacturers have recently developed shredder-windrowers especially to shred leaves very fine and reduce the autumn inoculum. The Perlim cooperative in the Limousin region of France has, for example, produced various new tools for litter management (Gomez, 2007). Several other manufacturers have developed new equipments for litter management (Jamar *et al.,* 2011).

Removing the leaves requires a special machine. The only machines available on the market are sweepers designed for parks (Gomez, 2007; Jamar *et al.,* 2011).

Burying leaves on the row during ridging supplements the shredding or sweeping of leaves in the inter-row. It should not be neglected, as fallen leaves on the row account for the greater part of the leaves on the ground and their removal has a beneficial effect on reducing the scab inoculum. Arboriculturists can carry out earth removal with a disc vineyard plough just before leaf fall, followed by ridging after leaf fall is complete to destroy the last weeds and bury the leaves lying on the row that cannot be shredded or collected up (Gomez, 2007).

I.1.4.4   Reduction of the ascosporic inoculum using chemical compounds

Several studies have shown the effects of sanitary practices on reducing the saprophytic phase of *V. inaequalis*. Such practices include urea treatments (Burchill, 1968, Ross and Burchill, 1968; Sutton *et al.*, 2000; Vincent *et al.*, 2004) or fungicide application to infected fallen leaves (Curtis, 1924; Plasman, 1953; Gadoury *et al.,* 1989).

The oldest study, conducted in New Zealand in 1924, showed that three soil treatments with lime sulphur in autumn reduced the incidence of scab by 60% (Curtis, 1924).

Using urea in autumn speeds up leaf decomposition (Sutton *et al.*, 2000; Holb *et al.*, 2006). Urea is believed to have a twofold action: (i) favourable effect on the microorganisms responsible for leaf decomposition and fungi antagonistic to *V. inaequalis*, (ii) direct inhibiting effect on *V. inaequalis* (MacHardy, 1996). It can be applied either directly to leaves still on the tree or to fallen leaves decomposing on the ground. However, urea treatments are not permitted in organic farming (European Regulation (EC) no. 889/2008).

Autumn application of dolomitic lime to infected leaves on the orchard floor reduces the percentage of apple and pear leaves with perithecia, the number of perithecia per leaf and the number of asci per perithecium of *Venturia inaequalis* and *V. pirina*. Applying 5 tonnes dolomitic lime per ha (containing 22.7 and 11.8% calcium and elementary magnesium, respectively) reduces the ascospore dose the following spring by more than 88% in the case of pears and 92% in apples (Spotts *et al.*, 1997).

The use of copper in autumn to control scab or canker (*Nectria galligena)* should be limited, because it adversely affects the microorganisms responsible for leaf decomposition and earthworms (Rousseau, 1995; MacHardy, 1996; Paoletti *et al.*, 1998).

I.1.4.5   Reduction of the ascosporic inoculum using biological agents

Five approaches using biological agents to reduce or eliminate the scab inoculum have been explored: (i) using microorganisms to confer resistance to *V. inaequalis* infections, (ii) using microorganisms antagonistic to *V. inaequalis*, (iii) using microorganisms to break down apple leaf litter in autumn and winter, (iv) using earthworms to clear dead leaves from the ground, and (v) using rotational grazing in the orchard.

Using microorganisms to confer resistance to V. inaequalis *infections*:

The effects of several strains of *Trichoderma harzianum* as a biological agent for controlling various cultivated plant diseases have been shown many times (Harman, 2000). For example, De Meyer *et al.* (1998) showed the resistance-inducing effect of *Trichoderma harzianum* T39 applied in the soil or on tomato, lettuce, pepper, bean and tobacco leaves in *Botrytis cinerea* control.

Some works showed that pathogens can cause less damage in plants infected with mycorrhizae than in uninfected plants (Strullu and Plenchette*,* 1992; Singh *et al.*, 2004). The presence of mycorrhizal fungi in plants is known to cause biochemical changes and trigger the production of various defence substances such as phytoalexins, lignin, callose, chitinase and other specific proteins, but whereas the general protective effects of mycorrhiza are not disputed, no satisfactory explanations have yet been produced for the mechanisms involved (Singh *et al.*, 2004).

The resistance-inducing effect of mycorrhizae or *Trichoderma harzianum* in apple scab control is not described in any study.

In a 1984 study quoted by MacHardy (1996), involving four microorganisms, neither *Sphaeropsis malorum*, nor *Erwinia amylovora*, nor *Pseudomonas fluorescens*, nor *Phytophtora cactorum* had any resistance-inducing effect on McIntosh plantlets inoculated with *V. inaequalis*.

Using microorganisms antagonistic to V. inaequalis:

The microflora occurring on the surface of the leaves is considered very effective against *V. inaequalis* in the first few weeks after leaf fall (MacHardy, 1996). Disease and pest control strategies therefore need to take account of this microflora. Substantial changes in the yeast, fungal and bacterial content on the surface of apple leaves have been observed up to seven months after the final application of a conventional protection scheme (Andrews, 1983).

Cross *et al.* (1968) showed that treating apple leaves with 5% urea in autumn stimulates fungus sporulation and increases bacterial and yeast populations twenty-fold, and may therefore help to discourage the development of *V. inaequalis* perithecia, notably by increasing the antagonistic microflora.

Two antagonistic fungi are potentially useful as biological control agents: *Chaetomium globosum* and *Athelia bombacina*. (MacHardy, 1996). *C. globosum* applied during the secondary infection season could be beneficial in that it reduces the size and number of lesions, the conidial density and the conidial germ tube germination rate and elongation. *A. bombacina* applied just before leaf fall reduces ascospore production (Young and Andrews, 1990) and also appears to play a part in the process of leaf decomposition and processing by earthworms (Miedtke and Kennel, 1990). Although these microorganisms have proved effective in scab control, their application is still very limited, given that (i) it is often hard for them to survive on the leaves and they do not survive for long, making repeated treatments necessary, (ii) their overall effectiveness is no more than partial, and (iii) the cost of use is high (Bosshard *et al.,* 1987; MacHardy, 1996).

Various studies under controlled conditions have shown the antagonistic effect of other microorganisms. Some strains of *Trichoderma longibrachiatum* have been identified as antagonistic towards *V. inaequalis*, reducing ascospore production from naturally infected leaves (Palani and Lalithakumari, 1999). More recently, Fiss *et al.* (2003) showed that several fungi (*Auerobasidium botrytis, Cladosporium spp*) and several epiphytic yeast strains from the apple tree plyllosphere inhibit *V. inaequalis* germination and mycelial growth on apple tree plantlets by up to 80%.

Several studies have identified *Microsphaeropsis ochracea*, which occurs naturally on dead leaves, as a good antagonist of *V. inaequalis* (Carisse *et al.,* 2000; Carisse and Dewdney, 2002). August and September are the best times to apply this microorganism in the orchard, resulting in a 95 to 99% reduction in spring ascospore production compared with untreated controls. This method did not alter the ejection distribution over time, and so standard infection models based on the usual method of ascospore ripening can be used

(Carisse and Rolland, 2004). In a recent study, Vincent *et al.* (2004) observed a reduction in spring ascospore production of about 81 and 85% following autumn application of *A. bombacina* and *M. ochracea*, respectively.

Using microorganisms to decompose apple leaf litter in autumn and winter:

The microflora occurring on the surface of leaves may interfere indirectly with the saprophytic phase of *V. inaequalis* by altering and breaking down dead leaves.

Miedtke and Kennel (1990) showed that leaves treated with *A. bombacina* in autumn, for instance, were 60% softer and 10 to 50% lighter in spring compared with untreated leaves.

However, no attempts have been made to speed up leaf decomposition in commercial orchards by applying soil microorganisms that can break down dead leaves. The only attempts made to speed up dead leaf decomposition have involved applying nutrients (such as urea) to the dead leaves in autumn or spring with the aim of promoting leaf decomposition through the alteration and growth of the microflora that occur naturally on dead leaves (Burchill 1968; Cross *et al.*, 1968).

Using earthworms to clear dead leaves from the soil:

The main natural biological agents for removing dead leaves from apple orchard floors in winter and early spring are earthworms, *Lumbricus terrestris* (Raw, 1962; Niklas and Kennel, 1981, Spotts et al., 1997; Holb et al., 2006). In grassed-down orchards the number and weight of leaves buried are directly linked to the weight of *L. terrestris* in the soil. In some orchards containing between 275 and 367 kg of *L. terrestris* per ha, the earthworms can bury up to 184 kg of leaves per ha, which is equivalent to 90% of the total autumn leaf fall. At the same time, this is a big contribution to the turnover and redistribution of organic matter in the soil (MacHardy, 1996).

Leaf burial by earthworms is inversely proportional to the quantity of surface vegetation, hence the need to keep the grass short in winter and carry out mechanical tillage. Tillage, which can be carried out in order to bury leaves in autumn, should be regarded as an alternative method, as it interferes with the work of the earthworms. Bare soil adversely affects earthworm activity because of the greater chilling effect. Below 2°C, earthworm burial activity slows down considerably. Grass strips are favourable to earthworms in summer as they provide a regular food supply (MacHardy, 1996).

Soil fauna, and earthworms in particular, seem to prefer urea-treated leaves, mainly because they soften up after urea treatment (Helling and Larink, 1998; Paoletti *et al.*, 1998). However, some fungicides, such as those containing copper, benzimidazols (benomyl and thiophanate-methyl), dodine and captafol, commonly applied to fallen leaves as a conventional control method, delay or prevent leaf decomposition by their adverse effects on earthworm populations (Cook and Swait, 1975; MacHardy, 1996; Paoletti *et al.* (1998).

Using rotational grazing in the orchard

Allowing small animals and poultry, e.g. sheep, pigs, geese or hens, to forage under fruit trees could offer various advantages, some of which could have a positive impact on scab control: (i) controlling weeds and maintaining a close-cropped plant cover without either mechanical tillage or mowing, in other words, without using fossil fuels, (ii) consumption and trampling of fallen leaves, promoting decomposition, (iii) supply of organic matter of animal origin and urea, (iv) control of voles, (v) codling moth control by eating windfall apples, (vi) stimulation of soil biological activity, (vii) increasing biodiversity. All these potential impacts need still to be validated in practice.

Such methods involving rotational grazing in intensive orchards are relatively untried to date, but some studies have shown their advantages, both in Europe and in the United States (Nunn et al., 2007). In Sweden, for example, the organic farming regulations require livestock to be grazed for several months each year and orchards are a good source of green spaces for small animals like pigs, sheep, etc. (Gustafson and Stern, 2003). Arboriculturists could indeed work with local livestock farmers to allow grazing at certain times of year.

I.1.5. Scab control and protection with fungicides

I.1.5.1 History

Although apparently committed to parasitism, *V. inaequalis* nevertheless has a short saprophytic stage in the dead leaves that were infected before leaf fall. The saprophytic stage is critical to the survival of *V. inaequalis*. It is closely synchronised with the apple tree's dormant period, and this is one of the main reasons *V. inaequalis* is a first-rate apple tree parasite.

However, the apple tree's dormant period is also the most sensitive time for *V. inaequalis*. Sexual reproduction links one growing season to the next. The first few weeks after leaf fall, when the sexual stage has to be initiated before the first frosts occur, are the most critical time for the parasite's survival. If the sexual stage does not occur then, the life cycle will come to an end. Preventing or interrupting that vital, vulnerable phase in the pathogen's life cycle thus appears to be an obvious control strategy.

So why are control schemes based almost exclusively on the use of fungicides to protect trees from primary and secondary infections, rather than on using practices focussing on control at that critical stage of the pathogen's life cycle? For the answer, one must go back to the beginnings of scab control schemes (MacHardy et al., 2001).

Apple scab first became a major problem in the second half of the 19th century, when large areas began to be planted for intensive apple production (MacHardy et al., 2001). The problem became acute in Europe at the end of the 19th century, after several years of weather conditions favourable to the disease. This also happened to be when Bordeaux

mixture was developed in France for controlling downy mildew of grape. So Bordeaux mixture was tried in scab control and several applications in spring were found to result in good control of the disease. The success of Bordeaux mixture was to shape scab control strategy throughout the 20th century (MacHardy et al., 2001).

MacHardy (1996) divides the history of scab control into four main periods: (i) first scab control schemes, the discovery of chemical fungicides against *V. inaequalis* and the start of development of protective fungicide schemes based chiefly on the use of copper (1880-1920), (ii) the arrival of inorganic fungicide schemes that were less phytotoxic than copper (such as sulphur and lime sulphur), based on epidemiological studies and efforts to improve fungicide performance by removing the saprophytic stage (1920-1950), (iii) the development of warning systems based on the Mills system (including validation of Mills' infection curves and better climate data recording instruments to determine infection periods), with proliferation of synthetic organic fungicides and improved curative fungicidal control (1950-1980), (iv) development of 'integrated' control methods (1980-2000).

Before 1920, Bordeaux mixture was the main weapon for chemical control of scab. Other substances were available such lime sulphur and crude powdered sulphur formulations (MacHardy, 1996). Because of the phytotoxic effects of copper, Bordeaux mixture came to be partly substituted by sulphur-based products.

The work of Hamilton (1931) and Mills (1947) marked the period from 1920 to 1950. Direct control of scab was then based very much on using lime sulphur and elementary sulphur. The damage caused to the apple trees by lime sulphur was often serious, and the only acceptable substitute at the time was elementary sulphur, which has the big drawback of having to be timed precisely according to spells of spring rain (Mills, 1947). Attention was then directed to developing fungicides that were less phytotoxic and more effective than in organic fungicides (copper, sulphur and mercury). The discovery of the fungicidal properties of dithiocarbamate in 1934 heralded the age of synthetic organic fungicides (MacHardy, 1996).

From 1950 onwards, a battery of synthetic fungicides with several new modes of action became available, and new spraying schemes were developed to exploit their preventive, curative and eradicant properties (MacHardy, 1996). Within the range of fungicides developed, Szkolnick (1978) established four main times for treatment application based on the fungicidal efficacy of the active substances. The first of these is called 'protection' i.e. before infection; the second is called 'post-infection', or just after infection; the third is called 'presymptom', i.e. applied until symptoms appear; and lastly, the fourth, called 'post-symptom', where a treatment can be applied after symptoms have appeared (Figure I.7).

The arrival of new fungicides was accompanied by improvements to spraying technologies including, in particular, the first air-blown sprayers in 1950, which enabled concentrated products to be applied and treatment volumes to be reduced (MacHardy, 1996).

Scab control since 1980 has been characterised by four main factors: (i) the development of new systemic sterol biosynthesis inhibitor fungicides ('DMI fungicides'), (ii) development of resistance to benzimidazole group fungicides (Olivier, 1986), and later to all main chemical groups including anilinopyrimidins, strobilurins, sterol biosynthesis inhibitors and also to some multi-site contact group products such as guanidines (Koller et

al., 2004; Micoud and Remuson, 2006), (iii) updating of fungicidal schemes based on infection forecasts and, lastly, (iv) improved weather recording equipment as an aid to identifying infection periods (MacHardy, 1996).
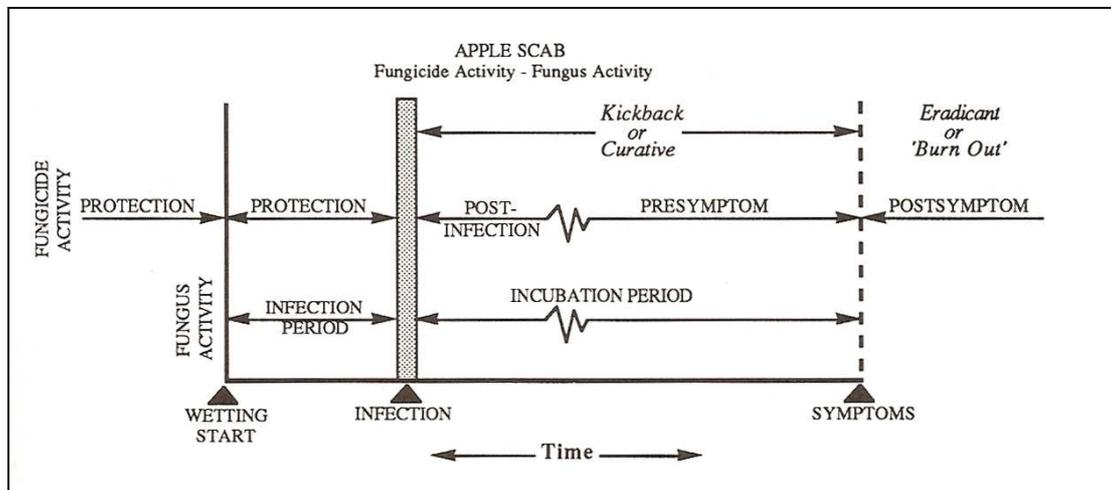
Figure I.7 − The type of activity of fungicides applied as sprays to apple trees for control of apple scab in relation to fungus (*V. inaequalis*) activity during the infection, incubation and postsymptom periods proposed by Szkolnick (Szkolnick, 1978).

I.1.5.2 Integrated Fruit Production

Chemical control: from the nineteen-fifties, spectacular progress by the chemicals industry resulted in a range of plant protection products coming on to the market that seemed to have solved the problem of crop pest control. The need to improve yields and the appearance of the fruit led to the systematic, and sometimes excessive ('insurance' treatment) use of multipurpose products.

However, these practices led to impasses:

- pest and disease resistances, necessitating further applications and research into new substances
- shifting of problems, with new pest species proliferating
- increased risks of residues

With some control methods having proved ineffective and faced with the risks of residues on fruit, new directions were tried in an endeavour to reduce plant protection treatments.

Integrated Production (IP) is a concept of sustainable agriculture developed in 1976 which has gained international recognition and application. The concept is based on the use of natural resources and regulating mechanisms to replace potentially polluting inputs. The agronomic preventive measures and biological/physical/chemical methods are carefully selected and balanced taking into account the protection of health of both farmers and

consumers and of the environment. The principles and objectives of IP evolving during the 1980s have been compiled, analyzed and formulated by an IOBC panel of experts in 1992, and first published in 1993 (IOBC/WPRS Bull. Vol. 16 (1), 1993). The document and vision was updated in the 2nd edition of 1999 and in the 3rd Edition 2004 (Boller *et al.,* 2004). The 2004 IOBC Standard for Integrated Production covers ecological, ethical and social aspects of agricultural production as well as aspects of food quality and safety. It is presently one of the highest international food production standards and unique in the way comprehensive sustainability ambitions are coupled with effective and practicable approaches on the farm. The current set of IP guidelines and related tools has proven helpful and inspirational for farmers' organisations looking for a feasible way to work with integrated production in the premium food segment.

IPM (Integrated Pest Management) is the part of IP focusing on pest and disease management. Integrated plant protection is, in principle, the combined, managed use of all available methods for controlling crop pests in order to keep their noxiousness low enough so that the damage caused is economically tolerable. In this concept of crop protection, the priority is the deliberate use of natural pest infestation limiters. Chemicals are used only as a last resort, when all the available control methods have been exhausted (Boller *et al.*, 2004). IOBC (International Organisation for Biological and Integrated Control of Noxious Animals and Plants) has published crop specific IP-guidelines for a large number of crops: pome fruits (Malavolta and Cross, 2009), stone fruits, arable crops, grapes, soft fruits (berries), olives, citrus and field grown vegetables. Full details are available on the site http://www.iobc-wprs.org.

A Regional Government Order passed in 1996 regulates the approval of the integrated production method for pome fruits, the control bodies and the growers practising that method. Full details are available on the site http://agriculture.wallonie.be

Integrated plant protection has made tremendous progress in terms of tree nutrition, plant protection product spraying quality, soil management, restriction on post-harvest treatments, selection and rate of application of active substances used in disease and pest control and timing of pest treatments, based on an assessment of the actual risk they pose. This risk must be assessed at plot level by observing and monitoring population levels and the presence and activity of natural enemies, with reference to tolerance thresholds. The conditions of use (crop, rate of application, time to harvest, compatibility with other pesticides) of each pesticide must be observed. The rates of application must be adapted to the volume of trees to be treated. Moreover, the treatment has to be applied at the right time, taking into account the time when the pest is vulnerable, the beneficials present, the weather conditions and the presence of flowering plants in the plot itself and in its immediate vicinity (Bosshard *et al*. 1987, Malavolta and Cross, 2009). Therefore, products should be selected with a view to (i) minimising the effects of fungicidal treatments on beneficial natural enemies and on the environment, (ii) limiting the number of treatments and increasing treatment effectiveness by appropriate timing, taking into consideration infection periods and propitious weather conditions for diseases, (iii) maintaining treatment effectiveness by alternating the active substances to prevent long-term pathogen and pest resistance to plant protection products from developing.

In practice, though, scab control in integrated production is still mainly a matter of chemical control, focussing on direct control, omitting basic prophylactic measures and

aiming, for example, to use resistant varieties and reduce the primary inoculum in autumn. Of the 45 main active fungicidal substances authorised in fruit tree disease control in France, about thirty synthetic active substances are approved for 'integrated control' (Aversenq, 2007). In Belgium, growers practising integrated control have some fifteen approved active substances at their disposal for apple scab control (Vermaete, 2000). These substances belong to a limited number of families or chemical groups: anilinopyrimidins, strobilurins, sterol biosynthesis inhibitors and multi-site contact products [phtalimides (captane, folpel); dithocarbamates (mancozeb, maneb, propineb, thiram, ziram); quinones (dithianon); guanidines (dodine); sulphamides (tolylfluanide); sulphur; copper]. The products approved for integrated pome fruit production in Belgium are classified according to three lists: green, yellow and orange. The green list contains products that can be used when their use is justified; the yellow list contains products that can be used only if none of the products on the green list is satisfactory for justified, effective use; and the orange list contains products that may be used only after the need for them has been proved, and with the authorisation of the supervisory body.

Thanks to the curative effect of sterol synthesis inhibitor group fungicides, anilinopyrimidins, strobulirins and guanidines, scab control has been targeted according to regional warnings based on data provided by infection detecting equipment.

Nevertheless, the resistance developed by *V. inaequalis* is giving rise to growing concern and the control strategies used against this fungus to date need to be fundamentally revised (Micoud and Remuson, 2006;). Resistance has already been acquired to the three main chemical groups (Koller *et al*., 2004, Köller *et al.,* 2005) and also to some multi-site group products, for example the instances of dodine resistance in the USA (Remuson *et al*. 2007). To reduce the development of resistance, irrespective of the orchard location, there are two basic principles that should always be strictly observed: alternating chemical groups with different methods of action, and limiting applications to three per season for each product group, either alone or in combination (Micoud and Remuson, 2006). Also, curative strategy, based on the use of fungicides with curative effects, must be limited and are currently not more recommended.

The noxiousness or economic thresholds are the basic building blocks of any integrated disease control programme. Without them, the principles of integrated control cannot be applied in order to optimise management tactics (MacHardy, 1996). Why has the concept of thresholds never become a major component of scab control programmes?

The main reason is that, unlike arthropods which are easy to observe and quantify, *V. inaequalis* spores are microscopic and hard to collect, identify and count. Until recently no procedures were available for determining *V. inaequalis* population densities in orchards. Moreover, growers, phytopathologists and industry consultants were for a long time firmly convinced that the harvest would contain an unacceptable level of scabby fruit unless the first sensitive parts of the plant received fungicidal protection, irrespective of the extent ascospore inoculum in spring (MacHardy, 1996).

I.1.5.3   Evaluation of the primary infection risks

When evaluating the primary infection risks on the basis of climate data, two prior parameters must be taken into account: (i) the quantity of primary inoculum present in spring, and (ii) the earliest ascospore discharge date (MacHardy, 1996). Then, throughout the primary infection season, the ascospore ripening rate in the perithecia until they are depleted (cf. the models developed by Gadoury and MacHardy (1982b) in § II.1.2.1). Additional data such as (i) the start, progress and intensity of ascospore flight, (ii) cultivar susceptibility, and (iii) the course of the phenological stages are all relevant when assessing infection risks.

Weather data collecting equipment in production orchards plays an essential part in detecting infection risk periods. This equipment, or weather stations, should be sited as close to the orchards as possible, and if possible in the orchard, one to two weeks before bud break. The detectors automatically measure the air temperature, relative humidity, rainfall, leaf wetting duration and, optionally, wind speed and direction. These data are then used to calculate the infection risks according to the Mills tables (cf. § I.1.2.4). When leaf wetting exceeds 10 hours, the average temperatures in the period have to be taken into consideration to determine the infection density with reference to the Mills table. If the conditions for light, moderate or heavy infection are met, a curative fungicide should be applied in the next few days (Viret, 2003).

At bud break, the bud wetting duration may be longer than that measured by the equipment sensor. The first two treatments should therefore be preventive ones (Viret, 2003). Various sensors for measuring wetting durations are commercially available, with minor differences in reactions. Caution is therefore required when interpreting this parameter.

The big innovation is the availability of GSM or GPRS stations that do not depend on the conventional telephone network for access. Small solar panels give the new stations an autonomous power supply (Rouzet and Pueyo, 2006).

As far as regional warning systems are concerned, the current aim is to refine the models from evaluating a regional risk to a more detailed analysis, closer to plot level (Rouzet and Pueyo, 2006).

I.1.5.4   The RIMpro apple scab simulation model

Numerous simulation models are currently available for assessing *V. inaequalis* primary infection risks (Trapman and Polfliet, 1997; Rossi et al., 2007). RIMpro is the scab model most commonly used in Europe at present. It is used by some 200 to 250 growers and experimental stations and 25 public or private warning services. The model is the basis for agricultural warnings in Quebec, Belgium, Denmark, France, the Netherlands, Sweden and some parts of Italy. RIMpro provides hour-by-hour data on the situation and is considered by most users to be a reliable model (Giraud and Trapman, 2006).

The RIMpro Scab model was developed by Marc Trapman, consultant to a network of arboriculturists in the Netherlands, within the framework of a team of researchers, experimenters and technicians. The group's initial aim was to produce an ascospore

infection simulation tool that would provide a better epidemiological approach than that offered by Mills curves alone, that could run at any weather station and that would be easy for growers and arboricultural consultants to use (Trapman and Polfliet, 1997). Details of this model are available on the site www.biofruitadvies.nl.

Since the first version came out in 1993, the model has been constantly developing in step with progress in scientific knowledge about scab. In the successive versions up to the 2007 edition, the model took into account:

-   The effects of light on ascospore discharge, that is to say, the fact that discharge takes place mostly by day,
-   The effects of temperature and wetting on the proportion of ascospores discharged,
-   Delayed ascospore ripening during dry spells in spring,
-   Leaf degradation on the ground affecting the available ascospore stock,
-   The parameters influencing ascospore and conidial survival during germination that are essential for managing the periods of interrupted wetting that may, or may not, halt the calculations,
-   The revised Mills table curves for ascospore infections, according to MacHardy and Gadoury (1989) and Stensvand *et al.* (1997). The Stensvand curve is used for secondary conidial infection on leaves, and the Schwabe curve for that on fruits,
-   Ascospore ripening is modelled from the New Hampshire curve, but will be adapted according to the geographical situation in future.

On launching the Scab model, the opening screen shows a graph depicting the current situation with the following main data: ascospore discharges, the state of ascospore ripening, the timing of the rainfall and periods of leaf wetting and the RIM value (Figure I.8). Forecasting is usually done by extrapolating the temperature and humidity data for the previous 24 hours, and by extending the wetting.

From the menu, another type of graph can be produced which is useful for displaying the risks of secondary contamination on leaves and fruit on the basis of Mills data. Another possible option is the weather data graph. This shows cumulative rainfall by rainy spell, illustrating any product leaching risk.

The level of ascospore infection, which could be translated in terms of risk, is represented by a value called RIM (Relative Infection Measure), which corresponds to the quantity of ascospores that have germinated and infected the apple tree leaves. This in fact answers the frequently asked question as to the relationship between the percentage of ascospores discharged and the scab risk in the orchard. A RIM value between 0 and 100 is considered low infection, between 100 and 300 as moderate infection and >300 as heavy infection.

In the studies carried out at the Laimbourg Research Station in South Tyrol, Italy, since the early days of RIMpro (Giraud and Trapman, 2006), the incidence of contaminating events in the orchard was measured on untreated apple trees grown in tubs which were moved along rails to expose them only to a given contamination before removing them to a chilled tunnel, where they were protected from subsequent contamination. The percentage of scabbed leaves per tub-grown tree was then noted. The biological discharges were measured with the aid of an ascospore trap. Overall, it was observed that no relationship existed

between the discharged ascospore volume and the level of tree infection, but that the latter correlated perfectly with the RIM value, which is now considered a benchmark for assessing scab risks.

Another graphic option allows fungicidal cover in several plots to be modelled. The treatments are input and the program produces residual curves for each treatment that can be superimposed on the discharge and RIM graphs (Figure I.8). The breakdown of the active substances is based on physical and chemical data supplied by the manufacturers (active ingredient half-life, rainfall necessary for 50% leaching, curative effect or not, etc.), the weather conditions and leaf growth (configuration necessary). This option, which is inherently interesting, is still at the experimental stage pending validation of all the data, and the proportion of fungicide on the leaves that determines when the product ceases to be effective is not yet known.

Trials are being set up using the RIM value as an action threshold (for preventive treatment, with the aid of the weather forecasting module) to reduce the number of treatments for non-susceptible cultivars under low autumn inoculum conditions.
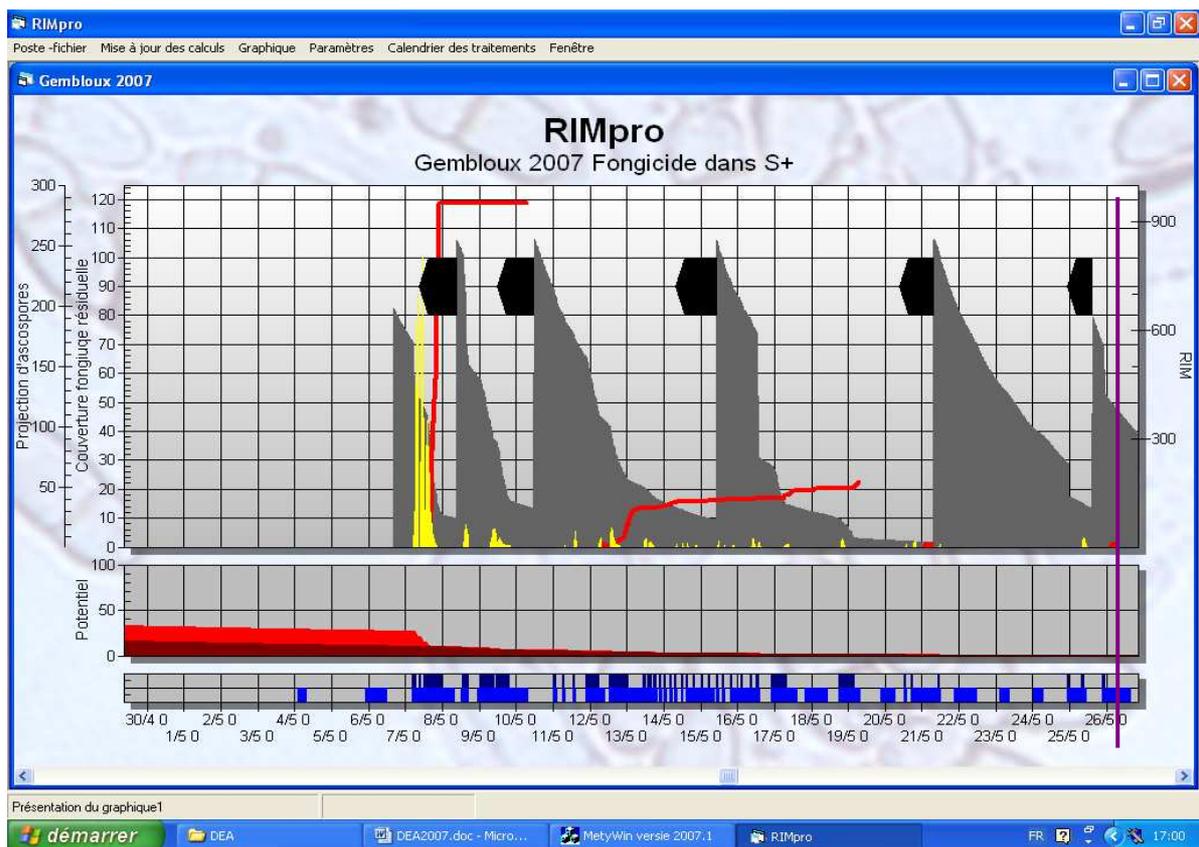
Figure I.8 : Graph showing applied fungicide cover (grey) on a given plot (S+) superimposed on ascospore discharge graphs (yellow) and RIM infections (red line). Degradation is simulated according to active substance degradation, growth and leaching parameters (dark blue). The back arrow symbolises protection by the curative effect (black). The potential ascospore dose is represented in the middle box either in red (ripe) or in brown (unripe). Periods of leaf wetting are

shown in light blue. The data extrapolated on the basis of weather forecasts can be seen to the right of the purple line.

I.1.5.5   Scab warning system based on climatic forecasts

Infections by fungal microorganisms are associated with specific climatic conditions. The simulation models take these infection conditions into account and translate the climate data into an infection risk. In the case of scab warnings, the practice to date has been to use climate data provided by a network of stations. The main problem with this warning system is that the infection conditions are already in place before the warning goes out to the arboriculturists and, consequently, treatments with curative products are by then the only means of halting the infection (Creemers, 2005). A model incorporating the recorded climate data and the weather forecasts would go some way towards overcoming the drawbacks of the 'curative' protection system. However, there are two prerequisites: firstly, the weather forecasts need to be sufficiently reliable and, secondly, it must be possible to calculate a realistic leaf wetting period from the weather forecasts.

In the launch configuration of the RIMpro model ('Advisor' version) a 'weather forecast' window can be opened allowing four-day forecasts to be input (with a three-hour time interval, for example) according to the local weather forecasts available. The advantage of this is that a rainfall event the next day or the day after, for instance, can be simulated in order to see what is likely and anticipate a critical situation by applying a preventive treatment.

Creemers (2005) considered the regional weather forecasts supplied by Belgium's IRM to be insufficiently detailed with regard to radiation and rainfall. The number of hours' rain and the time of day when rainfall occurs are more relevant to forecasting scab infections than the amount of rainfall. If it rains for four hours or more per day, the forecasts provide a good estimate. The less reliable results recorded for shorter forecast periods of rainfall are probably attributable to the localised nature of the showers (Creemers, 2005).

In the case of scab, the duration of leaf wetting is a key parameter. Now, this factor is very closely linked to the type of plant and so is not available in the data supplied by the IRM. It therefore has to be calculated using a model incorporating temperature, rainfall, relative humidity, wind speed and radiation as factors. No such model yet exists for the apple tree in Belgium (Creemers, 2005).

In integrated production, because of the curative effect (fungicidal action during the fungus incubation phase, between penetration of the leaves and the emergence of new sporulating scabs) of anilinopyrimidin, strobulirin, sterol biosynthesis inhibitor (SBI) and guanidine group fungicides, scab control can be targeted according to the indications provided by weather data collecting equipment (Olivier, 1986; Lefeuvre, 1995; Viret, 2003). The curative effect lasts for 1-2 days with guanidines, 2-3 days with anilipyrimidins and strobilurins and 3-4 days with SBIs (Viret, 2003). This type of treatment provides protection for 6 to 12 days, depending on the conditions. In spring, when the plants are growing rapidly, the protective effect lasts for no more than 6 to 8 days. After that, the detector data should be examined anew with a view to the timing of the next treatment. However, the

curative strategy is currently not more recommended because of the resistance developed by *V. inaequalis* (Creemers, 2005).

In organic farming, apart from the curative effect of lime sulphur (Holb *et al.,* 2003), all the other substances available should be used in preventive treatments or check treatments, i.e., timed before or at the beginning of the infection period, before the fungus penetrates the leaf. Hence the special value, in organic farming, of using models that can utilise and interpret data supplied by local weather forecasts.

I.1.5.6   Protection products in organic fruit production

The designation 'organic farming' in Belgium is governed by the following regulations:

- Council Regulation (EC) no. 884/2007 of 28 June 2007, which describes all the specific features of organic farming and sets out the aims and principles.
- Commission Regulation (EC) no. 889/2008 dated 5 September 2008, which establishes the conditions of application.
- The Regional Government Order dated 28 February 2008 on the method of production and labelling of organic products.

According to the foregoing, operators (producers, processors, importers) in organic farming must notify their activity and undertake to comply with the technical specifications. Belgium has two control bodies that are recognized and approved to perform inspections and issue organic farming certificates, namely Certisys sprl (http://www.certisys.eu) and Integra (Blik) sprl (http://www.integra-bvba.be).

The European specifications for organic farming were first established in 1991 and are currently contained in Regulations (EC) no. 884/2007 and 889/2008, available on the site http://europa.eu.int. Apart from various recommendations regarding prophylactic measures for disease control (use of resistant cultivars, basic sanitary procedures, cultural practices, etc.), organic farming restricts the types and quantities of plant protection products which producers can use for direct pest control.

Of the protection products that can be used in organic farming, seven types of substance have fungicidal properties and are potentially available for apple scab control. These comprise two organic substances of plant origin (lecithin and vegetable oils) and five mineral substances of various origins (copper, sulphur, lime sulphur, potassium permanganate and mineral oil). For environmental reasons the use of copper is limited to 6 kg of metal copper per ha per year, and will soon be limited to 4 kg (cf. new European Regulation due to come into force on 14 June 2011 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC).

National legislation relating to authorisation of plant protection products differs from one European country to another. In some countries like Denmark and Netherlands, for example, the use of copper as fungicide in arboriculture is totally prohibited, whereas copper

is authorised in Belgium (http://www.phytoweb.be). Lime sulphur was banned in Belgium back in 1970, but is still used in Italy, the Netherlands, France and Switzerland.

I.1.5.7 Sulphur and copper

Fungicidal properties of sulphur and copper: both these substances, which have long been known for their fungicidal properties, occur naturally in the environment (Dämmgen *et al*., 1998). Sulphur has been used in agriculture since time immemorial to 'disinfect' crops (Williams and Cooper, 2004). Back in 1805 a British researcher, Forsyte, mentioned sulphur powder as useful in controlling powdery mildew. Sulphur used in agriculture is obtained from (i) deposits frequently associated with oil-bearing strata or located in the vicinity of extinct volcanoes, (ii) gas scrubbing and (iii) ore extracts and sulphur-containing fuels (pyrites, coal, lignite and oil).

Sulphur and copper are the two main active substances authorised for apple scab control in organic production in Belgium. These two contact fungicides have a mainly preventive action (Hamilton, 1931; Mills, 1947; McCallan, 1967; Pezet *et al*. 1986; Tweedy 1981; Ellis *et al*., 1994, 1998; Tate *et al*. 2000; Holb *et al.,* 2003). Managed use of sulphur in agriculture is considered to have little adverse effect on the environment (Williams and Cooper, 2004). The quantities of sulphur used in organic apple growing in temperate climates may in some cases be substantial, even exceeding 100 kg per ha per year (Holb and Heijne*,* 2001).

Phytotoxic effects of sulphur and copper: copper and sulphur can both be phytotoxic (Vyas, 1988; Palmer *et al*. 2003, Holb *et al*. 2003). The phytotoxicity of sulphur and copper varies according to the cultivars, weather conditions, vegetative stage, rate applied and formulation. The phytotoxicity of sulphur depends on the temperature at the time of use: the same plant will be completely unaffected at 20°C but may be burned at 30°C. The more perfectly divided and regularly distributed the particles, the greater the danger of burning (Vyas, 1988). Caking should be avoided. Applied at the same rate, wettable sulphurs are more phytotoxic than those used in powder form. Russeting of the fruit limits the use of copper on susceptible varieties (Palmer *et al.,* 2003; Rousseau, 1995).

Fertilising effects of sulphur: sulphur, which is as old as the earth, played a key role in the development of the first organic molecules involved in the emergence of life on earth. Sulphur in all its forms (SO_4, SO_3, S, H_2S) is essential for living cells and plays an essential part in the biochemical processes concerned in vital cell functions (Dämmgen *et al*. 1998). Several authors have noted that sulphur treatments were followed, in various crops, by increased yields (Tweedy, 1981; Pezet *et al*., 1986). More recently, it has been shown that sulphur deficiencies, largely due to the decrease in atmospheric sulphur, were responsible for yield losses (Dämmgen *et al*. 1998; Williams and Cooper, 2004).

Dubuis *et al.* (2005) showed that sulphur deficiencies reduce the antimicrobial potential and make oilseed rape plants more susceptible to diseases.

Effects of sulphur on arthropods: the predacious mite *Typhlodromus pyri* Scheuten (Acari: phytoseiidae) is extremely useful in biological control of phytophagous mites such as *Panonychus ulmi* Koch and *Aculus schlechtendali* (Nalepa) (Duso *et al.,* 2003). Sulphur

compounds may adversely affect phytoseiid mites (Dabrowski, 1970; Kreiter *et al.,* 1998; Prischmann *et al*., 2005). However, other studies report predacious mite populations tolerant to sulphur compound treatments, probably through the development of new, tolerant strains (Schruft *et al*., 1992; Polesny *et al.,* 1996; Markoyiannaki-Printzioui *et al*., 2000). Dabrowski (1970) noted that *T. pyri* populations in apple orchards were lower in the first year of treatment with sulphur compounds, but increased three- to five-fold in the third year, showing acquired sulphur resistance. Sulphur is non-toxic to bees.

Secondary effects of copper on arthropods: copper fungicides are considered neutral for hemipterous bugs, ladybirds, Hymenoptera and predacious mites, but bioaccumulation causes some earthworm mortality in the soil. Repeated use of large quantities of copper on perennial crops may in the long term have adverse consequences for microorganisms and earthworms in the soil (Flemming and Trevors, 1989; Rousseau, 1995; Paoletti *et al*., 1998). European Directive 86/278/EEC proposes a maximum limit of 50 to 140 mg copper per kg of dry soil. Earthworm populations are considered unaffected at levels below 80 mg copper per kg of dry soil (Rousseau, 1995).

Mammalian toxicology of sulphur and copper: Sulphur is practically non-toxic to humans and animals according to the World Health Organisation (WHO) and Environmental Protection Agency (EPA) of the United States: "product unlikely to present acute hazard in normal use" (Tomlin, 1994). However, it could be irritating to skin, eyes and mucous membranes. Sulphur is non-toxic to fish. Degradation proceeds primarily by microbial reduction in and on plants as well as in soils. Slight oxidation to the volatile oxides could occurs in the environment. Considering copper hydroxide, the acute oral LD_{50} for rats is 1000 mg/kg and acute percutaneous LD_{50} for rabbits is >3160 mg/kg. For copper sulphate, oral intake leads to nausea. Copper from the hydroxide and oxychloride are considered as a slightly hazardous product by the WHO (toxicity class III) but copper from sulphate is considered as moderately harzardous (toxicity class II). Copper is toxic for fish, LC_{50} (48h) for carp is 2.2 mg/l. After animal ingestions, most of the copper is excreted with the faceas. Small amounts may be incorporated in natural proteins. In soil, copper is partly washed down to lower levels, partly bound by soil components.

I.1.5.8   Lime sulphur

Lime sulphur mixtures are prepared by combining boiling sulphur with lime suspended in water. Lime polysulphides are formed. The method is as follows: bring 68 litres of water to the boil in a tin-coated iron or brass boiler (not red copper) with a capacity of 100 litres. Dip a stick and mark the initial liquid level. While the water is coming to the boil, moisten the flowers of sulphur to a pasty consistency (10 kg per 6 litres water). Add the quick lime (6 kg) and small amounts of water, stirring all the time. The mixture heats up considerably on its own. As soon as the lime is completely disintegrated, pour the pasty mixture of sulphur and lime into the boiling water and allow boiling for three-quarters of an hour. Stir from time to time to help the sulphur mix with the lime. When the liquid level drops, add hot water to bring it back to the initial quantity of 68 litres. After boiling, allow the liquid to cool and the suspended matter will settle. Decant the reddish-brown surface liquid. This is the lime sulphur or California mixture. The mixture decays in contact with air. Correctly prepared, the mixture will have a density of 20° on the Baumé scale. The rate used in

summer treatment is 5%. However, industrial mixtures at 30° and 32° Be (density 1.26) are currently available at very attractive prices. Calcium polysulphide is considered to be the active substance in lime sulphur (Peeters, 1935).

The use of lime sulphur is currently a special issue, as (i) this long-known substance has curative properties, unlike elementary sulphur and copper, which have only a preventive action (Goldsworthy, 1928; Hamilton, 1931; Mills, 1947; McCallan, 1967; Tweedy 1981) and (ii) this substance is authorised in several EU countries but not yet in Belgium, despite its favourable ecotoxicological profile. Some recent studies have shown lime sulphur to have a curative effect in scab control (Kelderer, 2001; Holb *et al*., 2003; Montag *et al*., 2005). It is sometimes considered phytotoxic in some conditions of use (Ellis *et al*. 1998, Smilanick et Sorenson 2001, Holb *et al*., 2003, Palmer *et al.,* 2003).

I.1.5.9   Alternative protectant products

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; Heijne *et al.,* 2007). Bicarbonates of sodium, potassium and ammonium, in particular, are known to have fungicidal properties. Palmer *et al* (1997) showed the antagonistic effects of these salts on *Botrytis cinerea* growth on an *in vitro* crop. The possibility of using bicarbonates to control plant diseases has been shown notably by Homma *et al.* (1981), Ziv and Zitter (1992), Horst *et al.* (1992), Reh and Schlösser (1995), Palmer *et al.* (1997) Osnaya-Gonzalez *et al.* (1998), Smilanick *et al*., (1999) and Smilanick and Sorenson (2001). A small body of research is currently available highlighting the effectiveness of bicarbonate salts in apple scab control (Beresford *et al.,* 1996; Schulze and Schönherr, 2003; Montag *et al.,* 2005; Ilhan *et al*., 2006; Tamm *et al*., (2006), Heijne *et al*., 2007). Ihlan *et 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). No publication really deals with the mechanisms of action of the bicarbonate ions used as a plant protection product, except for Homma *et al.* (1981) and Palmer *et al.* (1997), who clearly show the effectiveness of the bicarbonate ion, independently of its effects on the pH.

Plant extracts: several authors have shown certain plant oils to be effective in controlling fungal diseases involving various leaf pathogens in cultivated plants (Clayton *et al.,* 1943; Cohen *et al*., 1991; Northover and Schneider, 1993; Steinhauer and Besser, 1997; Osnaya-Gonzalez *et al.,* 1998).

As many saponins have fungicidal properties and often occur in healthy plants, these substances are regarded as determinants of plant resistance to fungal attack (Osbourn, 1996). Saponins are glycosides with four different types of radical: triterpene, spirostanol, alkaloid and furostanol. They have the ability to destroy cells containing sterols. A recent study shows that a product containing *Yucca schidigera,* which is rich in saponin, has fairly pronounced anti-scab properties (Heijne *et al*., 2007; Bengtsson et al., 2009).

Soda and potassium silicates: these are conventionally recommended in organic farming manuals. Silica is an important constituent of epidermal tissues and supporting tissues, but its effects on scab are not described in any publication. On the other hand, several studies confirm the prophylactic effects of silica against numerous cultivated plant pathogens (Belanger *et al.,* 1995). Silica's mode of action is still being debated, although recent results have shown that it has the ability to trigger plant defence mechanisms (Belanger *et al.,* 1995). A specific Swiss formulation, named Myco-Sin, have been reported to be effective against apple scab (Tamm *et al*., 2004).

Lime: in the professional world of organic arboriculture, the application of lime to leaves is often recommended, notably because of its favourable effects in protecting against fungal diseases. Despite this, very few studies describing this property are mentioned in the literature. Four authors have revealed the inhibiting effects of calcium hydroxide on scab (Washington *et al*. 1998, Schulze and Schönherr 2003, Palmer *et al.* 2003; Grimm-Wetzel and Schönherr, 2005). Palmer *et al.* (2003) showed that the use of copper combined with slaked lime limits the incidence of scab on fruit to a level comparable with the 'IPM' programme (<2%), whereas copper or lime used on their own, in the same proportions, cannot reduce the incidence of scab to below 5.5 and 6.7%, respectively. According to Grimm-Wetzel and Schönherr (2005), large-scale application of lime in spring, during the primary infection season, limited the occurrence of scab on the fruit to below the 1% threshold.

Potassium permanganate: this is a highly oxidizing potassium salt which burns organic matter and has a curative fungicidal action on powdery mildew of grapevine, in particular. It acts as an antiseptic for numerous diseases of the vine and fruit trees, in winter treatments. Its action is immediate and very short-lived; all of the vegetation must be thoroughly wetted. It is very phytotoxic when used as a treatment on vegetation and the recommended application rate is not more than 300 g/hl. It is also very corrosive to the treatment equipment (Peeters, 1935).

I.1.5.10 Induced systemic resistance

Induced systemic resistance (ISR) is a phenomenon whereby resistance to infectious disease is systematically induced by local infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds (Kuć, 2001). The activity of the inducing agents is not due to antimicrobial activity *per se*. However, antimicrobial agents can induce resistance, and they give protection from the time of application until ISR is fully expressed. Events are put in motion and compounds are synthesized and accumulated which may contribute to resistance (van Loon *et al*., 1998; Kuć, 2001,).

Resistance to plant disease is often specific and metabolites and receptors contributing to this specificity may have specific structures. However, simple, structurally-unrelated compounds induce systemic resistance in unrelated plants to diverse pathogens including fungi, bacteria and viruses (Hammerschmidt *et al*., 2001). Both resistance and induced systemic resistance are associated with the rapid accumulation of the same structurally unrelated putative defence compounds that have diverse functions. It has been suggested that

cultivar (race)-specific resistance is initiated by the specific interaction of a pathogen product (or pathogen induced product) and a plant receptor. However, restricted infection by pathogens can result in ISR and many different compounds can cause ISR (Table I.3). It is thus evident that there are both specific and non-specific routes to the master switch for ISR and there may be more than one master switch. Adding to the complexity of resistance and ISR are the observations that different compounds and pathways may mediate different biochemical resistances. This makes the possibility of finding additional effective agents for ISR and disease control highly promising (Kuć, 2001, van Loon *et al*., 2006; Booler and Felix, 2009; Kauffmann et al., 2009).

Natural compounds used as elicitors of induced systemic resistance offer new prospects for controlling pome fruit tree diseases. This new method of plant protection for pome fruit tree diseases has a potential response to the very high use of pesticides in commercial production (Lateur, 2002).

Table I.3 - Main agents with induced resistance effect in plants (from Lyon and Newton, 1997; Kuć 2001)

Biotic elicitors
- fungi, bacteria, viruses, nematodes, insects
- fungal, bacterial and plant cell wall fractions, intercellular plants fluids and extract of plants, fungi, yeasts, bacteria and insects (poly- or oligo-saccharides, glucans, chitosans)
- glycine, glutamic acid, α and β-aminobutyric acids, D-phenylanaline, D-alanine and DL-tryptophane
- salicylic acid, acetylsalicylic acid, gallic acid and vanillic acid
- D-galacturonic acid, acide oxalic acid and polyacrylic acid
- oleic acid, linoleic acid, linolenic acid and arachidonic acid
- jasmonique acid, ethylene, riboflavin
Abiotic elicitors
- potassium and sodium phosphate, ferric chloride and silica, potassium phosphonate, aluminium phosethyl, boric acid, copper sulfate
- paraquat, sodium chlorate, nitric oxide, prohexadione-calcium
- 2,6-dichloroisonicotinic acid, acibenzolar-S-methyl, propenazole
- ultraviolet (UV) light

A lot of studies have demonstrated induced resistance in various host plant-pathogen relations. Elicitors which induce resistance in plants are from various origins: non pathogenic micro-organisms such as fungus, bacteria (Harman, 2000; De Meyer *et al*., 1998; van Loon *et al*., 1998), mineral substances (Kuć, 2001), synthetic chemical substances such as salicylic acid derivative (3,5-dichlorosalicylic acid, ADCS) (Ortega *et al*., 1998; Métraux, 2001), organic substances of biological origin such as proteins, lipids and oligosaccharides (Cohen *et al.,* 1991), as well as physical agents, changes or stress (Kuć, 2001).

The use of elicitors which induce resistance for the control of apple scab was demonstrated from chemical compounds such as the carpropamide, the prohexadione-Ca of BASF and the acibenzolar-S-méthyl (ASM) from Novartis Bayer (Ortéga *et al*., 1998;

Oostendorp *et al.*, 2001; Bengtsson *et al.*, 2009), but also from some natural compounds such as *Laminaria digitata* (Klarzynski *et al.*, 2000; Creemers, 2001; Lateur, 2002) or chitinase of *Trichoderma atroviride* (Faize *et al.*, 2003).

In recent field trials, conducted in both 2006 and 2007, established apple (*Malus* cv. Golden Delicious) and pear (Pyrus communis 'Williams' Bon Chretien') was used to assess the efficacy of three commercially available systemic inducing resistance products, Messenger (a.i. Harpin protein from *Erwinia amylovora* bacteria), Phoenix (a.i. Potassium phosphite) and Rigel (a.i. Salicylic acid derivative) applied at four different growth stages of tree development (bud break, green cluster, 90% petal fall, early fruitlet) against the foliar pathogens *V. inaequalis* and *V. pirina* which cause apple and pear scab respectively. A conventional synthetic fungicide (penconazole) used within the UK for apple and pear scab control was included for comparison. When the above compounds were applied at three or more growth stages efficacy as scab protectants was confirmed. The synthetic fungicide penconazole provided the greatest protection against apple and pear scab in both the 2006 and 2007 field trials. There was little difference in the magnitude of scab protection conferred by each ISR agent. Results suggest application of at least three sprays during bud break to early fruitlet formation with an appropriate ISR agent may provide a useful addition to existing methods of apple and pear scab management under field conditions (Percival *et al.,* 2009). Another recent study reported the induced resistance effects of an extract of *Yucca schidigera* on the control and infection process of the apple scab pathogen (Bengtsson et al., 2009).

However, few examples were reported of the use of elicitors of natural origin which induce systemic resistance for controlling apple scab. Many factors today limit their practical use. Their efficacy is only partial in the presence of high-yielding plants that presently have a level of resistance that is inadequate for disease control under high pathogen pressure. Much work has to be done to improve the formulation and to determine doses and rates of application and the right phonologic application times. Furthermore, they are often submitted to the normal high standards of plant protection product regulations which are long, very expensive and not adapted to compounds which can have a very complex composition (Lateur, 2002). On the other hand, this new method of plant protection presents many potential advantages: (i) using relatively simple, inexpensive, non-toxic natural compounds with a good image, (ii) polyvalent and broad field of action, (iii) non-specific and multi-sided action which offers a good durability of action, (iv) systemic action in the plants for a relatively long period of time and (v) the possibility of controlling difficult bacterial diseases and, more surprisingly, viral diseases. The multiple advantages presented offer valuable prospects for a better, environmentally friendly way to control pome fruit diseases in the near future.

I.1.6. Conclusion

Modern agricultural practices that have the effect of eroding the genetic diversity of cultivated apple trees have become very favourable to the development of *V. inaequalis*, which has the potential to recombine genetically each year (unlike its host) and thus to improve its parasitic fitness over time. The host-parasite system appears to have become unbalanced as a result of various human activities (MacHardy *et al.,* 2001).

Reproductive success is a key measure of parasitic and biological fitness, and three reproductive strategies help to ensure the reproductive success and thus the survival of *V. inaequalis* (MacHardy *et al.*, 2001).

Strategy 1: establishment of primary infection, i.e., ensuring that the ascospores created by new genotype combinations are deposited (at random) on susceptible tissues when the infection conditions are favourable. This takes place over a period of several weeks, from bud break until the maximum quantity of susceptible tissues has been produced, on the one hand, and the ripe ascospores have expanded, on the other.

Strategy 2: dispersability of diverse biotypes for greater probability of sexual reproduction, i.e., ensuring that the two different 'biotypes' necessary for sexual reproduction are present on the same leaf. This is achieved by producing conidia which burst out from lesions and disperse towards susceptible tissues, mainly in the same canopy. The incubation period is relatively short (2-3 weeks) and this secondary cycle can last until leaf fall.

Strategy 3: production of new combinations of the successful genotypes that had successfully infected the host during the growing season, some of which may make the fungus more parasitically and/or biologically suitable. This is accomplished when opposite mating types on the same leaf mate soon after leaf fall to produce immature pseudothecia.

Management practices used for apple scab control are aimed at disrupting these three reproductive strategies (MacHardy *et al.*, 2001). Two basic practices have been used to disrupt or halt *V. inaequalis*' three reproductive strategies: ***preventive practice***, aiming to reduce the ascospore dose in the saprophytic phase and to make use of the apple tree's natural resistance; and ***defensive practice***, aiming to protect the tree from ascospore and conidial infection. Preventive tactics include chemical, biological and physical methods for either attacking the fungus in the leaf litter or decomposing or removing the leaf litter, coupled with selecting cultivars with disease resistance characteristics. Defensive tactics include fungicidal treatments from bud break, in spring, until summer and sometimes until harvest (Figure I.9).
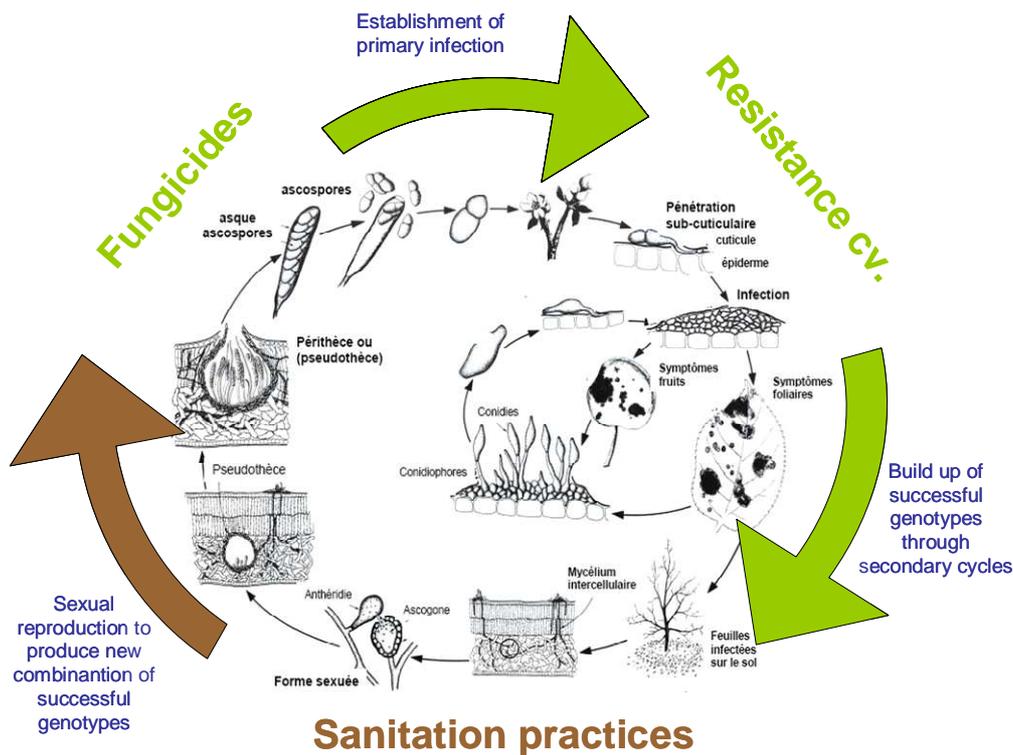
Figure I.9: Parasitic and saprophytic phases in the life cycle of *Venturia inaequalis* illustrated to show the three reproductive strategies and practices for controlling each reproductive strategy (redrawn and adapted from MacHardy *et al.,* 2001).

The most advanced scab control programmes incorporate both strategies, preventive and defensive (MacHardy, 2000a and b). Research over the past 20 years has had two major effects on the management of anti-scab programmes: the transition from a regional management programme to a management programme at 'individual orchard' level; and the transition from an essentially defensive programme to a more balanced programme which is both prophylactic and defensive (MacHardy *et al*., 2001). The 'individual orchard' level approach to protection management resulted from a growing awareness of the impact of the 'potential ascospore dose' and the associated risk of infection, which may vary considerably from one orchard to another, along with the new practice of siting weather stations in production orchards, enabling infection periods to be pinpointed at local level.

Full understanding of these concepts and implementation of these methods will have favourable consequences for the seasonal rate of fungicide application, the cost of fungicidal protection and the use of fungicide programmes that are compatible with the natural enemies and anti-resistance strategies.

Organic farming is faithfully modelled on these modern concepts of scab control strategies, but other constraints are still constraining its development.

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Chapter II

Objectives and outline of the thesis

II Chapter II : Objectives and outline of the thesis

Scab caused by *Venturia inaequalis* (Cke.) Wint. is the main disease of the apple tree, especially in temperate climate areas. Most present-day cultivars are highly susceptible to this disease, and losses directly attributable to the disease can compromise entire harvests unless protective treatments are applied. The conventional approach is to apply treatments regularly from the start of bud break (in March) until just before harvest in order to obtain unblemished fruit fit for marketing. Currently, in commercial apple organic growing, controlling apple scab presents major difficulties because few active substances are authorised in this context and new European restrictions are being imposed that will drastically limit the quantities of copper that can be used each year, although copper is the main and the most effective active substance available.

This thesis aims to define innovative scab control strategies that are in accordance with the organic production method and that also minimise the use of plant protection products, particularly copper. This will be achieved by establishing scientific bases for the most effective methods of controlling this fungal disease, while keeping the environmental impact to a minimum.

The specific aims of the study are (i) to identify fungicides of natural origin as alternatives to copper, (ii) to establish a treatment timing strategy for controlling primary infections, (iii) to find the best combination of active substances for use in scab control schemes that minimises the use of copper, phytotoxicity and adverse effects on natural enemies, (iv) to help to define application methods for the most effective plant protection products, and (v) to ensure that the cultural practice adopted is favourable overall to the biological activity of the soil.

This study starts from the hypothesis that scab can be controlled if a way can be found for controlling the primary infections that occur in spring, from late March to mid June, and are caused by ascospore contamination. In that way, the likelihood of secondary infections being caused in summer is minimised, as is the possibility of producing sufficient inoculum the following season.

The first step in the search for these new control tools suitable for organic production is to carry out experiments on plantlets grown under glass in controlled conditions. The most promising results need then be validated in field conditions, in an experimental orchard established at Gembloux in March 2002 specifically aimed at achieving the aforementioned aims. This orchard contains a series of components necessary for a balanced agro-ecological system, while at the same time complying with modern production conditions similar to those prevailing among professional growers.

In the first experimental part of this work (Chapter III) new scab control agents of natural origin, identified in controlled experimental conditions, are described for the first time. The best time to apply these substances, in relation to artificial inoculation, is also established.

In the second part of the thesis (Chapter IV) the effectiveness of compounds containing sulphur, copper and potassium bicarbonate in controlling primary scab infections in orchards is assessed over two growing seasons. This work is based on a managed treatment application strategy called 'during infection', applied and tried out in an orchard for the first time. Field experiments are conducted in an experimental orchard planted at Gembloux in 2002 (Table II.1, Figures II.1 and II.2).

The third part of the work (Chapter V) analyses the long-term impact of the organic apple growing method and the application of 10 scab treatment schemes on crop quality and quantity, in relation to eight apple cultivars. This study covers a period of 6 years, from the second to the seventh year of production in the orchard (i.e., from 2003 to 2008). In particular, it shows the effects of plant protection schemes on various production parameters, in relation to the degree and type of cultivar resistance.

In all the previous experiments, all the plant protection product treatments were applied with a tunnel sprayer, minimising drift. To establish whether the results of these experiments were linked to the particular properties of the tunnel sprayer, the next part of the study compares the spray distribution quality in the tree canopy when using the tunnel sprayer with the pattern produced by a standard axial flow sprayer (Chapter VI).

The penultimate part of the work (Chapter VII) describes the impact of 7 years of organic production on four biological soil parameters that may be connected with the soil's ability to digest dead leaves in autumn and winter and thus, ultimately, with the level of scab inoculum occurring in spring.

Finally, the conclusions emerging from this research are discussed and proposals for further work are made (Chapter VIII)

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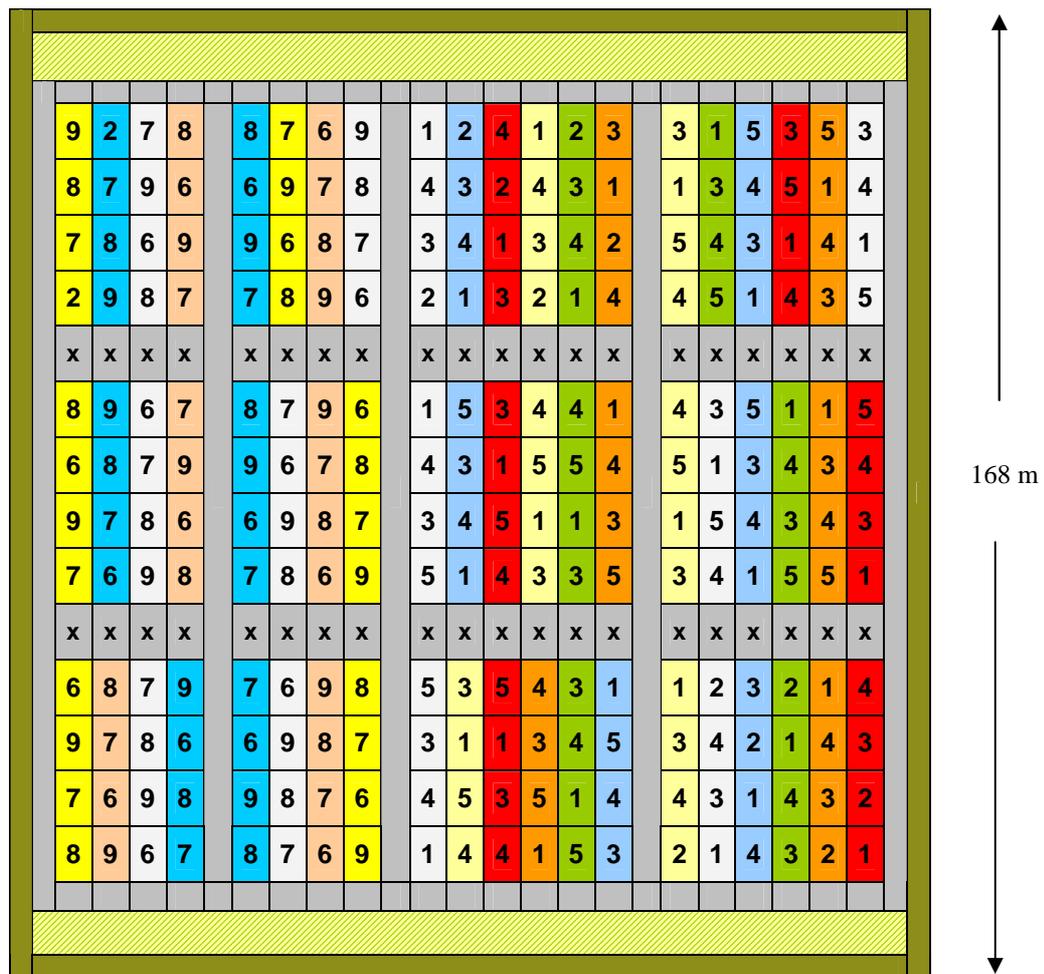
 Figure II.1: « Split-plot » experimental design of the organic orchard planted in March 2002 at Gembloux, Belgium. Each number mentioned on the figure indicates the six trees of a single cultivar: 1 = 'Pinova', 2 = 'Reinette Hernaut', 3 = 'Reinette des Capucins', 4 = 'Pirouette-Rubinstep', 5 = 'Reinette de Waleffe', 6 = 'JN(20/33/58)', 7 = 'Topaz', 8 = 'Initial', 9 = 'Zvatava'. Each « x » indicates a single cv. 'Alkméne' tree. Each colour indicates a specific spray programme against apple scab (Table II.1). Grey parts indicate tree edges composed of *Sambucus nigra, S. aurea, S. laciniata* and *Corylus avellana* with an extra of 20 species of flowers forming optimal ecological zones. Yellow zones indicate mowed grass. Peripheral zones in green are *Alnus glutinosa* edges.

Table II.1 – Legend of the figure II.1 (see chapter V for treatment explanations)

Orchard	Code	Colour	Description		Cultivars
1	RE1		Sulphur + others	1	**'Pinova'** *(Clivia [Oldenburg-Cox] x G. Del.)*
1	AR1		Mainly Armicarb	2	**'Reinette Hernaut'** *(Belgian old Cultivar)*
1	SF1		Sulphur + others	3	**'Reinette des Capucins'** *(French old cultivar)*
1	LS1		Lime sulphur	4	**'Pirouette'** *(Clivia [Oldenburg-Cox] x Rubin)*
1	CS1		Sulphur + copper	5	**'Reinette de Waleffe** *(Belgian old Cultivar)*
1	UC1		Untreated Control	x	**'Alkmene'** *(Oldenburgxx Cox)*
2	CS2		Sulphur + copper	6	*JN (20/33/58) (Delbard x Prima Vf)*
2	SF2		Sulphur	7	**'Topaz'** *(Rubin x Vanda)*
2	RE2		Sulphur + high copper	8	**'Initial'** *(Gala x Red Free Vf)*
2	UC2		Untreated Control	9	**Zvatava** *(Golden Delicious x hybride Vf)*
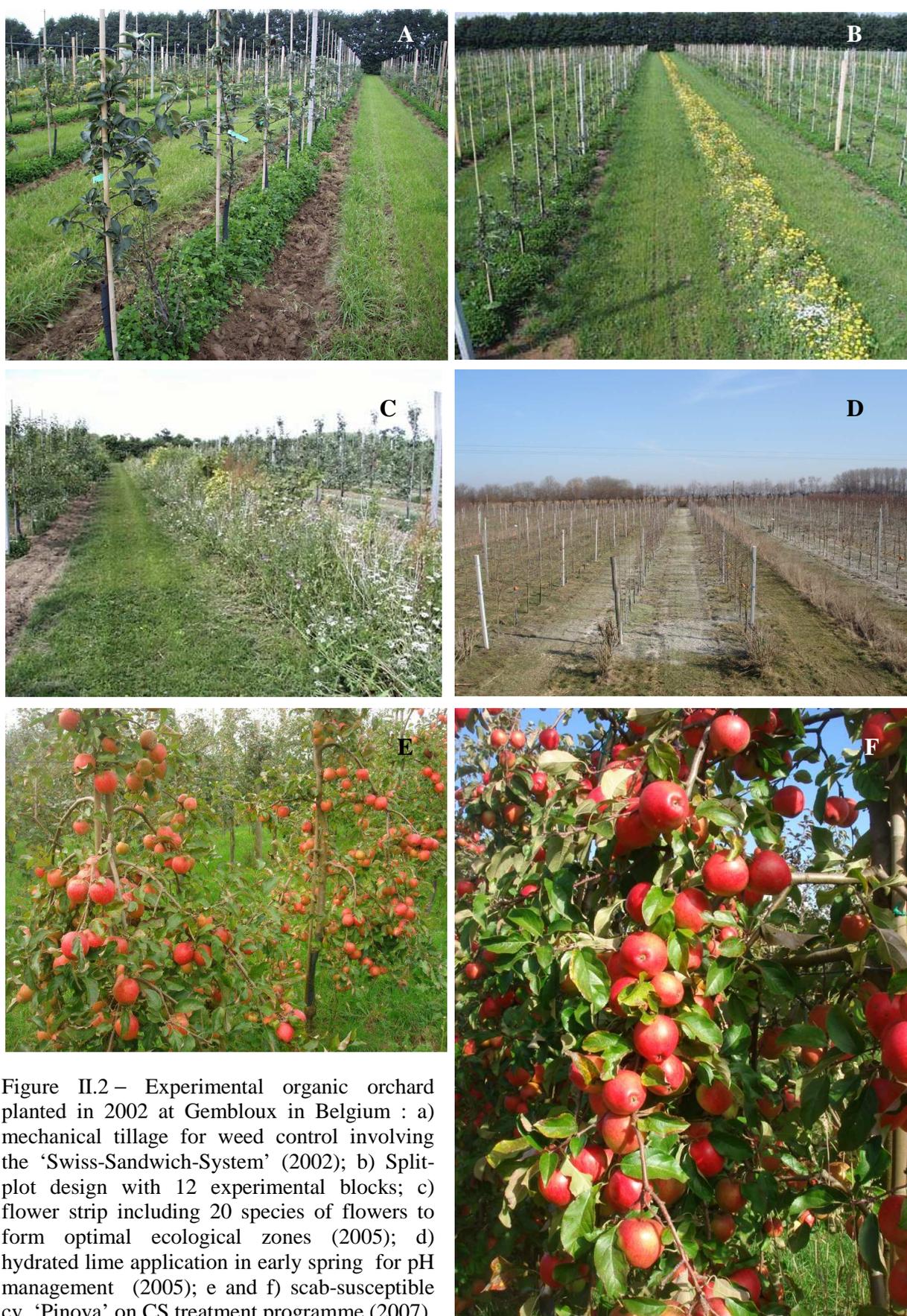
Figure II.2 − Experimental organic orchard planted in 2002 at Gembloux in Belgium : a) mechanical tillage for weed control involving the 'Swiss-Sandwich-System' (2002); b) Split-plot design with 12 experimental blocks; c) flower strip including 20 species of flowers to form optimal ecological zones (2005); d) hydrated lime application in early spring for pH management (2005); e and f) scab-susceptible cv. 'Pinova' on CS treatment programme (2007).

Chapter III

Control of apple scab *(Venturia inaequalis)* with bicarbonate salts under controlled environment

Foreword

This thesis is divided into chapters that have been written to be read individually. Each chapter includes the following sections: abstract, introduction, material and method, results, discussion and references.

In this chapter, greenhouse experiments conducted on seedling under controlled conditions are presented. At the end of this chapter, a recapitulative table provides information on the effectiveness of 60 products that were experimented during this thesis. Among these 60 products, only a few alternative products tested were putative promising as copper replacements for scab control (Table III.5). In particular, the results obtained under controlled conditions show that bicarbonate salts are a promising way for controlling apple scab. For that reason, this chapter is focused specifically on the study of scab control with several bicarbonate formulations.

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Table III.5 has been added to the thesis after acceptance of the manuscript "Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment" by the *Journal of Plant Diseases and Protection* editors. Therefore, the corresponding table is not present in the original publication.

III Chapter III : Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment

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Abstract

The effectiveness of potassium bicarbonate against *Venturia inaequalis*, the cause of apple scab, was studied. *In vitro* experiments with sodium, ammonium and potassium bicarbonate, as well as potassium phosphate used at 1% (w/v), reduced colony growth of *V. inaequalis* by 99, 98, 90 and 64%, respectively. Under controlled conditions in greenhouse experiments, a single spray of 0.5 or 1% (w/v) aqueous solution of sodium or potassium bicarbonate applied on young apple seedlings, 24 h before or 24 h after scab artificial inoculation, significantly controlled the disease. Greater effectiveness of potassium bicarbonate was recorded when the period of time before or after the inoculation was reduced. A significant increase of the fungicide activity of potassium bicarbonate was observed when salt was mixed with mineral oils. However, combining potassium bicarbonate with vegetable linseed oil and grapefruit seed extract did not increase its efficacy whereas these two vegetable products used alone reduced significantly scab infections. Formulated potassium bicarbonate, under the trade name Armicarb®100 and containing surfactant compounds, was more effective than bicarbonate alone. A phytotoxicity effect of potassium bicarbonate was observed with a 0.75% dose. The potential and limitations of potassium bicarbonate used to control apple scab in the field are discussed.

Key words: alternative fungicides, Armicarb, copper, linseed oil, organic farming, natural substances.

III.1. Introduction

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint. is the most important apple disease, causing significant economic losses in many of the world's apple production areas, particularly in rainfed agricultural areas where intensive fungicide control is necessary for commercial apple production. With the cultivation of susceptible commercial apple cultivars, apple scab control is becoming more difficult, such that losses caused by apple scab would be about 70% if no control measures were taken. 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). Prediction systems have been developed for apple scab and used successfully to assist in timing fungicide applications (MACHARDY 1996; TRAPMAN and POLFLIET 1997; JAMAR and LATEUR 2005). There is, however, a growing concern globally over the continuous use of synthetic chemicals on food crops because of their potential effects on human health and on the environment. Pathogen resistance is another factor militating against the continuous use of synthetic fungicides (BENAOUF and PARISI 1998; ANONYMOUS 2004; KOLLER et al. 2004).

Bicarbonate salts are one of several alternative control options that have recently received attention. These 'biocompatible' chemicals are particularly interesting because they have fungicidal properties combined with a very low mammalian and environmental toxicity profile. Bicarbonates are generally regarded as safe by United States Environmental Protection Agency (EPA) and therefore will be much easier to register. They are common food additives allowed in many applications by European and North American regulations. The actual use of these products in the control of plant disease is, however, still limited.

Bicarbonates have been shown to control a wide range of fungi, including food spoilage organisms and plant pathogens. The fungicidal effects of the carbonate and bicarbonate salts of ammonium, potassium and sodium have been reported on soil-borne pathogens, including *Sclerotium rolfsii* Sacc. (PUNGA and GROGAN 1982). HOMMA et al. (1981b) found sodium bicarbonate to be inhibitive to powdery mildew on cucumber and green mould on citrus, and the addition of surfactants to improve the effectiveness of sodium bicarbonate against green mould on citrus. HORST et al. (1992) showed that rose powdery mildew *(Sphaeroteca pannosa)* and blackspot *(Diplocarpon rosae)* were significantly controlled by weekly sprays of 0.5% (w/v) aqueous solution of either potassium or sodium bicarbonate used alone or with 0.5% or 1.0% (v/v) Sunspray oils. ZIV and ZITTER (1992) found pronounced detrimental effects of bicarbonates on the *in vitro* growth and disease incidence of several cucurbit foliar pathogens: *Alternaria cumerina, Colletotrichum arbiculare, Didymella bryoniae*, and *Yulocladium cucurbitae*. PALMER et al. (1997) reported the inhibitory effects of bicarbonates on the *in vitro* colony growth of *Botrytis cinerea* and examined the contribution of pH and buffering capacity of these compounds. The pre-harvest application of 2% potassium bicarbonate on bell peppers significantly reduced post-harvested gray mould, caused by *B. cinerea* (FALLIK et al. 1997). Sodium bicarbonate controls post-harvest green mould, caused by *Penicillium digitatum* on citrus fruit, and is in common commercial use (SMILANICK et al. 1999). MLIKOTA GABLER and SMILANICK (2001) demonstrated the potential of carbonate and bicarbonate salts for the control of post-harvest gray mold on grapes. A novel fungicide (Armicarb SP) containing potassium bicarbonate reduced defoliation in citrus caused by *Mycosphaerella citri*. (MCGOVERN et al. 2003). Potassium bicarbonate salt reduced powdery mildew *(Microsphaera pulchra)* in flowering dogwood in the field (MMBAGA and SAUVE 2004).

There are very few studies on the bicarbonate effects on apple scab: SCHULZE and SCHÖNHERR (2003) reported that potassium carbonate prevents spore germination and kills the germ tubes of apple scab (*V. inaequalis*) at a concentration of 4.3 g/l. A successful field trial with potassium carbonate to reduce fruit and leaf scab has been conducted by GRIMM-WETZEL and SCHÖNHERR (2005). More recently, ILHAN et al. (2006) reported the effectiveness of sodium bicarbonate alone or in combination with a reduced dose of tebuconazol for controlling apple scab under field conditions.

The objective of this study was to evaluate the effectiveness of bicarbonates used alone or combined with horticultural oils for the control of apple scab in order to develop a successful strategy using environmentally friendly substances compatible with the organic production system. This is the first study we know of that demonstrates the effectiveness of potassium bicarbonate for controlling apple scab (*V. inaequalis*) on *in vivo* plantlets raised from seeds.

III.2. Materials and methods

III.2.1. Plant material

Experiments were carried out with highly susceptible apple seedlings from open-pollinated trees of cv. 'Golden Delicious' that had probably been pollinated by cv 'Gala', two highly scab susceptible cultivars. After dry storage, the seeds were stratified in moist peat at 2°C for 80–90 days. The apple seeds were raised in commercial potting soil mixture under greenhouse conditions at 18°C ± 2°C and 80% relative humidity in a 12-h light regime as described by OLIVIER and LESPINASSE (1980). Four-week-old plants at the four-leaf stage were used for the experiment.

III.2.2. Controlled inoculation

A mixture of strains of *Venturia inaequalis* isolated from diseased leaves from unsprayed orchards of various cultivars in central Belgium was used for the experiments. Dry leaves were conserved in the deep freeze at –18°C. The inoculum was prepared as described by SZKOLNICK (1978). For infection experiments, conidia were collected in distilled water and the suspension was adjusted to 1.5×10^5 living conidia ml^{-1}, using a heamocytometer. Quantitative seedling inoculations were carried out with an automatic bench sprayer machine in laboratory. The conidial suspension was sprayed at the 'just-before-run-off' stage. Immediately after inoculation, the plants were incubated in a dew chamber at 100% relative humidity for 48 h at 18°C to provide optimal infection conditions. The treatments were randomised within the mist chamber in a complete block design.

III.2.3. Fungicide preparations

The chemicals tested included potassium bicarbonate (99.5% KHCO3, from Sigma-Aldrich sa, Belgium), sodium bicarbonate (99.5% NaHCO3, from Sigma-Aldrich sa, Belgium), Armicarb®100 (85% KHCO3 from Helena Chemical Company, USA) and both 'Candit' (a synthetic fungicide containing 50% Kresoxim-methyl, from BASF, Belgium) and Thiovit jet (80% micronised sulphur, from Syngenta Agro S.A.S., France) as the positive control. The treatments included bicarbonate salts alone or combined with emulsified linseed oil (from Vandeputte Oleochemicals Belgium), Citripur grapefruit seed extract (containing 33% grapefruit seed extract without Benzethonium from Pro-vera sprl, Belgium) and Oviphyt mineral oil (a refined petroleum distillate marketed by Belchim

Benelux). Armicarb is registered in US by the EPA and labelled as a biocompatible fungicide.

III.2.4. Performance of bicarbonate salts at varying concentrations for foliar disease control

In the first experiment, freshly prepared aqueous solutions containing 0.25, 0.5 and 1% of $KHCO_3$, 0.25, 0.5 and 1% of $NaHCO_3$, and 0.25, 0.5 and 1% of Armicarb were sprayed from a bench sprayer machine in the laboratory onto the upper surface of seedling leaves until just before run-off. A solution containing 0.02% Candit (50% Kresoxim-methyl) was used as a reference treatment. Water-treated samples were used as the control. There were 160 seedlings per treatment (4 replicates of 40 seedlings for each treatment). Pre-inoculation protective treatments were applied once 24 h before inoculation and post-inoculation curative treatments were applied 24 h after inoculation when the germination period had ended and the infection period had begun (MACHARDY 1996). The plants were then placed on the greenhouse bench at 18°C and 80% relative humidity for 3 weeks to promote plant and disease development. Disease incidence was assessed 21 days after inoculation by estimating the scab severity on the most infected leaf of the plant (LATEUR and POPULER 1996; LATEUR and BLAZEK 2002).

III.2.5. Use of potassium bicarbonate and oils for foliar disease control

The set-up of this experiment was as described above except for the following modifications. $KHCO_3$ was used as an active ingredient at a concentration of 0.5% (w/v). It was applied alone or mixed with soluble vegetable linseed oil, grapefruit seed extract or mineral oil at 0.5% (v/v). The choice of linseed oil and grapefruit seed extract was based on unpublished results that had been obtained in our laboratory. Armicarb was used as a comparative commercial formulated potassium bicarbonate. A sulphur solution (0.2% w/v) was used as a reference treatment. In this experiment, all treatments were applied with a hand sprayer until runoff 24 h before or 12 h after inoculation with *V. inaequalis*.

III.2.6. Effect of treatment timing on potassium bicarbonate effectiveness

In a third set of experiments, the plants were sprayed once up to run-off with a freshly prepared salt solution of KHCO3 0.85% (w/v) plus Tween-20 0.05% (v/v) as a surfactant compound, Armicarb 1% (w/v) and Thiovit 0.25% (w/v). According to previous assessment, Tween-20 at 0.05% did not express any inhibitory effects on apple scab. The treatments were applied 48, 24 and 3 h before the inoculation or 3, 24 and 48 h after inoculation. The seedling treatments, inoculation, incubation and experimental design were carried out as described above.

III.2.7. Phytotoxicity studies

In this experiment, solutions of sodium and potassium bicarbonates alone or combined with 0.5% mineral oil were prepared in distilled water at 0.5, 0.75, 1.0 and 2.0% (w/v). For comparative purposes, solutions of Armicarb were prepared with the same active ingredient dose. Each solution was applied with the bench sprayer machine in the laboratory onto the upper surface of 40 healthy seedlings until just before run-off. The plants were 4 weeks old at the time of treatment. Visible leaf phytotoxicity (necrotic area) was recorded on the 10th day after treatment. A qualitative assessment was conducted on the third and fourth leaf of each seedling. Leaf phytotoxicity was scored thus: - = no damage; + = 0 to 2%; ++ = 2 to 5%; +++ = 5 to 20%; and ++++ = >20% of the leaf surface damaged.

III.2.8. In vitro effect of bicarbonate salts on mycelial growth of V. inaequalis

Two monoconidial strains of *V. inaequalis* isolated from Belgium apple cultivars were used to evaluate the bicarbonate inhibition of *in vitro* colony growth. The first strain was isolated from *Malus floribunda* and the second provided by 'Golden Delicious' (strain EU-B-04 INRA Anger). Malt extract at 25.0 g l^{-1} and agar at 20.0 g l^{-1} (Roberts and Crute 1994) were mixed with Na, K, NH_4^+ bicarbonate or K_2HPO_4 at three concentrations (100, 1000 and 10.000 ppm) and were autoclaved for 20 min, then incubated at 60°C and then poured onto sterile plastic Petri plates. Non-autoclaved Captan at 10 and 100 ppm was used as a control. The *V. inaequalis* conidia were transferred to solidified plates with a heat-sterilized glass rod; the plates were then sealed with Parafilm. The media pH was not adjusted after amending with bicarbonate. Six plates differentiated by treatments and by strain were inoculated and then incubated at 18°C under dark conditions. The colony diameter was determined by measuring the average radial growth at 7, 14, 21 and 28 days. The control consisted of pathogen grown on standard malt agar.

III.2.9. Experimental design and data analysis

All the greenhouse experiments were arranged in a completely randomized split-plot design with four replicates of 40 seedlings for each treatment and repeated at least twice. The percentage data were transformed into arcsine angles before performing an analysis of variance. The data were analysed using statistical SAS software and the Student-Newman-Keuls test was applied as a mean variance analysis. All statistical analysis was conducted at a significance level of *P*<0.05. For *in vitro* experiments, six replicates per strain and treatment were conducted and the experiment was repeated twice. The Student-Newman-Keuls multiple range test at *P*<0.05 was also used to establish the differences among treatments.

III.3. Results

III.3.1. Effect of bicarbonate salts at varying concentrations under greenhouse conditions

All sprays of aqueous solutions of $NaHCO_3$ and $KHCO_3$ applied on apple plantlets 24 h before or 24 h after artificial inoculation with a conidial suspension of *V. inaequalis* significantly reduced the scab severity on the leaves (Table III.1). Similar effects were recorded with both Na and $KHCO_3$, although $KHCO_3$ performed slightly better than $NaHCO_3$. When applied 1 day before inoculation, sodium and potassium bicarbonates at 1% (w/v) reduced scab severity to rates of 6.9 and 4.9%, respectively, compared with the infection rate of the controls that ranged from 41 to 43.8%, respectively. Similar values were recorded when treatments were applied 1 day after inoculation. The results indicated that Armicarb was more effective than $KHCO_3$ alone at the same active ingredient (a.i.) rate. Slight phytotoxicity was observed only when bicarbonates and Armicarb were used at a 1% a.i. dose.

Table III.1 − Effect of $KHCO_3$, $NaHCO_3$ and Armicarb applied at increasing concentrations on apple scab development on artificially inoculated apple seedlings. The chemicals were applied 24 h before or 24 h after the conidial suspension was added.

Treatment	a.i. dose %	Leaf area covered with scab (%)		Phytotoxicity [a]
		Pre-inoculation treatments	Post-inoculation treatments	
Water control	…	41.0 a [b]	43.8 a	-
$NaHCO_3$	0.25	30.6 b	33.9 b	-
$KHCO_3$	0.25	31.6 b	33.0 c	-
Armicarb	0.25	19.5 cd	22.7 c	-
$NaHCO_3$	0.5	19.3 cd	16.6 d	-
$KHCO_3$	0.5	17.9 d	15.6 d	-
Armicarb	0.5	06.5 e	08.4 e	-
$NaHCO_3$	1.0	06.9 e	06.5 e	+
$KHCO_3$	1.0	04.9 ef	04.1 ef	+
Armicarb	1.0	02.2 f	01.6 f	+
Kresoxym-methyl	0.01	01.0 f	01.0 f	-

[a] - = no phytotoxicity., + = slight phytotoxicity.
[b] Means of four replicates, each replicate including 40 seedlings. Two leaves per seedling were assessed. Means followed by the same letter are not significantly different according to the Student-Newman-Keuls multiple range test at P<0.05. The experiment was repeated three times and similar results were recorded.

III.3.2. Effect of potassium bicarbonate when mixed with oils

$KHCO_3$ at 0.5% applied 24 h before or 12 h after inoculation significantly reduced scab severity with an effectiveness of 69% compared with the water control. When $KHCO_3$ treatments were combined with vegetable oils, no significant increase of the fungicide

activity of $KHCO_3$ was observed. However, significant improvement in the effectiveness of $KHCO_3$ was observed when it was mixed with 0.5% (v/v) mineral oil (Table III.2). Apple scab was reduced by 88 and 86% compared with the controls on plants sprayed with Armicarb 24 h before or 12 h after inoculation, respectively. The effectiveness of $KHCO_3$ mixed with mineral oil at 0.5% (v/v) and Armicarb, were fairly similar. Linseed oil and grapefruit seed extract at 0.5% used alone significantly reduced infection, whereas mineral oil used alone did not reduce infection significantly. In another set of experiments, our observations indicated that the effectiveness of linseed oil increased as the concentration increased (data not shown).

Table III.2 − Effect of pre-inoculation and post-inoculation application of potassium bicarbonate alone or combined with a vegetable oil, a mineral oil and a vegetable extract on the severity of apple scab in the greenhouse.

Treatments [a]	pH	Leaf area covered with scab (%)[c]	
		Pre-inoculation [b]	Post-inoculation
Water control	7.6	52 a	52 a
0.5% MPO	7.8	48 a	46 a
0.5% VGE	7.6	26 b	23 bc
0.5% VLO	7.7	17 cd	20 bcd
0.5% $KHCO_3$	8.5	15 d	16 d
0.5% $KHCO_3$ + 0.5% MPO	8.6	08 e	07 e
0.5% $KHCO_3$ + 0.5% VLO	8.5	17 cd	19 cd
0.5% $KHCO_3$ + 0.5% VGE	8.5	17 cd	18 cd
0.5% Armicarb	8.5	05 e	06 e
0.25% Sulphur control	7.7	02 e	02 e

[a] MPO = mineral paraffinic, VLO = soluble vegetable linseed oil, VGE = soluble vegetable grapefruit seed extract.
[b] The pre-inoculation and post-inoculation treatments were made, 24 h before and 12 h after inoculation, respectively.
[c] Means followed by the same letter are not significantly different according to the Student-Newman-Keuls multiple range test at $P<0.05$

III.3.3. Effect of treatment timing on potassium bicarbonate effectiveness

The effects of single spray of aqueous solutions of $KHCO_3$, Armicarb or sulphur applied from 48 h before inoculation to 48 h after inoculation are given in Figure III.1. In this set of experiments, $KHCO_3$ at 0.85% reduced apple scab by 95 and 94.5% when applied 3 h before or 3 h after inoculation, respectively. Control of apple scab was significantly better with Armicarb than with $KHCO_3$ when the treatments were applied 48 h before inoculation. From 24 h before inoculation to 24 h after inoculation the effectiveness of $KHCO_3$ and Armicarb were as affective as sulphur. When the treatments were applied 48 h after inoculation, the control of the disease was significantly reduced. Hence, $KHCO_3$ was more effective when treatments were applied just before or just after artificial inoculation. Any phytotoxicity on leaves from either $KHCO_3$ or Armicarb was observed at this concentration (0.85% w/v).
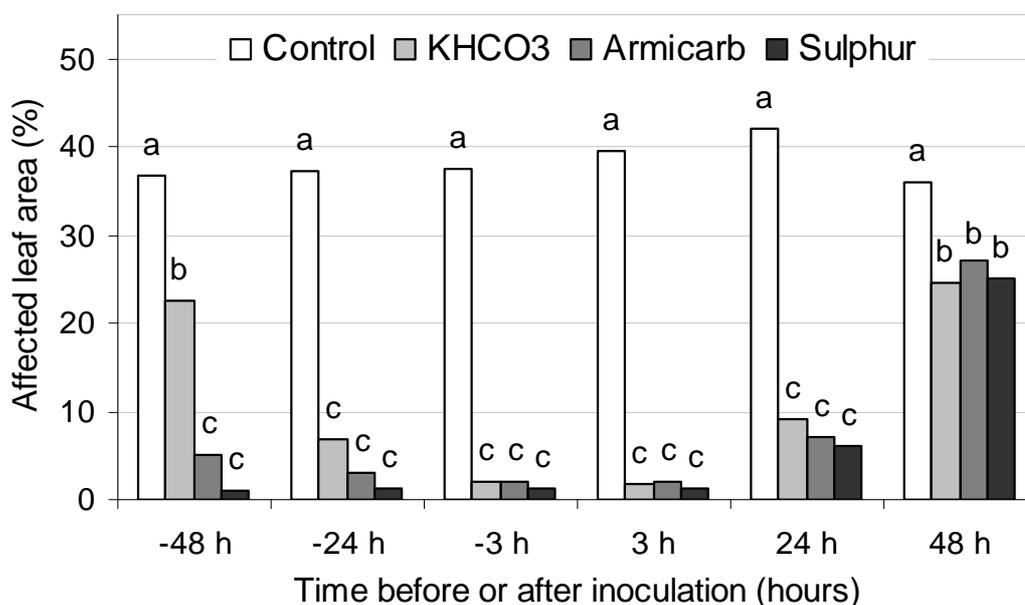
Figure III.1: Effect of a single foliar spray of 0.85% potassium bicarbonate, 1% Armicarb and 0.3% wettable sulphur solutions on apple scab severity (leaf area infected). Freshly prepared salt solutions of $KHCO_3$, Armicarb and wettable sulphur were sprayed until run-off on the upper surface of each leaf of the plants 48, 24 or 3 h before inoculation and 3, 24 or 48 h after inoculation. The numbers are means of 160 plants per treatment, including four replicates. Different letters denote a significant difference ($P<0.05$) among treatment means according to the Student-Newman-Keuls multiple range test.

III.3.4. Phytotoxicity studies

Phytotoxicity in the form of beige to light brownish necrotic areas was noted on healthy apple leaves treated with 0.5, 0.75, 1 and 2% of $NaHCO_3$ and $KHCO_3$ used alone or combined with oil and Armicarb (Table III.3). Tests with 4-week-old seedlings showed that phytotoxicity symptoms were related to the concentration of the bicarbonate salts used. The importance of injury level is directly correlated to the level of salt concentration. With all the treatments, at 0.5% no injury at all was observed on young leaves. A few beige necrotic spots appeared on leaves treated with $NaHCO_3$ and $KHCO_3$ when used at concentrations of 0.75%. When bicarbonates salts were mixed with mineral oil leaves were far less susceptible to phytotoxic reactions. At a 0.75% concentration, neither bicarbonate plus oil (0.5% v/v) nor Armicarb showed any injury symptoms. Armicarb was less phytotoxic than potassium bicarbonate at the same active ingredient dose. In all cases, it was noted that the application of bicarbonate solutions that resulted of distinct droplets on the leave surface produced more phytotoxicity compared with a better standard leaf coverage like a continuous film.

Table III.3 − Phytotoxicity of bicarbonates on apple seedlings under greenhouse conditions.

Treatment	Active ingredient concentration (% w/v)			
	0.5	0.75	1	2
KHCO$_3$	-	+	++	+++
NaHCO$_3$	-	+	++	+++
KHCO$_3$ + MO 0.5%	-	-	+	++
NaHCO$_3$ + MO 0.5%	-	-	+	++
Armicarb	-	-	+	++

Phytotoxicity was recorded on the 10th day after treatment on healthy leaves of 4-week-old seedlings. A qualitative assessment was conducted on the third and fourth leaves of each seedling. - = no damage, + = 0 to 2%, ++ = 2 to 5%, +++ = 5 to 20% of the leaf damaged.

III.3.5. In vitro screening

The in vitro results show that potassium and sodium bicarbonates reduced the growth development of *V. inaequalis* colony as the bicarbonate concentration increased (Table III.4). At 1000 ppm, both NaHCO$_3$ and KHCO$_3$ significantly reduced colony growth in comparison with malt agar controls, but NH$_4$HCO$_3$ did not express any fungicide effect. No colony expansion was measurable with NaHCO$_3$, KHCO$_3$ and NH$_4$HCO$_3$ at 10.000 ppm. At low concentration, potassium phosphate dibasic stimulated colony growth and at 1000 ppm some inhibition was recorded. At 10.000 ppm, potassium phosphate reduced colony growth but was less effective than bicarbonate salts. Similar pH for all bicarbonate agar solutions were measured: 5.9, 6.4, 7.2 and 7.9 at 0, 100, 1000 and 10.000 ppm, respectively. A higher pH was observed for the phosphate agar solutions: 6.4, 7.4 and 8.2 at 100, 1000 and 10.000 ppm, respectively.

Table III.4 − Mean diameters (mm) of *Venturia inaequalis* colonies 28 days after placement onto malt extract agar amended with KHCO$_3$, NaHCO$_3$ or NH$_4$HCO$_3$ applied at increasing concentrations

Treatment	100 ppm	1000 ppm	10.000 ppm
Control	16.6 b	16.7 b	16.6 b
K$_2$HPO$_4$	22.8 a	16.4 b	06.0 d
KHCO3	15.0 b	10.0 c	01.8 e
NaHCO$_3$	17.8 b	10.2 c	00.2 e
NH$_4$HCO$_3$	15.6 b	16.4 b	00.4 e
Fungicide	00.1 e	-	-

Mean of 12 replicates. Values followed by the same letters are not significantly different according to the Student-Newman-Keuls multiple range test at P<0.05.

III.4. Discussion

The results presented in this paper describe the ability of $NaHCO_3$ and $KHCO_3$ to reduce the growth (*in vitro* and *in vivo*) of *V. inaequalis*. The ability of bicarbonate salts to reduce *in vitro* fungal development and disease incidence in several disease systems had already been reported (PUNJA et al. 1982; HORST et al. 1992; ZIV and ZITTER 1992; REH and SCHLÖSSER 1995; OSNAYA-GONZALEZ et al. 1998; MLIKOTA and SMILANICK 2001; MMBAGA and SAUVE 2004). Controlling apple scab with bicarbonate salts has previously been demonstrated in an *in vitro* study using isolated cuticles (SCHULZE and SCHÖNHERR 2003) and in three field studies conducted by BEREFORDS et al. (1996), GRIMM-WETZEL and SCHÖNHERR (2005) and ILHAN et al. (2006).

The mechanisms of fungistatic or fungicidal activity of bicarbonate salts have not been conclusively established. Because K_2HPO_4 was much less effective than $NaHCO_3$ and $KHCO_3$ in our *in vitro* experiments, it seems clear that bicarbonate anions are involved directly in the reduction of *V.inaequalis* colony growth. As reported by PALMER et al. (1997), bicarbonate anion appears to be the active portion of the bicarbonate salts even if cations might have some minor effects, as demonstrated by differing sensitivities to various bicarbonate salts. The same author reported that other bicarbonates salts, such ammonium bicarbonate, were more effective than sodium or potassium bicarbonate salts in controlling the colony growth of *Botrytis cinerea*.

Bicarbonates might have several modes of action against fungi, including buffering and action to raise the pH level and the osmotic pressure of cells at the leaf surface, both factors leading to detrimental conditions for fungal spore (PALMER et al. 1997). MLIKOTA GABLER and SMILANICK (2001) demonstrated that bicarbonate solutions were less toxic when tested at pH 7.2 than at a higher pH in the case of controlling post-harvest gray mold (*Botrytis cinerea*) in grapes. Bicarbonate concentration in solution is directly related to the pH of that solution. Bicarbonates are ineffective under acidic conditions because carbonic acid predominates in solutions below ph 6.5. H_2CO_3 is unstable and decomposes into carbon dioxide and water. As the pH increases to pH 8.5, the concentration of bicarbonate increases. Above pH 8.5, bicarbonate concentration decreases and the level of carbonate rises. However, high pH on leaf and fruit surfaces was shown to be effective as a control strategy for scab (WASHINGTON et al. 1998).

Our results and those described by HORST et al. (1992) show that the effectiveness of disease control by bicarbonates can be improved when bicarbonates are used in combination with horticultural oils. This improved effectiveness was attributable to the following factors: bicarbonate ions, the fungicidal characteristics of oils, the improved leaf coverage ability and the spreader-sticker characteristics of oils that keep the bicarbonate ions on foliar surfaces. Therefore, in order to enhance the stability on leaves and hence scab control effectiveness, bicarbonates could be mixed with mineral oils and with surfactant supplies in a well-buffered alkaline solution. This raises the question of whether the greater activity observed with Armicarb was due to the active ingredient itself ($KHCO_3$) or to other components of the unspecified formulation.

The remarkable reduction of apple scab with a single foliar spray of linseed oil and grapefruit seed extract was shown in our work. Other authors have reported detrimental effects of various vegetable oils on the disease incidence of several plant foliar pathogens

(COHEN et al. 1991; NORTHOVER and SCHNEIDER 1996; STEINHAUER and BESSER 1997; OSNAYA et al. 1998). Grapefruit seed antimicrobial effects as well as grapefruit seed effectiveness against apple scab had been reported (TRAPMAN 2004) althought its relation to the preservative substances contained has been established earlier (WOEDTKE 1999). CLAYTON et al. (1943) showed that oils from cottonseed, corn, linseed, peanut and soybean were fungicidal against *Phytophtora tabacina* and that the oils from castor bean, coconut, olive and palm were non-fungicidal. They concluded that linoleic acid "occurs in large amounts in most of the fungicidal oils, but not to any extent in the no fungicidal oils". They also concluded that there were "strong indications that linolenic acid (in linseed oil) is associated with positive fungicidal activity". These conclusions contrast with NORTHOVER and SCHNEIDER (1993) who showed that against three foliar pathogens there was no difference in fungicidal activity between two groups of oils which had either a high or a low linoleic acid concentration. Corroborating evidence was obtained by COHEN et al. (1990) using water sonicates of free unsaturated fatty acid instead of oils. Against *Phytophtora infestans*, they found that linoleic and linolenic acids were fungicidal, whereas oleic acid was not fungicidal. Linseed oil was more effective than paraffinic oil when used alone in our experiment, whereas linseed oil mixed to $KHCO_3$ did not have positive additive effect, unlike parraffinic oil. This might be related to the negative effect on the pH of the linseed mixed solution.

The effectiveness of bicarbonates salts in controlling scab on apple, as reported here, together with the improvement of several disease controls using bicarbonate salts (HOMMA et al. 1981; HORST et al. 1992; ZIV and ZITTER 1992; SCHULZE and SCHÖNHERR 2003; SMILANICK et al. 2005), suggest that these simple compounds are good 'biocompatible' fungicides. The compounds are ubiquitous in nature, naturally present in human food, available to the general public for non-pesticide uses, and available for normal functions in human, animal, plant and environmental systems. The US EPA and the European Commission DG Health & Consumer Protection ruled that $NaHCO_3$ and $KHCO_3$ are exempt from residue tolerances. Bicarbonate salts have minimal environmental or worker safety issues associated with their use; they pose a minimal ingestion hazard because of their very low toxicity to animals.

Some issues need further examination, however, before the technology can be recommended for commercial adoption against apple scab. The fact that effectiveness of potassium bicarbonates fell when the time before or after inoculation increased indicates the short longevity activity of these salts when applied alone on the upper surface of the leaves.

Potassium bicarbonate acts as a contact fungicide and is not likely to be systemic or curative. Therefore, it is very important to apply such compounds with a very high foliar coverage quality. Armicarb is formulated with a surfactant system that increases its coverage ability. Consequently, the performance of Armicarb is much better than that of the pure potassium bicarbonate salt. The timing of their application is crucial since *V. inaequalis* cause deep-seated fungal infections after germination in contrast with *Podosphaera leucotricha*, the causal agent of powdery mildew. As showed in this study, a long-lasting action of potassium bicarbonate cannot be expected. Bicarbonate salts are quickly converted into an ineffective compound and are highly water soluble, and they will be washed off the leaves by a small amount of precipitation. They will therefore require frequent spray applications considering the presence of ascospores in the orchards and infection risk periods determined by modern local warning systems.

The practical relevance of this work on the use of bicarbonate salts for controlling apple scab includes the following points: (i) $NaHCO_3$ and $KHCO_3$ used at up to 0.5% were effective in controlling apple scab in greenhouse seedlings inoculated with a *Venturia inaequalis* suspension; (ii) the stability and performance of Armicarb is much better than that with straight $KHCO_3$; (iii) the addition of mineral oil to $KHCO_3$ improved its effectiveness in controlling apple scab; (iv) $NaHCO_3$, $KHCO_3$ and Armicarb could not be used at up to 0.75% without a phototoxic risk on seedling leaves under greenhouse conditions. Additional research is necessary to determine the effectiveness of $KHCO_3$ under field conditions, with an appropriate treatment formulation, timing and frequency. The results of field trials will be the subject of a future paper.

III.5. Acknowledgments

III.6. References

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Table III.5 : Main products experimented on apple seedling and corresponding efficacy for scab control under controlled environments

Products		Main active ingredient		Application	Efficacy	
Market name	**Firm**	**Type**	**%**	**Concentration of the product %**	**Pré-inoculation 24 h**	**Post-inoculation 24 h**
Copper based products						
Kocide WG	Griffin Europe	Copper hydroxide	40	0,25		
Kocide 2000	Griffin Europe	Copper hydroxide	35	0,25		
Kocide Opti	Dupont de Nemours	Copper hydroxide	30	0,25		
Cuivrol	Samabiol	Copper sulfate + oligoél.	18	0,5		
Cuprex	Hermoo Belgium nv	Copper oxychlorid	50	0,25		
Amino-cuivre	Agriclean sarl	Copper + ac. aminés (20,5%)	5	1		-
KB Bouillie Bordelaise	Scotts Belgium bvba	Copper sulfate	20	0,5		
Chelal Kubig	BMS Micro-Nutriments nv	Copper hydroxide	10	1		-
Héliocuivre	Samabiol sarl	Copper sulfate	40	0,25		-
Sulphur based products						
Thiovit Jet	Syngenta Crop Protection nv	Wettable sulphur	80	0,3		
Fertisoufre	UFAB	sulphut + Plant extract	38	0,6		
Bouillie Nantaise	Biomat sa	Calcium polysulfide	14	2		
Bouillie sulfocalcique	Polisenio	Calcium polysulfide	23	1		
Myco-San	Andermatt Biocontrol AG	Wettable sulphu + Myco-Sin	41	0,6		
Mycrosulfo	Cerexagri sa	Wettable sulphur	80	0,3		
Aminosoufre	Agriclean sarl	Wettable sulphur	40	0,6		
Héliosoufre	Samabiol sarl	Wettable sulphur	80	0,3		
Mineral substances based products						
Bicarbonate de K	Sigma-Aldrich	Bicarbonate	99,5	1		
Bicarbonate de Na	Sigma-Aldrich	Bicarbonate	99,5	1		
Bicarbonate de NH₄	Sigma-Aldrich	Bicarbonate	99	1		
Armicarb® 100	Helena Chemical Company	Bicarbonate	85	1		
KMnO₄	Sigma-Aldrich	Potassium permanganate	99,7	0,2		
Fertifeuille Lithothamne	UFAB	Calcium Hydroxide	45	2		
Supercalco 95	Carmeuse Belgium sa	Calcium Hydroxide	97	1		
Argile verte surfine	Strath sa	Green clay	100	2		
NAPF	Andermatt Biocontrol AG	Poudre de roche	100	2		
Myco-Sin	Andermatt Biocontrol AG	Clay, diatome earth, *Equisitum*	-	0,5		
Surround WP	Tessenderlo Kerley Inc.	Kaolin	95	1		
Phytoclean	Agriclean sarl	Potassium phosphonate	53	0,5		
Phosphite de K	Sigma-Aldrich nv	Potassium phosphite	99	0,5		
Acide phosphoreux	Sigma-Aldrich nv	Phosphorous acid	99	0,5		
Fructospor	Hermoo Belgium nv	Potassium phosphite	99	0,5		
DermaProx	ABT nv	Oxygen peroxyde	57	1		
Sun Spray 7E	Protex nv	Parafinic oil	8,5	1		
Calcitec	Monseu	Calcium carbonate	98	2		
Silicate de K	Sigma-Aldrich nv	Potassium silicate	30	1		
Silicate de Na	Sigma-Aldrich nv	Sodium Silicate	27	1		
Silicate de Ca	Sigma-Aldrich nv	Calcium silicate	100	0,5		
Siliforce	Agro-solutions sa	Orthosilicic acid	0,7	0,2		
Ulmasud B	Biofa Agrar Gmbh	Acid rock powder	100	1		
Organic substances based products						
Rimulgan®	Temmen gmbh	Castor oil	100	1		-
Temazad®	Temmen gmbh	Neem oil	100	1		
Micula	Temmen GMBH	Rapeseed oil	100	1		
Veomix R262002	Vandeputte Oleochemicals	Emulsified linseed oil	100	1		
Citripur	Pro-vera sprl	grapefruit seed extract	33	1		
Forever Young	Pro-vera sprl	Aloe Vera extract	-	2		
Nutrifeuil SL	Horpi-systems sa	Nettle liquid manure	-	2		
Nutrisol SL	Horpi-systems sa	Purin de prêle	-	2		
Agribioprop SC	Agriclean sarl	Arnica, *Equisit.* , propolis, fennel	-	0,3		
IRF 84 SC	Samabiol sarl	Seaweed extract (Polysacharid)	100	1		
Yucca AG Aide liquide	Desert King International	*Yucca schidigera* extract	99,5	1		
QL AGRI 35	Desert King International	*Quillaja saponaria* extract	99,7	1		
Quillaja Dry 100	Desert King International	*Quillaja saponaria* extract	100	1		
Quiwet 541 Powder	Nor-Natur	*Quillaja saponaria, Chenopodium* , .	100	1		
Norponin BS Liq	Nor-Natur	*Yucca schidigera*	99,7	1		
Quiponin BS Liq.	Nor-Natur	*Quillaja saponaria*	99,7	1		
Teawet TQ-Liquid	Nor-Natur	*Chenopodium, Camellia, Trigonella*	99,5	1		
Prev-B2	Samabiol sarl	Orange peel extract	100	1		
Salix purpurea	CRA-W	*Salix purp. water extract*	100	10		
Avena sativa	CRA-W	*Avena sativawater extract*	100	10		
Acide salicylique	Sigma-Aldrich nv	salicylic acid	99,5	0,05		
Betalgine	Cofathim	Seaweed dry extract + oligoel.	-	0,2		
Biotic agents based products						
Stimulase	Biophytec	*Trichoderma harz.* extract	-	0,3		
Bio-Fungus WP	DCM	*Trichoderma harzianum*	10⁸ ufc/g	1		
Levures Lyophilisées	Fusagx	Yeast	10⁸ ufc/g	1		
Synthetic products						
Candit	Basf Belgium sa	Kresoxim-méthyl	50	0,02		

Efficacy : [red] > 90 , [orange] from 75 to 90 % , [dark yellow] from 50 to 75 % , [yellow] from 25 to 50 % , [pale yellow] fom 0 to 25 %

Chapter IV

The during-infection spray strategy for primary scab control in organic apple production

Foreword

In this Chapter, the effectiveness of compounds containing sulphur, copper and potassium bicarbonate in controlling primary scab infections in orchards is assessed over two growing seasons. This work is based on a managed treatment application strategy called 'during infection', applied and tried out in an orchard for the first time.

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IV Chapter IV: A during-infection spray strategy using sulphur compounds, copper and a new formulation of potassium bicarbonate for primary scab control in organic apple production

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Abstract

In a field experiment conducted over two growing seasons, the effectiveness and phytotoxicity of inorganic fungicides such as sulphur, lime sulphur, copper, silicon and Armicarb (a new formulation of potassium bicarbonate) was compared with water for the control of primary apple scab infections in Belgium on high, medium and low scab-susceptible cultivars (cvs. 'Pinova', 'Pirouette' and 'Reinette des Capucins', respectively). In order to drastically reduce the amount of fungicide applied in the orchard, two approaches were used: (i) a strategy involving spraying during the infection process, before fungal penetration and (ii) a tunnel sprayer machine for treatment applications. Under field conditions that were highly favourable for disease, low rates of elemental sulphur (31.8 and 38.6 kg ha-1 year-1 in 2005 and 2006, respectively) combined with low rates of copper (2.1 kg ha-1 year-1 in both years) provided the best scab control and reduced scab severity on the fruits of cv. 'Pinova' by 97 and 98% compared with water control in 2005 and 2006, respectively. Lime sulphur was much more effective than wettable sulphur and appeared to be efficient at temperatures below 10°C, but its effectiveness against apple scab decreased if the treatments were applied 12–24 h later than in the 'during-infection' spray strategy. Armicarb used alone significantly reduced apple scab severity on the leaves and fruits of the three cultivars compared with water control. Its effectiveness was as good as wettable sulphur applied using the same timing and dosage. Silicon reduced apple scab on fruits very slightly, but not on leaves. The amounts of wettable sulphur, lime sulphur, copper, silicon and potassium bicarbonate used in this experiment to control apple scab were not phytotoxic, did not increase fruit russet, did increase the yield of each cultivar and did not affect summer density of the beneficial *Typhlodromus pyri*. The potential and limitations of 'during-infection' spraying as a protection strategy against apple scab in organic farming are discussed.

Key words: alternative control, disease management, lime sulphur, natural substances, polygenic resistance, *Typhlodromus pyri*, *Venturia inaequalis*

IV.1. Introduction

Over the past decade, public concern about pesticide residues on fruit and in the environment have generated much interest in organic apple production. One of the main components of successful organic apple production in a humid environment is the control of scab (*Venturia inaequalis* [Cooke] Winter). Only a few approved chemical compounds are available for disease control under organic guidelines, based mainly on sulphur and copper. Copper is effective against apple scab but, for environmental reasons, a new EU Council Regulation (EC No 473/2002) allows only a reduced input of copper fungicides; in some countries the use of copper fungicides is no longer allowed. Because no new options have been proposed, the remaining ones for apple scab control are the use of elemental sulphur and lime sulphur products (Holb et al., 2003). Sulphur compounds are often less effective than copper-based compounds, especially under cold weather conditions, and apple scab control needs large amounts of sulphur compounds to compensate for copper. Several studies have shown that the repeated application of large amounts of sulphur compounds has ecotoxicological and phytotoxic side-effects (Mills, 1947; Tweedy, 1981; Holb et al., 2003; Palmer et al., 2003). Therefore, organic apple growers under humid climate conditions have to contend with the results of using large amounts of sulphur compounds which could lead to leaf phytotoxicity, reduced fruit quality (Holb et al., 2003) and undesirable effects on beneficial fauna (Kreiter et al., 1998). Typically, in an organic apple orchard, 10–26 sprays are applied against apple scab in each season, depending on cultivar susceptibility, weather conditions and the amount of inoculum (Holb, 2006).

Various strategies have been proposed to reduce fungicide applications on apple. Several studies have shown that early warning systems based on disease forecasting models that give timely information of apple scab infection periods have the potential to limit the use of fungicides (MacHardy, 1996; Trapman and Polfliet, 1997; Hindorf et al., 2000). An after-infection programme can significantly reduce fungicide applications for scab control (Funt et al., 1990; Holb et al., 2003). However, organic growers have not widely adopted this new technology, including the after-infection spray approach, probably because of the lack of (i) compounds with curative properties and (ii) an accurate local warning system. Most sulphur compounds have poor curative properties; the exception is lime sulphur, which might have good curative properties against apple scab (Holb et al., 2003; Montag et al., 2005). Although the use of lime sulphur is permitted under EU regulations for organic production (EEC No 2092/91), it is currently not allowed to be used in Belgium. Therefore, the 'during-infection' spray strategy, involving spraying during the infection process, would be a promising scab control approach using compounds with poor curative properties and requiring fewer treatments than preventive control.

Another promising way to significantly reduce the use of fungicides for scab control would be to expand the cultivation of scab-resistant cultivars (Ellis et al., 1998; MacHardy et al., 2001). Monogenic resistance cannot be considered durable. New scab races, virulent to the *Vf* gene, exist in most European countries (Gessler et al., 2006). Apple breeding programmes based on polygenic resistance are therefore of greater interest now (Lateur et al., 1999; Gessler et al., 2006).

Other options for controlling apple scab may be to use natural substances as fungicides that have no known adverse affects on the environment and human health. Bicarbonates are

one of several control options now attracting attention. They are common food additives allowed in many applications under European and North American regulations and they have been used against several plant pathogens (Horst et al., 1992) and recently against apple scab (Beresford, 1996; Schulze and Schönherr, 2003; Ilhan et al., 2006; Jamar and Lateur, 2007; Jamar et al., 2007). The use of soluble silicon in horticulture, as a protective agent against several fungal pathogens, has also been reported (Belanger et al., 1995).

The objectives of this study were: (i) to evaluate the relative effectiveness of inorganic fungicides such as wettable sulphur, lime sulphur, copper, potassium bicarbonate and silicon for primary scab control, and (ii) to evaluate the effectiveness of the 'during-infection' spray strategy using reduced amounts of fungicide. Effectiveness, phytotoxicity and effects on yield, fruit quality and *T. pyri* populations were studied on high, medium and low scab-susceptible cultivars in a modern apple orchard system.

IV.2. Materials and methods

IV.2.1.   Orchard design and equipment

The study was conducted in 2005 and 2006 in a well-maintained experimental apple orchard (3.5 x 1.5 m) planted in 2002 at Gembloux, Belgium. A split-plot design based on six randomized blocks was used. Each block comprised six rows (plots) of 18 dwarf trees. The plots consisted of six trees of cv. 'Pinova', six trees of cv. 'Rubinstep-Pirouette' and six trees of cv. 'Reinette des Capucins' grafted onto M9 rootstocks. The cultivars were randomized to subplots within the plots. The cvs. 'Pinova', 'Pirouette' and 'Reinette des Capucins' were reported to be high, medium and low scab-susceptible cultivars, respectively (Jamar and Lateur, 2007).

The trees were grown according to the organic production standards (Anon., 2007). The orchard soil was heavy loam containing 1.2% C and each year it received 1000 kg ha^{-1} of organic fertilizers (5% N) and 1000 kg ha^{-1} of hydrated lime for pH enhancement. Any significant soil nutrient limitation was registered. Orchard maintenance included a centrifugal training system (Simon et al., 2006). The trees reached an average of 3 and 3.25 m in 2005 and 2006, respectively. For weed control under the tree rows, a cover-crop machine was successfully used four times a year. Grass alleys between the tree rows were kept short. Leaf analysis revealed B, Zn, Mn deficiency, so six correcting foliar treatments were applied during the growing season in both years.

Potential infection periods, based on Mills criteria, were recorded in the field using a METY computer-based weather recorder (Bodata Co. Ltd, Dortrecht, The Netherlands) connected to a RIMpro scab warning system (Trapman and Polfliet, 1997) from 15 March to harvest in both 2005 and 2006. The scab warning system calculated the infection periods based on the hourly detected meteorological data, the modified Mills table (MacHardy and Gadoury, 1989), the simulation of ascospore release and the effect of previously used sprays. Furthermore, the local climatic forecasts were daily registered in the RIMpro for infection risk extrapolations.

IV.2.2.   Treatments

The experiment was conducted on 648 trees, involving six experimental spray programmes of treatment in both years. Each treatment was applied to 108 trees (36 trees per cultivar). The treatments were randomised in plots within each of the six blocks. Each treatment combination occurred once in each block. The experimental orchard could be considered as homogeneous at the beginning of the experiment. The treatments were applied with a tunnel sprayer (Munckhof, 5961 CV Horst, The Netherlands) to prevent spray-drift and to reduce pesticide dispersal. In order to achieve various treatments in a single run, the sprayer was fitted with six individual tanks.

The six spray programmes in both years were as follows: (1) water control (Control); (2) 1.6% potassium bicarbonate (0.8% during flowering) (PB); (3) 1.6% sulphur from wettable sulphur (0.8% during flowering) + 1% calcium hydroxide (WS); (4) 1.6% sulphur from lime sulphur (0.8% during flowering) (LS); (5) 0.1% potassium silicate in 2005 (PSi) and 1.6% sulphur from lime sulphur (0.8% during flowering) under delayed spray timing in 2006 (LSd); and (6) 1.6% sulphur from wettable sulphur (0.8% during flowering) + 0.16% copper from the hydroxide form before flowering (WSCu) (Table IV.2). The same treatments were applied in the same plots in both years except for potassium silicate (PSi), which was replaced by delayed lime sulphur (LSd) application in 2006. Calcium hydroxide was added to the wettable sulphur to obtain as much calcium in the wettable sulphur treatments (WS) as in the lime sulphur treatments (LS and LSd).

All treatments were applied at a low spray rate of 300 l ha-1. The treatment timings, defined as the number of hours multiplied by the mean temperature in degrees Celsius (degree-hours) between the onset of rain (associated with infection) and the time of application, were registered for each treatment in both years (Table IV.1). In both 2005 and 2006, the treatments were applied during each potential primary infection period identified by the RIMpro scab warning system. They were applied during the infection process, after ascospore inoculation and before hypha penetration (< 300 DH). Thus, treatments were applied just before or at the beginning of the infection risk periods detected by the RIMpro scab warning system. To anticipate whether the primary infection periods were associated with the infection periods forecast by the RIMpro, the extrapolation system using the short-term weather forecasts was used. The treatments were applied on 30 May 2005 and on 9 April, 30 May and 15 June 2006, although RIMpro did not consider primary infection risk on those days; potential infections were then based on revised Mills criteria. A delayed spray programme, consisting of spraying 12–24 h after the 'during-infection' spraying, was also implemented in 2006 with lime sulphur (LSd).

Two additional treatments were applied on two secondary infection risk periods during the summer (on 11 and 18 July in 2005 and on 12 July and 14 August in 2006). All spray programmes received two post-harvest applications of 0.2% copper (from the hydroxide form) in both 2005 and 2006, mainly for European canker (*Nectria galligena*) control. Insect control was the same for all treatments and followed standard European organic guidelines (Anon., 2007).

The applied products included: wettable sulphur (Thiovit jet, 80%, Syngenta Agro, Saint Cyr l'Ecole Cedex, France), copper hydroxide (Kocide WG, 40%, Griffin Europe, Zaventem, Belgium), lime sulphur (Polisolfurio di Calcio, 23% of elemental sulphur,

Polisenio, Lugo, Italy), calcium hydroxide (Supercalco 95, 97.7%, Carmeuse, Seilles, Belgium), potassium silicate (Soluble potassium silicate, 34%, Sigma-Aldrich, Bornem, Belgium) and potassium bicarbonate (Armicarb®100, 85%, Helena Chemical Company, Collierville, TN, USA) (Table IV.2).

Table IV.1 − Degree-hours (DH) between the onsets of the rain period associated with primary infection risks and the time of spraying.

2005					DH	2006					DH
Scab infections						Scab infections					
Date	F[a]	T[b]	Mills	RIM[d] value	Early spray	Date	F	T	Mills	RIM value	Early spray	Delay. spray
27 March	C3	9.4	L[c]	93	62	30 March	C3	9.1	S	123	90	90
14 April	D	9.8	M	1018	85	09 April	D	7.8	L	0	50	194
17 April	E	5.7	S	1044	125	14 April	D	7.9	S	396	269	386
25 April	E2	9.9	S	138	220	01 May	E2	6.2	M	71	170	269
29 April	F2	11.6	S	197	180	08 May	F2	10.7	M	13	189	302
03 May	G	10.1	M	113	253	18 May	H	12.4	S	92	129	219
08 May	H	6.1	M	44	270	20 May	HI	9.7	L	160	258	376
30 May	I	8.1	M	0	290	22 May	I	10.6	S	165	242	364
						30 May	I	7.2	M	0	215	335
						15 June	J	14.7	S	0	228	372

[a] F = Tree growth stages according to the Fleckinger-growth stage scale : C3, green leaf tip; D, green bud; E, early tight cluster; E2, tight cluster; F2, full bloom; G, petal fall; H, first fruit set; I, fruit setting; J, fruit swelling.
[b] T = mean temperature between the onset of the rain associated with the infection and the time of spraying.
[c] L = low, M = moderate, S = severe infections according to the revised Mills criteria.
[d] Infection risks (day values) according to RIMpro 2005 and 2006, respectively.

The common formulation of lime sulphur products contains a mixture of calcium polysulphide and a small amount of calcium thiosulphate. The Polisolfurio di Calcio contains 23% of elemental sulphur which was considered as the active ingredient content in this paper. Armicarb®100 is a new formulation registered in the USA by the Environmental Protection Agency (EPA) and can be used in organic farming system in this country. It was chosen for this study for its effectiveness under greenhouse conditions and its adapted formulation for foliar applications (Jamar et al., 2007).

A recovery system that included a continuous recycling process in the tunnel sprayer led to an average of 30% being saved on the applied spray mixtures when spraying under moderate wind speed ($\leq$15 km h^{-1}) in a 6-years-old apple orchard. The amounts of active ingredients applied per ha and per year, including a 30% saving, are indicated in Table IV.2. The amount of elemental sulphur used annually, for the WS, LS, LSd, WSCu spray programmes, was 31.9 and 38.6 kg ha^{-1} in 2005 and 2006, respectively. For the WSCu spray programme, an additional 1.3 kg ha^{-1} of copper was applied during the growing seasons in both years. The rate of potassium bicarbonate in the PB spray programme was also 31.9 and 38.6 kg ha^{-1} in 2005 and 2006, respectively (Table IV.2).

Table IV.2 − Description of the spray programmes used in 2005 and 2006[a]

Spray programme	Trademark (Manufacturer)	Active ingredient (%)	Active ingredient application rate (%)	Total a.i. amount (kg ha^{-1} year^{-1})[b]	
				2005	2006
Control	Water control	- c	-	-	-
PB	Armicarb®100 (Helena Chemical Company, USA)	Potassium bicarbonate (85%)	1.6d (0.8)e	31.9	38.6
WS	Thiovit jet (Syngenta Agro, France)	Elemental sulphur (80%)	1.6 (0.8)	31.9	38.6
	Supercalco 95 (Carmeuse, Belgium)	Calcium hydroxyde (98%)	1.0 (1.0)	21.0	25.2
PSi	Potassium silicate solution (Sigma-Aldrich, Belgium)	Potassium silicate (34%)	0.1 (0.1)	1.3	-
LSd	Polisolfurio di Calcio (Polisenio, Italy)	Elemental sulphur (23%)	1.6 (0.8)	-	38.6
LS	Polisolfurio di Calcio (Polisenio, Italy)	Elemental sulphur (23%)	1.6 (0.8)	31.9	38.6
WSCu	Thiovit jet (Syngenta Agro, France)	Elemental sulphur (80%)	1.6 (0.8)	31.9	38.6
	Kocide WG (Griffin Europe, Belgium)	Copper (under hydroxide form) (40%)	0.16	1.3	1.3

[a] For each spray programme, ten and twelve treatments (300 l ha^{-1}) were applied during the growing seasons, from March to harvest, in 2005 and 2006, respectively, including one treatment during flowering and two treatments during summer in both years.

[b] Amount of active ingredient (a.i.) applied during the growing seasons, from March to harvest, including a 30% saving with the tunnel sprayer process. Each year, all spray programmes received two additional post-harvest applications of 0.2% copper, mainly for European canker (Nectria galligena) control.

[c] No values are applicable

[d] Application rate apart from flowering.

[e] Values in brackets are application rate during flowering; for the WSCu treatment, the Kocide WG was applied only before flowering.

IV.2.3. Scab incidence and severity assessment

Disease assessments were made on leaves and fruits in both years. For leaf severity assessments, 10 shoots per tree were recorded on 17 and 20 June in 2005 and 2006, respectively. Observations were made on 10 older leaves per shoot. A 1–9 scale was used whereby: 1 = no scab lesions; 2 = ≤ 1% infected leaves with at least one lesion; 3 = ≤ 5% infected leaves with at least one lesion; 4 = 5–50% infected leaves with at least one lesion; 5 = ≥ 50% leaves with lesions with ≤ 5% leaf area spotted; 6 = 5–25% leaf area spotted; 7 = 25–50% leaf area spotted; 8 = 50–75% leaf area spotted; and 9 = maximum infection, leaves black with scab (Lateur and Blazek, 2002).

The disease assessment on fruit was made on harvested fruits, from 15 to 31 October in both years. The percentage of diseased fruit was assessed on the whole yield collected per plot. Fruit incidence (FI) was calculated as the proportion of infected fruits with at least one scab lesion. Scab severity on fruits was assessed on the whole yield from each plot according to a scale of 1 to 6 based on a standard diagram method reported by Croxall et al.

(1952) whereby: 1 = no scab; 2 = 0–1%; 3 = 1–5%; 4 = 5–20%; 5 = 20–50%; and 6 = ≥ 50% fruit surface covered by scab. Fruit severity (FS) was defined as the mean proportion of the fruit surface covered by scab and was calculated using the following equation:

$$FS = \frac{n1 \times 0}{nt} + \frac{n2 \times 0.5}{nt} + \frac{n3 \times 2.5}{nt} + \frac{n4 \times 12.5}{nt} + \frac{n5 \times 35}{nt} + \frac{n6 \times 75}{nt}$$

where n1 to n6 represent the number of fruits in each category; nt represents the total number of fruits; and the coefficients 0, 0.5, 2.5, 12.5, 35 and 75 represent the median of the lower and upper boundaries of classes 1 to 6, respectively.

IV.2.4.   Yield, fruit quality and leaf phytotoxicity

Fruits were harvested on 20 and 21 September and 14 October in 2005 and on 21 and 25 September and 17 October in 2006 for cvs 'Pirouette', 'Reinette des Capucins' and 'Pinova', respectively. Yield was characterised by the weight of all harvested fruits and was classified into four size categories (<60; 60–70; 70–80 and >80 mm) when all fruits per plot were collected. The number of harvested fruits associated with yield was assessed for each plot. No hand thinning had been done during the growing season for any cultivars.

Fruit russet was registered for the whole yield, after harvest, from 15 to 31 October in both years. Fruit russet was assessed according to EPPO/OEPP standards based on a scale of 1 to 4 whereby: 1= no russet; 2 = < 10%; 3 = 10–30%; and 4 = 30–100% russet on the fruit surface area. The fruit russet severity index (FR) was calculated using the following equation:

$$FR = \frac{n1 \times 0}{nt} + \frac{n2 \times 5}{nt} + \frac{n3 \times 20}{nt} + \frac{n4 \times 65}{nt}$$

where n1 to n4 represent the number of fruits in each category; nt represents the total number of fruits; and the coefficients 0, 5, 20 and 65 represent the median of the lower and upper boundaries of classes 1 to 4, respectively.

The percentage and weight of the first-class fruit were determined. In our experiment, the first-class fruit was defined as fruits with a scab severity of < 1% (category 1 and 2), russet < 10% (category 1 and 2) and size > 60 mm (category 2, 3 and 4), irrespective of all other parameters.

Leaf phytotoxicity observations were made on five spur-leaf clusters per tree on 3 and 5 June in 2005 and 2006, respectively. Phytotoxicity was assessed on the whole leaf lamina following EPPO/OEPP standards. Leaf phytotoxicity assessments were rated on a 0–5 scale, as follows: 0 = no damage; 1 = leaf size 60–80% of normal size and no leaf necroses; 2 = leaf size less than 60% of normal size and with brown margins (less than 3% leaf necroses); 4 = leaf less than 60% of normal size and 3–6% leaf necroses; and 5 = bumpy small leaf and more than 6% leaf necroses, as described by Holb et al. (2003).

IV.2.5.   Effects on the predatory mite Typhlodromus pyri population

The predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), particularly useful for the biological control of phytophagous mites such as *Panonychus ulmi* Koch and *Aculus schlechtendali* (Nalepa), was imported from an IPM orchard into the experimental orchard in 2002. The introduction of *T. pyri* was achieved in August by hanging a 1-year-old branch from the IPM orchard inside each tree of the experimental orchard in order to allow a free propagation of the predatory mite. A regular distribution of the *T. pyri* population was registered in June 2003 and 2004. The residual effects of treatments, applied to control apple scab, on *T. pyri, P. ulmi* and *A. schlechtendali* densities were evaluated on cvs. 'Pinova', 'Pirouette' and 'Reinette des Capucins' in both 2005 and 2006. The treatments were the same as described earlier. Mite density assessments were made on 17 May, 10 June and 29 July in 2005 and on 15 May, 7 June and 31 July in 2006. For each assessment, 108 leaves per treatment (36 leaves per cultivar) were collected and observed in the laboratory. One leaf per tree was taken at shoulder height from a 1-year-old shoot in the external part of the canopy. Zeiss magnifying glass binoculars were used to count adult mites on the lower surface of each leaf.

IV.2.6.   Data analysis

The years were analysed separately for each variable. The percentage data were transformed in arcsine before performing an analysis of variance. No transformation was carried out for other measures. The data were analysed using SAS software version 9.1 (SAS Institute, Cary, North Carolina, USA) and the Student-Newman-Keuls multiple range test was applied to determine whether the differences between treatments were significant. All the statistical evaluations were conducted at a significance level of $P = 0.05$.

IV.3. Results

IV.3.1.   Infection periods

In 2005 and 2006 there were 8 and 10 Mills' infection periods, respectively, recorded from the end of March to mid-June in which one infection period occurred during flowering, whereas the RIMpro scab warning system identified only seven potential infection periods in both years. The primary infection periods, based on the revised Mills criteria, were severe in three and five instances, moderate in four and three instances and low in one and two instances in 2005 and 2006, respectively (Table 1). There was heavy disease pressure during the primary infection seasons, as revealed by the high scab infection rates recorded in untreated cv. 'Pinova' plots (Control) in both years (Table 3).

IV.3.2. Apple scab assessments

Apple scab symptoms present in the untreated plots in the orchard gave the following ratings for the cultivars: high, medium and low scab-susceptible for cvs 'Pinova', 'Pirouette' and 'Reinette des Capucins', respectively (Table IV.3).

Table IV.3 − Effects of low-rate treatments on apple scab on cvs. 'Pinova', 'Pirouette' and 'Reinette des Capucins' in 2005 and 2006 (June and harvested fruits assessments).

Treatmentsa	Pinova			Pirouette			Reinette des Capucins		
	LSvb	FI	FS	LSv	FI	FS	LSv	FI	FS
	(1-9)	(%)	(%)	(1-9)	(%)	(%)	(1-9)	(%)	(%)
2005									
Control	6.8 ac	84.5 a	6.2 a	3.1 a	45.6 a	1.2 a	1.7 a	0.0	0.0
PSi	6.3 a	81.3 a	4.5 b	3.0 a	46.3 a	0.8 ab	1.6 a	0.0	0.0
PB	1.9 b	55.3 b	1.8 c	1.4 b	28.1 b	0.4 b	1.2 b	0.0	0.0
WS	2.2 b	45.4 c	0.9 cd	1.5 b	24.4 bc	0.3 b	1.0 b	0.0	0.0
LS	1.8 b	27.0 d	0.4 d	1.2 b	15.1 bc	0.2 b	1.0 b	0.0	0.0
WSCu	1.1 c	18.4 e	0.2 d	1.1 b	11.0 c	0.1 b	1.0 b	0.0	0.0
F-test	***	***	***	***	***	*	*	ns	ns
2006									
Control	7.0 a	99.1 a	18.7 a	3.7 a	38.8 a	2.2 a	2.4 a	0.0	0.0
PB	4.9 b	81.3 b	7.7 b	2.5 b	21.3 b	0.7 b	1.2 b	0.0	0.0
WS	4.6 c	82.5 b	9.1 b	2.6 b	21.1 b	0.5 b	1.3 b	0.0	0.0
LSd	3.2 d	65.9 c	2.2 c	2.2 c	8.3 c	0.2 c	1.1 b	0.2	0.0
LS	2.7 e	45.0 d	1.2 c	2.0 d	4.2 d	0.0 c	1.1 b	0.0	0.0
WSCu	2.7 e	21.1 e	0.4 c	1.9 d	2.1 d	0.0 c	1.1 b	0.0	0.0
F-test	***	***	***	***	***	***	***	ns	ns

[a] Control = water treated plots, PSi = potassium silicate, PB = potassium bicarbonate, WS = wettable sulphur, LS = lime sulphur, LSd = lime sulphur in delayed spray timing, WSCu = wettable sulphur + copper hydroxide (see Table 2).
[b] LSv = leaf severity using a 1–9 scale, where 1 = no scab and 9 = maximum scab infection; June assessment shown here. FI = fruit incidence: proportion of total harvested fruits with at least one spot. FS = fruit severity: mean scabbed area or mean proportion of fruit surface covered by scab.
[c] Values within columns followed by different letters are significantly different (P≤0.05) according to the Student-Neuwman-Keuls multiple range tests. The significant F probability values, considered non-significant (ns) at P ≥ 0.05 and *, **, *** significant at P ≤ 0.05, 0.01, 0.001.

In both years, all the treatments significantly reduced apple scab compared with the untreated control for all cultivars. In both years, the combined copper and wettable sulphur treatments (WSCu) gave the best apple scab control on both leaves and fruits for all cultivars. With this treatment, the scab severity on fruits of the scab-susceptible cv 'Pinova' was reduced by 97 and 98% compared with water control in 2005 and 2006, respectively (Table IV.3). In 2005 and 2006, the lime sulphur treatments (LS) resulted in significantly lower scab damage on both the leaves and fruits of cv. 'Pinova' compared with wettable sulphur treatments (WS), while the same amount of elemental sulphur was applied. The delayed lime sulphur treatment (LSd) was less effective than the 'during-infection' lime sulphur treatment (LS), although the timing of the applications differed by no more than 0–

144 degree-hours (DH) (Table IV.1). For the medium scab-susceptible cv. 'Pirouette', lime sulphur treatments (LS) resulted in almost the same level of scab control achieved by the combined copper and sulphur treatments (WSCu). In most cases, for the scab-susceptible cv. 'Pinova', the combined sulphur and copper treatment (WSCu) gave better control than lime sulphur (LS).

In 2005 and 2006, potassium bicarbonate treatments (PB) significantly reduced apple scab severity on leaves and fruits compared with water control (Table IV.3). In most cases, potassium bicarbonate (PB) was as effective as the wettable sulphur treatment (WS), using the same amount of active ingredients for both treatments. In 2005, the potassium silicate treatments (PSi) at 0.1%, using the 'during-infection' spray strategy, did not reduce scab severity on leaves, but did reduce it very slightly on fruits.

IV.3.3. Yield, fruit quality and leaf phytotoxicity

None of the treatments adversely affected leaves in either year (no phytotoxicity, leaf size reduction or necrotic damage). All scores ranged between 0 and 0.3 on the 0–5 scale used (data not shown). None of the treatments adversely affected fruit russet compared with the untreated control in either 2005 or 2006. The average fruit russet severity index (FR) was 3.2 and 3.5% for cvs. 'Pinova' and 'Pirouette' and 5.8 and 6.2% for cv. 'Reinette des Capucins' in 2005 and 2006, respectively (data not shown). Fruit russet was slightly higher on cv. 'Reinette des Capucins'; this cultivar is genetically more prone to russet than the two other cultivars.

In most cases, compared with the untreated control, all treatments significantly increased overall yield per tree, reduced the proportion of fruits smaller than 60 mm and increased the amount of first-class fruit (Table IV.4). For cv. 'Pinova', the yield values on plots treated with WSCu were 2.6 and 4.6 times higher than in the control plots in 2005 and 2006, respectively. For cv. 'Reinette des Capucins', with a very low scab rate, the sulphur-based treatments (WS, LS, LSd, WSCu) also increased yield compared with the non-sulphur-based treatments (Control, PSi and PB), largely as a result of the effects on fruit number per tree rather than on mean fruit weight (Table 4). The lime sulphur treatment (LS) did not affect mean yields per ha compared with wettable sulphur treatments (WS and WSCu) (Table IV.4).

Table IV.4 − Effect of treatments on overall yield per tree, total fruit number per tree (FN), proportion of fruits < 60 mm (<60), and Class 1 yield (Class 1) of 4- and 5-year-old trees of cvs. 'Pinova', 'Pirouette' and 'Reinette des Capucins' in 2005 and 2006, respectively.

Treatments[a]	cv. 'Pinova'				cv. 'Pirouette'				cv. 'Reinette des Capucins'			
	Yield	FN	<60	Class 1[b]	Yield	FN	<60	Class 1	Yield	FN	<60	Class 1
	kg tree^{-1}		%	ton ha^{-1} (%)	kg tree^{-1}		%	ton ha^{-1} (%)	kg tree^{-1}		%	ton ha^{-1} (%)
2005												
Control	4.2 a[c]	31 a	12 a	1.7 (22) a	8.1	43	6.5 a	9.2 (60)	8.7 a	51 a	3	15.9 (96) a
PSi	7.3 ab	52 ab	11 a	3.5 (25) ab	8.1	42	3.0 ab	10.0 (65)	11.2 bc	64 abc	3	18.0 (95) a
PB	8.3 b	52 ab	2 b	8.0 (51) b	8.0	34	0.6 b	11.5 (76)	10.3 ab	60 ab	2	19.0 (97) a
WS	12.7 c	76 c	3 b	13.9 (59) c	10.2	53	2.0 b	15.3 (79)	13.3 c	79 c	3	24.3 (96) b
LS	11.7 c	68 bc	3 b	17.3 (78) c	9.2	42	1.0 b	15.6 (89)	12.8 c	70 bc	3	23.1 (95) b
WSCu	11.0 bc	69 bc	3 b	18.2 (87) c	9.7	43	0.5 b	17.7 (93)	11.5 bc	63 abc	5	20.8 (95) ab
F-test	*	*	**	***	ns	ns	**	ns	*	*	ns	*
2006												
Control	5.4 a	48 a	63 a	0.2 (02) a	13.8 a	90	10	17.6 (67) a	15.4 a	97 a	8	26.6 (91) a
PB	11.5 b	89 b	52 a	5.5 (25) b	16.2 ab	94	3	25.5 (83) b	15.4 a	84 a	5	27.4 (93) a
WS	12.4 b	123 bc	51 a	6.6 (28) b	17.7 abc	106	9	28.2 (84) b	19.5 ab	116 ab	8	34.1 (92) ab
LSd	19.4 c	154 cd	31 b	19.2 (52) c	19.2 bc	119	3	34.3 (94) c	23.6 bc	137 b	7	41.3 (92) bc
LS	23.1 d	185 d	31 b	28.1 (64) d	19.5 bc	116	6	35.2 (95) c	23.7 bc	148 b	5	42.3 (94) bc
WSCu	24.8 d	196 d	22 b	35.3 (75) d	21.2 c	124	3	38.7 (96) c	25.6 c	144 b	6	45.2 (93) c
F-test	***	***	**	***	**	ns	ns	***	**	**	ns	**

[a] For an explanation of treatments and dosages see Table IV.2.

[b] Class 1 includes fruits with scab severity < 1%, size > 60 mm and russet < 10%. The calculation base for yield (ton ha^{-1}) was 1900 trees per ha. Values in brackets are the proportion of Class 1 compared with total yield.

[c] Values within columns with different letters differ significantly according to the Student-Neuwman-Keuls multiple range tests (P≤0.05). F-test = ns (non-significant), *, **, *** significant at P ≤ 0.05, 0.01, 0.001.

IV.3.4. Effects on the predatory mite *Typhlodromus pyri* population

In both 2005 and 2006, winter observations of 2- and 3-year-old branches did not show any eggs of *P. ulmi*. Assessments of the leaves in the spring and summer did not reveal the presence of any *P. ulmi* populations under any treatments. July assessment of *A. schlechtendali* showed that all the sulphur treatments significantly reduced its density to 0.1 individuals per leaf compared with an average of 2 individuals per leaf from untreated plots in both years (data not shown). All the treatments slightly reduced the predatory *T. pyri* density level in June 2005 and in May and June 2006, but not in May and July 2005 or in July 2006 compared with the untreated control (Table IV.5). There was no significant difference in the *T. pyri* density level among the lime sulphur (LS, LSd), wettable sulphur (WS) and wettable sulphur combined with copper (WSCu) treatments in either 2005 or 2006.

Table IV.5 – Effects of treatments on adult *Typhlodromus pyri* density in May, June and July 2005 and 2006

Treatments[a]	Number of *T. pyri* per 100 leaves[c]					
	2005			2006		
	May	June	July	May	June	July
Control	44	83 a[d]	70	51 a	56 a	76
PB	48	69 b	64	37 b	38 b	66
PSi or LSd[b]	42	70 b	66	32 b	31 b	71
WS	40	43 cd	55	33 b	33 b	64
LS	47	55 c	63	28 b	22 b	69
WSCu	46	39 d	59	31 b	30 b	61
F-test	ns	***	ns	*	**	ns

[a] For an explanation of treatments and dosages see Table IV.2.

[b] PSi in 2005 and LSd in 2006.

[c] Values are the means of six replicates, each replicate including 18 leaves (six leaves per cultivar).

[d] Values within columns with different letters differ significantly according the Student-Neuwman-Keuls multiple range tests (P≤0.05). F-test = ns (non-significant), *, **, *** significant at P ≤ 0.05, 0.01, 0.001.

IV.4. Discussion

The results of this study clearly demonstrate that the 'during-infection' spray strategy, was effective in controlling apple scab with a reduced amount of fungicides such as sulphur, lime sulphur, copper and potassium bicarbonate. Our results show that it was possible to produce 75% of marketable fruit (35.3 ton ha^{-1} of Class 1) from the scab-susceptible cv. 'Pinova' from a 5-year-old orchard with no more than 38.6 kg of elemental sulphur and 2.1 kg of copper (from the hydroxide) per ha per year, whereas no more than 2% of marketable fruits (0.2 ton ha^{-1} of Class 1) were obtained from untreated plots (Table 4). These amounts of fungicides are about 70% below the amounts usually used to control apple scab in organic production under humid climate conditions (Ellis et al., 1994; Holb et al., 2003; Palmer et

al., 2003). Up to 110 kg ha^{-1} year^{-1} of elemental sulphur combined with 8 kg ha^{-1} year^{-1} of copper were used for scab control in organic apple production in The Netherlands (Holb and Heijne, 2001).

Fungicides were applied shortly after rainfall associated with primary scab infections. Treatments were applied during the infection process, sometimes on drying leaves. Ascospores had been discharged, susceptible tissue was present and, in most cases, the minimum temperature and leaf-wetness conditions for infection accorded with the revised Mills criteria, but penetration of the cuticle had not yet occurred (Table 1). Below 250 DH after rainfall, few ascopores reach the stadium of penetration, and a relevant portion will reach this stage only after 300 DH (Smereka et al., 1987; MacHardy, 1996). Sprays below 300 DH can be considered to be applied during the infection process (before infection), on germinating spores possibly already with appressoria, however not yet with the formation of the primary stroma which allows the fungus to be protected by the plant cuticle.

The poorer result registered with the delayed spray programme (LSd) showed that the timing of treatment application must be close to the onset of infection, even with lime sulphur.

The 'during-infection' spray strategy has several important advantages compared with the preventive (before rainfall) spray strategy; these include (i) less washing effect, (ii) greater treatment effectiveness, (iii) avoidance of unnecessary treatments (Funt et al., 1990; Holb et al., 2003) and (iv) in hot seasons, applying treatments during less sunny periods. However, there are potential problems with this strategy, such as spraying in windy weather being unsuitable for 'low-volume' spraying, delaying a spray during an extended rainy period and spray-timing within few hours after the onset of the rain.

The low rate of copper (0.16%) added to the wettable sulphur treatments before flowering gave far better scab control than sulphur alone. This suggests that dilute copper could be very effective under cold weather conditions in early primary scab infection periods. In both 2005 and 2006, the lime sulphur treatment was slightly less effective than the combined wettable sulphur and copper treatment. However, lime sulphur was more effective than wettable sulphur used alone, confirming the results of previous studies (Mills, 1947; Ellis et al., 1994; Holb et al., 2003).

The efficiency of bicarbonate salts in controlling apple scab, as reported here and in previous studies (Schulze and Schönherr, 2003; Ilhan et al., 2006; Jamar and Lateur, 2007; Jamar et al., 2007), together with the improvement of other disease-control treatments using bicarbonates salts (Horst et al., 1992), suggest that this compound could be introduced in apple disease management. The fact that the compounds are ubiquitous in nature, naturally present in human food, available to the general public for non-pesticide uses and required for normal functions in human, animal, plant and environmental systems imply that this simple compound is appropriate for organic production systems. However, potassium bicarbonate acts as a contact fungicide and is not likely to be systemic or curative. Greenhouse experiments have shown that 1% Armicarb is 95% effective in controlling apple scab, but a long-lasting effect cannot be expected (Jamar et al., 2007). Bicarbonates are quickly converted into ineffective compounds and are highly water-soluble, and they will be washed off the leaves by a small amount of precipitation. They therefore require frequent spray applications well-targeted in the infection risk periods. So far, no data are available on the effectiveness of potassium bicarbonate under low temperature conditions. Activity below 10°C is a prerequisite if copper is to be replaced. Our results indicated that applications of

Armicarb alone during the growing season were not effective enough against scab and suggested that it must be supplemented. In our experimental conditions, silicon had a very poor effect on apple scab. However, Belanger et al. (1995) reported that there is cumulative evidence that increased silicon absorption offers protection against various fungal diseases.

The impact of the disease was stronger in 2006 than in 2005 because (i) weather conditions were more favourable for scab infections in 2006 and (ii) several untreated and poorly treated plots in 2005 led to heavy disease pressure during the 2006 primary infection season. Since several sanitation practices were reported to reduce the potential ascospore dose (Holb, 2006), autumn leaf-shredding and early spring leaf-burying were carried out between the two growing seasons in order to limit the influence of the previous year. In addition, the risk of early scab epidemics initiated by over-wintered conidia is high in organic orchards (Holb et al., 2005a) and this could explain the relatively lower effectiveness of wettable sulphur and potassium bicarbonate used alone in the primary 2006 scab season compared with 2005. That means that the results registered in 2006 were probably influenced by treatments applied in the previous year. Some of the scab damage observed on harvested fruits possibly arose from secondary scab infection, as only two summer sprays were applied, especially in plots where primary scab control was partial (MacHardy, 1996; Holb et al., 2005b).

Although some authors have reported poorer leaf appearance with sulphur and copper treatments (Palmer et al., 2003; Holb et al., 2003), the amount of active substances used in our study to control apple scab did not induce any phytotoxic effects, plant damage or yield fall. However, in our experiment the use of copper was avoided during and after flowering, the treatments were applied with a tunnel sprayer at a low rate of about 300 l ha^{-1} and the treatment frequencies and fungicide doses were limited, particularly during flowering.

On the low scab-susceptible cultivar cv. 'Reinette des Capucins', sprays based on sulphur caused a significant increase in yield per tree (Table 4). Such positive effects of sulphur compounds on yields cannot be explained by the control of apple scab or other apple diseases such as powdery mildew (*Podosphaera leucotricha*), because the infection levels on untreated plots were very low, including at a later stage, in both years. These results contrast with earlier studies showing that sulphur applications reduced yield and fruit numbers (Mills, 1947; Holb et al., 2003; Palmer et al., 2003). The application of elemental sulphur to crops is increasingly advocated as a way of overcoming deficiency in this key nutrient, and sulphur deficiency has recently become a widespread nutrient disorder in crops, largely due to restrictions on fossil fuel burning (Schnug, 1998; Williams and Cooper, 2004). A chemical analysis of leaves from cv. 'Reinette des Capucins' collected on 25 June 2006, previously washed with acid solutions, showed that the leaf dry extracts from the WS, LS, LSd and WSCu treatments contained 0.38% of sulphur while the leaf dry extracts from the PB and Control treatments contained 0.30% of sulphur (*P<0.001*).

The absence of the phytophagous mite *P. ulmi* and the very low density of *A. schlechtendali* during the two growing seasons might be associated with the very high density of the predator *T. pyri* observed throughout the orchard in both years. The slight and temporary reduction of *T. pyri* on treated plots in June might be correlated with periods with higher treatment frequencies. The treatments might have had harmful effects on *T. pyri,* but the reduction of *T. pyri* during these periods of treatments might also be due to the decrease of prey availability. Sulphur compounds can have harmful effects on phytoseiids (Kreiter et al., 1998). However, other studies have reported predator mite population tolerance of treatments with sulphur compounds, probably due to the development of tolerant strains

with an acquired resistance to sulphur (Markoyiannaki-Printzioui et al., 2000). An earlier study (Beresford et al., 1996) showed that bicarbonate salts did not reduce predator mite numbers or disrupt biological mite control.

In this study it was clearly shown that (i) the 'during-infection' spray strategy using reduced amounts of either lime sulphur or wettable sulphur combined with copper was very effective against primary scab infections; (ii) copper and lime sulphur were efficient when temperatures were below 10°C; (iii) lime sulphur was more effective than wettable sulphur; (iv) lime sulphur effectiveness against apple scab decreased if the treatments were applied 12–24h later than in the 'during-infection' spray strategy; (v) potassium bicarbonate was effective against apple scab and as effective as wettable sulphur; (vi) the sulphur-based treatments increased yield even with a low scab-susceptible cultivar; (vii) inorganic fungicide doses and frequencies used to reduce apple scab severity on fruits by about 98% were not phytotoxic, did not adversely affect yield and did not affect summer *T. pyri* density; and (viii) the amount of copper for scab control could be reduced on medium and low scab-susceptible cultivars compared with high scab-susceptible cultivars.

As lime sulphur is not allowed in Belgium, copper is still needed for apple scab control under apple organic production in the country. Currently, lime sulphur appears to be the sole remaining option for replacing copper when temperatures are below 10°C in organic farming, and therefore scab management in Belgium would be compromised if there were new European or national regulations restricting the use of copper. The present study has demonstrated the potential of controlling apple scab with reduced and non-damaging amounts of inorganic fungicides using accurate timing of treatments and a spray machine.

IV.5. Acknowledgements

IV.6. References

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Chapter V

Apple scab control in organic production – impact on fruit quality and yield over six growing seasons

<u>Foreword</u>

Chapter 5 analyses the long-term impact of the organic apple growing method and the application of ten scab treatment schemes on crop quality and quantity, in relation to eight apple cultivars. This study covers a period of six years, from the second to the seventh year of production at the orchard (i.e., from 2003 to 2008). In particular, it shows the effects of plant protection schemes on various production parameters in relation to the degree and type of cultivar resistance.

This chapter has been published as a slightly modified version in:

Running title: A low-input strategy for scab control in organic apple production

V Chapter V : Primary scab control using a 'during-infection' spray timing and the effect on fruit quality and yield in Organic Apple Production

A low input strategy for scab control in organic apple production

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 Abstract

Organic apple production in Europe depends to a great extent on the use of copper fungicides for scab control (Venturia inaequalis). The objective of this 6-year study (2003−2008) conducted in Belgium was to determine measures for reducing the use of copper fungicides in organic apple production. The effectiveness of a 'during-infection' spray strategy using wettable sulphur (with or without copper), lime sulphur, potassium bicarbonate, silicon and five natural plant extracts (orange peel, soapbark, tea seed, quinoa seed and grapefruit seed) for controlling primary scab was investigated in a split-plot field experiment. Four apple cultivars that express a gradient of partial scab resistance were included: a high scab-susceptible cultivar (cv. 'Pinova'), a medium scab-susceptible cultivar (cvs. 'Pirouette') and two old cultivars expressing low to very low scab susceptibility (cvs. 'Reinette Hernaut' and 'Reinette Capucins'). Apart from these cultivars, four *Vf* scab-resistant cultivars (cvs. 'Initial', 'Topaz', 'Zvatava' and 'JN 20/33/58') were also included in the experimental orchard. In order to reduce the amount of fungicide required, two strategies were used: (i) a specific spray timing involving spraying during the infection processes, before fungal penetration, determined by the RIMpro software warning system, and (ii) a tunnel sprayer machine for optimal treatment applications. Depending on the year, a total of 8−12 applications were made annually. Under field conditions that were highly conducive disease, low rates of elemental sulphur (≤ 40 kg ha^{-1} year^{-1}) combined with low rates of copper (≤ 2.1 kg ha^{-1} year^{-1}) provided the best scab control and reduced scab severity on the leaves and fruits by 85−100%, depending on the year and cultivar, compared with the untreated control. In most cases, the lime sulphur spray treatment, which used more elemental sulphur but did not use copper, provided a similar level of scab control to the combined wettable sulphur and copper spray treatment. Sulphur, potassium bicarbonate and all plant extracts significantly reduced scab severity on leaves and fruits. In general, the treatments increased the yield of the high and medium scab-susceptible cultivars as well as that of the low and very low scab-susceptible cultivars. Under these experimental conditions, none of the treatments caused phytotoxicity, increased fruit russet or led to undesirable soil and fruit residues at harvest. The potential and limitations of 'during-infection' spraying as a protection strategy against apple scab in organic farming are discussed.

 Keywords: copper, disease management, lime sulphur, natural plant extract, potassium bicarbonate, scab resistance, *Venturia inaequalis*

V.1. 1. Introduction

Apple scab caused by *Venturia inaequalis* [Cooke] Winter is a major disease in apple production in the world's temperate zones (MacHardy, 1996). Most commercial apple cultivars are very susceptible to scab, and in commercial apple orchards very frequent fungicide applications (15−22 annually) are needed to control apple scab, depending on weather conditions, disease pressure and cultivar susceptibility (Holb et al., 2005b). Environmental considerations are becoming increasingly important and interest has therefore turned from conventional or integrated production to organic apple production, where management practices differ from those in conventional and integrated production. In addition, consumers increasingly demand apples free of any synthetic chemical residues. In organic apple growing contexts, only a few approved chemical compounds are available for disease control, based mainly on sulphur and copper (Holb, 2008). Prolonged use of copper-based compounds could result in copper levels exceeding the international official limit of 36 mg kg^{-1} soil in European orchards (Holb, 2008); these levels have been shown to induce a negative impact on soil ecology and earthworm populations (van Rhee, 1976, Paoletti et al., 1998). Consequently, some European countries, including The Netherlands and Scandinavian countries, have banned copper-based products and others have restricted copper use (EC, 2008).

The known remaining chemical option for apple scab control in organic farming is the use of elemental sulphur and lime sulphur products (Holb et al., 2003). Sulphur compounds are often less effective than copper-based compounds, especially in cold weather, and apple scab control needs large amounts of sulphur compounds to compensate for copper. Several studies have shown that the repeated application of large amounts of sulphur compounds has ecotoxicological and phytotoxic side-effects (Mills, 1947; Kreiter et al., 1998; Holb et al., 2003; Palmer et al., 2003).

Organic growers often adopt a preventive control strategy that requires more treatments and non useful treatments. Early warning systems based on disease forecasting models that give timely information about apple scab infection periods have the potential to limit the use of fungicides (MacHardy, 1996; Trapman and Polfliet, 1997; Hindorf et al., 2000, Jamar et al., 2008b). Mills (1944) reported that elemental sulphur is fully effective as a rain application only up to the time when infection occurs. In a 2-year study, a 'during-infection' spray strategy involving spraying during the infection process was developed to reduce the amount of fungicide used for scab control in an organic apple orchard, using compounds with poor curative properties (Jamar et al., 2008b; Jamar et al., 2010b).

An after-infection programme can significantly reduce fungicide applications for scab control (Funt et al., 1990; Holb et al., 2003). However, this technology, including the after-infection spray approach, has not been widely adopted by organic growers, probably because of the lack of (i) compounds with curative properties and (ii) an accurate local warning system, including weather forecast management. Most sulphur compounds have poor curative properties; the exception is lime sulphur, which might have good curative properties against apple scab (Mills, 1944; Holb et al., 2003; Montag et al., 2005). Although

the use of lime sulphur is permitted under EU regulations for organic production (EC, 2008), it is currently not allowed in Belgium.

As an alternative to using copper compounds, several natural substances have the potential to be used as fungicides that have a low eco-toxicologic profile (no known adverse effects on the environment or human health) and are acceptable and economically feasible in organic farming. Bicarbonates are one of several control options now attracting attention. They have been used against several plant pathogens (Horst et al., 1992; Beresford, 1996; Schulze and Schönherr, 2003; Ilhan et al., 2006; Jamar and Lateur, 2007; Jamar et al., 2007). Various natural plant extracts containing triterpenoid saponins, polyphenols and specific flavonoids have been reported to possess antifungal properties (Bahraminejad et al., 2008; Köhl et al., 2007; Bengstsson et al., 2009). Natural substances have also been reported as elicitors of resistance (Lateur, 2002) and specific mineral substances have been reported to be protective agents against several fungal pathogens in horticulture, e.g., silicon-based products (Belanger et al., 1995, Köhl et al., 2007).

Expanding the cultivation of low scab-susceptible or scab-resistant cultivars carrying the *Vf* gene would be another way of significantly reducing the use of fungicides for scab control (Ellis et al., 1998; MacHardy et al., 2001). However, new scab races, virulent to the *Vf* gene, have appeared in most European countries (Gessler et al., 2006), and these monogenic resistance cultivars therefore need to be carefully integrated into anti-breakdown strategies. Apple cultivars with polygenic resistance, deficient in most commercial cultivars, and new cultivars that combine monogenic and polygenic resistances are therefore of increasing interest for breeders and growers (Lateur et al., 1999; Gessler et al., 2006).

Although several field studies have evaluated the scab susceptibility of apple cultivars under unsprayed orchard conditions (Lateur et al., 1999), little information is available on the long-term reaction of apple cultivars to scab under a clearly defined spraying strategy in organic orchards involving various fungicide treatments and their impact on fruit yield and quality.

The aims of this 6-year study were to contribute to the reduction of copper in organic apple production by initially evaluating the relative effectiveness of five inorganic fungicides (sulphur, lime sulphur, potassium bicarbonates, silicon and copper) and five natural plant extracts (orange peel, soapbark, tea seed, quinoa seed and grapefruit seed) in primary scab control, and then evaluating over the long term the effectiveness of the 'during-infection' spray strategy using reduced amounts of fungicides. Effectiveness against scab, phytotoxicity and effects on yield and fruit quality were studied for high, medium and low scab-susceptible cultivars in a modern apple orchard system.

V.2. Materials and methods

V.2.1. Orchard design and equipment

The study was conducted over a period of 6 years, from 2003 to 2008, in two experimental apple orchards planted in 2002 at Gembloux, Belgium. The first orchard was

composed of one high scab-susceptible cultivar (cv. 'Pinova'), one medium scab-susceptible cultivar (cvs. 'Pirouette'), one low scab-susceptible cultivar ('Reinette Hernaut') and one very low scab-susceptible cultivar ('Reinette Capucins') (Jamar et al., 2008b). The second orchard was composed of four *Vf* scab-resistant cultivars (cvs. 'Initial', 'Topaz', 'Zvatava' and 'JN 20/33/58'). The trees were grafted on dwarfing rootstocks (interstem of cv. 'Golden Delicious') and planted in a single row system (3.5 x 1.5 m). A split-plot design based on six randomized blocks with six replicates was used in each orchard. Each block comprised six rows (plots) of 24 dwarf trees. The plots consisted of 24 trees of four cultivars. The cultivars were randomized to subplots within the plots in 4 mono-cultivar groups of 6 trees. Tree density was 1900 trees ha^{-1} in blocks, however, taking into account the presence of 20 % of ecological zones situated between blocks, the tree density in the whole experimental orchard correspond with 1500 trees ha^{-1}.

The trees were grown according to the organic production standards (EC, 2008). The orchard soil was a heavy loam containing 1.2% C and it received cattle compost twice and then in most years about 1000 kg ha^{-1} of organic fertilizers (5% N) and 2000 kg ha^{-1} of hydrated lime for pH enhancement (Table V.1). Any significant soil nutrient limitation was registered. Tree maintenance training included a centrifugal training system (Simon et al., 2006). The trees reached an average height of 3.25 m in 2005. For weed control under the tree rows, a mechanic cover-crop machine was used successfully four times a year. The grass in the alleys between the tree rows was kept short by mowing regularly. Leaf analysis revealed B, Zn and Mn deficiency. Therefore four correcting foliar treatments were applied during the growing season from 2004 to 2008.

From 15 March to harvest time in each year, potential infection periods, based on the Mills criteria, were recorded in the field using a METY computer-based weather recorder (Bodata Co. Ltd, Dortrecht, The Netherlands) connected to a RIMpro scab warning system (Trapman and Polfliet, 1997) from 15 March to harvest time each year. The scab warning system calculated the infection periods based on hourly recorded meteorological data, the modified Mills table (MacHardy and Gadoury, 1989), the simulation of ascospore release and the effect of previously used sprays. In addition, the local climate forecasts were registered every 6 hours using the RIMpro software for infection risk extrapolations.

Table V.1. − Organic fertilizers and amendments applied in the experimental orchard

	2002	2003	2004	2005	2006	2007	2008	Mean
Compost 0.5% N (t/ha)	30.0	-	-	25.0	-	-	-	7.9
Lin-waste 5/2/2 (t/ha)	0.5	1.0	1.0		1.0	0.8	1.0	0.7
Patentkali (t/ha)	2.0	-	0.3	-	-	-	-	0.3
Natural phosphate 50% (t/ha)	1.0	-	0.3	-	-	-	-	0.2
Hydrated lime 50% (t/ha)	2.0	1.0	2.0	2.0	2.0	2.0	2.0	1.8
Nitrogen unit (u.N/ha) [a]	57.5	67.5	72.5	62.5	50.0	63.8	45.0	59.8
Ca [b]	-	-	4.0	4.0	4.0	4.0	4.0	2.8
B, Mn, Zn [b]	-	-	4.0	4.0	4.0	4.0	4.0	2.8

[a] Estimation of nitrogen availability for the compost used = 30% year 1, 20% year 2, 15% year 3 and for the lin-waste used = 50% year 1 and 50% year 2
[b] Number of foliar treatment

V.2.2. Treatments

Each year, the experiment was conducted on 1440 trees and involved 10 experimental spray treatments each year. Each treatment was applied to 144 trees (36 trees per cultivar). The treatments were randomised in plots within each of the 12 blocks. Each treatment combination was applied once in each block. The experimental orchard could be considered as homogeneous at the beginning of the experiment. To prevent spray-drift and to reduce pesticide dispersal, the treatments were applied using a tunnel sprayer (Munckhof, 5961 CV Horst, The Netherlands). In order to complete the various treatments in a single run, the sprayer was fitted with six individual tanks.

In the first orchard, the six spray treatments were: (1) untreated control (UC1); (2) potassium bicarbonate-based compounds (AR1); (3) depending on the year, sulphur from wettable sulphur, silicon, delayed lime sulphur, soapbark, tea seed or quinoa seed (SF1); (4) depending on the year, products used in Integrated Fruit Production (IFP), sulphur, grapefruit seed or copper combined with orange peel (RE1); (5) sulphur from lime sulphur (LS1) and (6) sulphur from wettable sulphur combined with copper from the hydroxide form (CS1) (Table 2). Every year, except for SF1 and RE1, the same set of treatments was applied in the same plots (Table V.3). In the second orchard, there were four spray treatments: (1) untreated control (UC2); (2) sulphur from wettable sulphur, (SF2); (3) depending on the year, products used in IFP, a combination of copper and wettable sulphur (RE2); and (4) other combinations of copper and wettable sulphur (CS2) (Table V.3).

All treatments were applied at a low spray rate of 300 l ha^{-1}. In each year, the treatments were applied during the potential primary infection period identified by the RIMpro scab warning system. The treatments were applied during the infection process, after ascospore inoculation and before hypha penetration (Figure V.1). In practice, the treatment timings, defined as the number of hours multiplied by the mean temperature in degrees Celsius (degree-hours, DH) between the onset of rain (associated with infection) and the time of application, were always between 50 and 300 DH. Therefore, the treatments were applied just before or at the beginning of the infection risk periods detected by the RIMpro scab warning system. To anticipate infection periods, the extrapolation system of RIMpro, using the short-term weather forecasts, was used. In some cases, the treatments were applied even when RIMpro did not indicate a primary infection risk on those days; potential infections were then based on revised Mills criteria. A delayed spray treatment, consisting of spraying 12–24 h after the 'during-infection' spraying, was also implemented in 2006 with lime sulphur (lms delayed).

Between 2004 and 2008, two additional treatments were applied in two secondary infection risk periods in the summer. All the treatments included two post-harvest applications of 0.2% copper (from the hydroxide form) from 2002 to 2006, mainly for European canker (Nectria galligena) control. Limited insect control was uniformly applied for all treatments and followed standard European organic guidelines (EC, 2008).

The applied products included: wettable sulphur (Thiovit jet, 80%, Syngenta Agro, Saint Cyr l'Ecole Cedex, France), copper hydroxide (Kocide WG, 40%, Griffin Europe, Zaventem, Belgium), lime sulphur (Polisolfurio di Calcio, 23% of elemental sulphur,

Polisenio, Lugo, Italy), potassium silicate (soluble potassium silicate, 34%, Sigma-Aldrich, Bornem, Belgium), clay (Myco-Sin, 100%, Andermatt Biocontrol, Grossdietwil, Switzerland), orange peel extract (Prev-B2, 100%, Vivagro, Cestas, France), soapbark tree (*Quillaja saponaria*) extract (QL AGRI^{TM} 35, 99.8%, Desert King International, Manya Circle, USA), tea (*Camellia oleifera*) and quinoa (*Chenopodium quinoa*) seed extracts (Teawet^{TM} TQ Liquid, 99.8%, Nor-Natur, Hvidovre, Denmark), grapefruit seed extract (Citripur, 33%, Pro-vera, Braine l'Alleud, Belgium), potassium bicarbonate (potassium bicarbonate, 99.5%, Sigma-Aldrich, Bornem, Belgium) and potassium bicarbonate (Armicarb$^®$100, 85%, Helena Chemical Company, Collierville, TN, USA) (Table 2).

The Polisolfurio di Calcio contained 23% of elemental sulphur, which was considered as the active ingredient content in this study. Armicarb$^®$100 is a new formulation registered in the USA by the Environmental Protection Agency (EPA) and can be used in organic farming systems in Belgium. Armicarb, Citripur, Prev-B2, Teawet TQ Liquid and QL AGRI 35 were chosen for this study for their effectiveness under greenhouse conditions and their adapted formulation for foliar applications (Jamar, 2007; Jamar et al., 2007).

A recovery system that included a continuous recycling process in the tunnel sprayer led to saving an average of 30% of the applied spray mixtures when spraying under moderate wind speed (≤15 km h^{-1}) in a 6-year-old apple orchard (Jamar et al., 2010c). The amount of active ingredients applied per ha and per year therefore need also to include a 30% product saving.
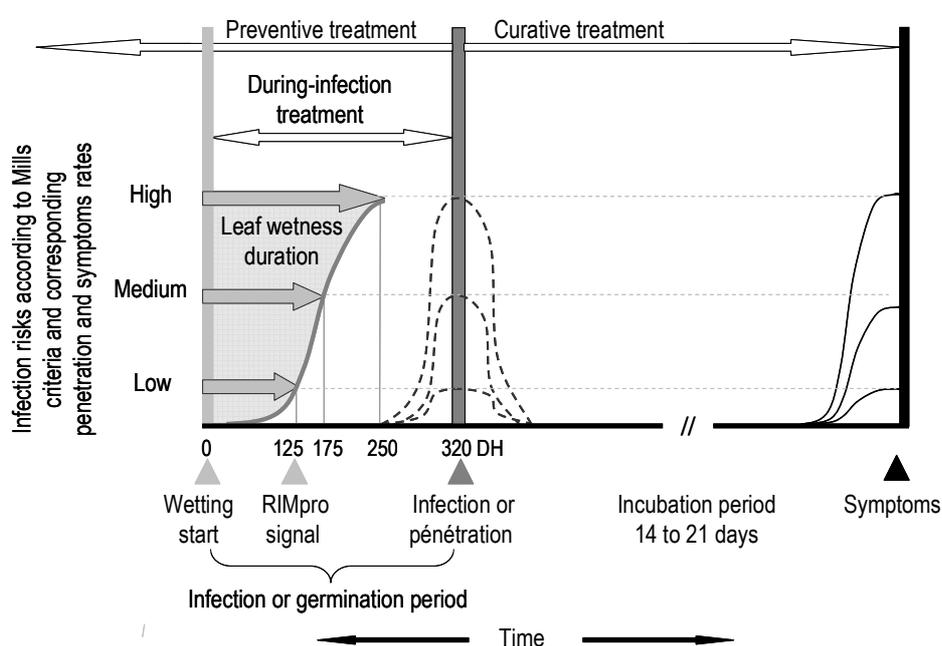

Figure V.1. Schematic illustration of the 'During-infection' spray timing (treatment applied 0–320 degree-hours [DH] after the wetting start) in relation to leaf wetness duration, infection risks according to Mills criteria, fungus (*Venturia inaequalis*) activity and the approximate RIMpro infection starting-point

Table V.2 −Treatments and active ingredient application rate

Code	Trademark (Manufacturer)	Active ingredient a.i. (%)	a.i. application rate (%) [a] [b]
arm	Armicarb®100 (Helena Chem. Co., Us)	Potassium bicarbonate (85%)	1.6 (0.8)
cit	Citripur (Pro-vera, Be)	Grapefruit seed extract (33%)	0.15 (0.15)
cop	Kocide WG (Griffin Europe, Be)	Copper (hydroxyde) (40%)	0.16 (0.04)
lms	Polisolfurio di Calcio (Polisenio, It)	Elemental sulphur (23%)	1.6 (0.8)
myc	Myco-Sin (Andermatt Biocontrol, Ch)	Clay, *Equisetum arvense* (100%)	1.0 (1.0)
pbi	Potassium bicarbonate (Sigma-Al., Be)	Potassium bicarbonate (99%)	1.6 (0.8)
prv	Prev-B2 (Vivagro, Fr)	Orange peel extract (100%)	0.5 (0.5)
qui	QL AGRI 35 (Desert King Int., US)	Soapbark tree, *Q. saponaria* (100%)	1.0 (1.0)
sil	Potassium silicate (Sigma-Aldrich, Be)	Potassium silicate (34%)	0.1 (0.1)
sul	Thiovit jet (Syngenta Agro, Fr.)	Elemental sulphur (80%)	1.6 (0.8)
tea	Teawet-TQ-Liquid (Nor-Natur, Dk)	Tea & quinoa seed extract (100%)	1.0 (1.0)

[a] Application rate apart from flowering and values in brackets are application rate during flowering
[b] All treatments were applied at a low spray rate of 300 l ha^{-1}

Table V.3 − Spray treatments applied from 2003 to 2008 in the two experimental orchards

	2003	2004	2005	2006	2007	2008
Orchard 1 (cvs. 'Pinova', 'Pirouette', 'R. Hernaut' and 'R. Capucins')						
UC1 (Untreated Control)	-	-	-	-	-	cop
AR1	pbi−myc [a]	pbi	arm	arm	arm	cop−arm
SF1	sul	sil	sil	lms delayed	qui	cop−tea
RE1	ipm	ipm	sul	sul	cit	cop−prv
LS1	lms−sul	lms−sul	lms	lms	lms	lms
CS1	cop−sul	cop−sul	cop−sul	cop+−sul	cop+−sul	cop+−sul
Number of treatment [b]	3 + 3 + 6	3 + 2 + 5	4 + 1 + 3	4 + 1 + 5	0 + 0 + 8	3 + 1 + 5
Orchard 2 (cvs 'Initial', 'Topaz', 'Zvatava' and 'JN 20/33/58')						
UC2 (Untreated Control)	-	-	-	-	-	-
SF2	sul	sul	sul	sul	sul	sul
RE2	ipm	ipm	cop+−sul	cop+−sul	cop++−sul	cop++−sul
CS2	cop−sul	cop−sul	cop−sul	cop−sul	cop+−sul	cop+−sul
Number of treatment	3 + 3 + 5	3 + 2 + 4	4 + 1 + 3	4 + 1 + 5	0 + 0 + 8	3 + 1 + 5

[a] For treatment explanations, see Table V.2. The 'ipm' (Integrated Production Management) includes dodine, captane, kresoxym méthyl, dithianon, pyriméthanil and difénoconazole at recommended doses. 'Cop' includes treatments only before flowering; 'cop+' includes additional 0.04% copper treatments from flowering; and 'cop++' includes additional treatments at 0.04% copper from flowering to fruit-tipping-over stage and at 0.08% copper from fruit-tipping-over stage. 'Prv' and 'tea' include treatments only after flowering, and all others include treatments before and after flowering. In 'cop−sul', 'cop+−sul' and 'cop++−sul' treatments, 'sul' was associated with 'cop' if the temperature rose above 10°C
[b] Number of treatments applied during the primary scab infection seasons, from March to June (first, second and third values = before, during and after flowering, respectively). From 2004 to 2008, all spray treatments included two additional summer applications of 2% sulphur (for scab control) and from 2002 to 2006 two additional post-harvest applications of 0.2% copper (for European canker 'Nectria galligena' control)

V.2.3. Scab incidence and severity assessment

Each year, disease assessments on the leaves and fruits were made. For leaf severity assessments, 10 shoots per tree were recorded about 60 days after flowering. Observations were made on 10 older leaves per shoot. A 1–9 global scab intensity scale was used whereby: 1 = no scab lesions; 2 = ≤ 1% infected leaves with at least one lesion; 3 = ≤ 5% infected leaves with at least one lesion; 4 = 5–50% infected leaves with at least one lesion; 5 = ≥ 50% leaves with lesions and with ≤ 5% leaf area spotted; 6 = 5–25% leaf area spotted; 7 = 25–50% leaf area spotted; 8 = 50–75% leaf area spotted; and 9 = maximum infection, leaves black with scab (Lateur and Blazek, 2002).

Disease assessment on fruit was made on harvested fruits from 15 to 31 October each year. The percentage of diseased fruit was assessed for the whole yield collected per plot. Fruit incidence (FI) was calculated as the proportion of infected fruits with at least one scab lesion. Scab severity on fruits was assessed for the whole yield from each plot based on a scale of 1 to 6 following the standard diagram method reported by Croxall et al. (1952) whereby: 1 = no scab; 2 = 0–1%; 3 = 1–5%; 4 = 5–20%; 5 = 20–50%; and 6 = ≥ 50% fruit surface covered by scab. Fruit severity (FS) was defined as the mean proportion of the fruit surface covered by scab, and it was calculated using the following equation:

$$FS = \frac{n1 \times 0}{nt} + \frac{n2 \times 0.5}{nt} + \frac{n3 \times 2.5}{nt} + \frac{n4 \times 12.5}{nt} + \frac{n5 \times 35}{nt} + \frac{n6 \times 75}{nt}$$

where n1 to n6 represent the number of fruits in each category; nt represents the total number of fruits; and the coefficients 0, 0.5, 2.5, 12.5, 35 and 75 represent the median of the lower and upper boundaries of classes 1 to 6, respectively.

V.2.4. Yield, fruit number and size, and phytotoxicity

The fruits were harvested from 1 September to 15 October, depending on cultivar maturity. When all fruits per plot were collected, yield was determined according to the weight of all harvested fruits and was classified into four size categories (<65; 65–75; 75–85 and >85 mm). The number of harvested fruits associated with yield was assessed for each plot. No hand or chemical thinning had been done during the growing seasons for any of the cultivars.

Fruit russet was assessed on the whole yield, after harvest, according to EPPO/OEPP standards based on a scale of 1 to 4 whereby: 1= no russet; 2 = < 10%; 3 = 10–30%; and 4 = 30–100% russet on the fruit surface area. The fruit russet severity index (FR) was calculated as described by Jamar et al., 2008b. Leaf phytotoxicity observations were made on five spur-leaf clusters per tree in June. Leaf phytotoxicity was assessed on a 0–5 scale, as described by Holb et al. (2003) and following EPPO/OEPP standards.

V.2.5. Copper residues in soil and fruits

Since soil microbial activity and earthworm abundance are particularly sensitive to copper, chemical soil parameters and soil copper content (EDTA and ammonium acetate extraction method), in particular, were assessed every 2 years in the experimental orchards, in untreated control (UC) plots as well as in copper-treated (CS) plots. Each assessment included five replicates per treatment.

In 2006, on fruits of the cultivars 'Pinova' and 'Topaz', fungicide residues were assessed on treated fruits (CS) in comparison with untreated fruits (UC). Analyses were made using an overall fruit or peel mineral analysis at harvest and included 10-fruit sub-samples replicated six times for each treatment and cultivar.

V.2.6. Data analysis

Year factors was analysed separately for each variable. The percentage data were transformed in arcsine before performing an analysis of variance. No transformation was carried out for other measures. The data were analysed using SAS software version 9.1 (SAS Institute, Cary, North Carolina, USA) and the Student-Newman-Keuls multiple range test was applied to determine whether the differences between treatments were significant. All the statistical evaluations were conducted at a significance level of $P = 0.05$.

V.3. Results

V.3.1. Apple scab assessments

From 2003 to 2008, the RIMpro scab warning system identified a maximum of 10 potential infection periods per year in Gembloux, with 0–2 infection periods occurring during the flowering period. Between 2003 and 2008, the number of primary infection periods per year, based on the revised Mills criteria, were classified as severe in 3–5 instances, moderate in 2–6 instances and low in 2–5 instances. There was heavy disease pressure in the primary infection seasons, especially in 2005, 2006 and 2008, as revealed by the high scab infection rates recorded in the untreated cv. 'Pinova' plots (Figure 2). In 2007 the primary apple scab infections were particularly low due to a warm, dry spring.

Based on apple scab symptoms present in the untreated plots in the orchard (Figure 2), as well as apple scab symptoms present in other local untreated orchards including cvs. 'Gala' and 'Golden Delicious' (Lefrancq and Lateur, 2009), the following susceptibility ratings for the cultivars can be proposed: high to very high scab-susceptible cultivars for cvs. 'Pinova', 'Initial', 'Zvatava' and 'JN 20/33/58'; medium scab-susceptible cultivars for cvs. 'Pirouette' and 'Topaz'; and low scab-susceptible cultivars for cvs. 'Reinette Hernaut' and 'Reinette Capucins'. Very few scab infections were recorded on the untreated Vf scab-resistant cultivars up to 2007, except for the cv. 'Initial', but in 2008 the scab infections were very severe on all the Vf scab-resistant cultivars, indicating that the Vf scab gene

protection had been completely broken down by new virulent races except for cv. 'Topaz', which still expressed good residual resistance after the *Vf* gene breakdown (Figure V.2). Under our conditions, the treatments did not completely prevent the propagation of these new virulent scab races between 2006 and 2008 (Figures V.3 and V.6).
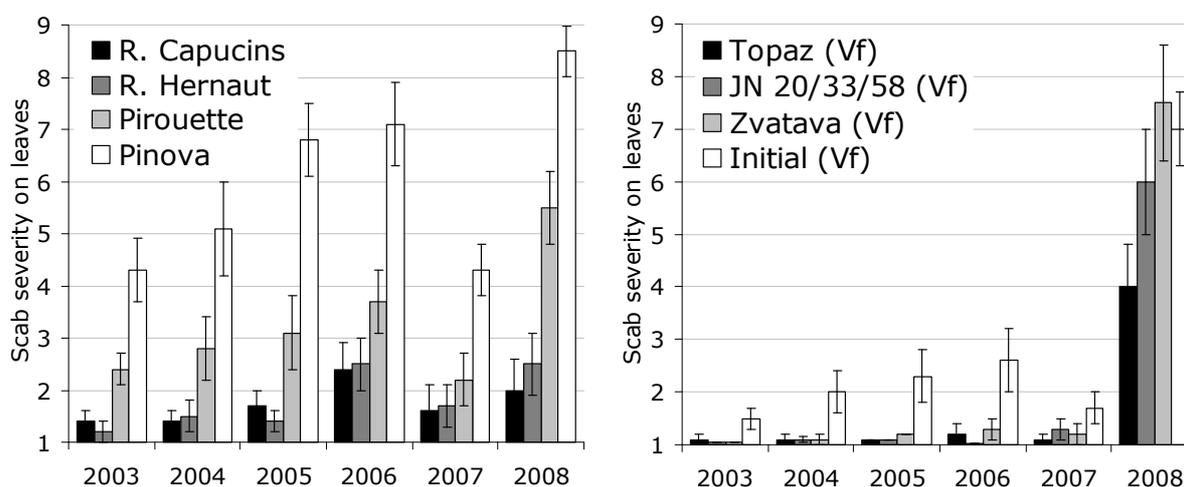

Figure V.2. Evolution of overall scab severity on leaves (1−9 scale: 1 = no scab, 9 = maximum scab infection) assessed in untreated control plots 60 days after flowering, for each cultivar from 2003 to 2008. Error bars denote standard error of the mean (*n* = 6).

A total of 8−12 treatments were applied annually, depending on the year. In each year, all the treatments significantly reduced apple scab compared with the untreated control for the high scab-susceptible cvs. 'Pinova' and 'Initial'. In each year, for cvs. 'Pinova' and 'Initial', the combined copper and wettable sulphur treatments CS1, CS2 and RE2 gave the best apple scab control on both the leaves and fruits (Figures V.3 and V.4). For the CS spray treatments, the amount of elemental sulphur and copper used annually were less than 40 and 2.1 kg ha^{-1}, respectively. In both 2003 and 2004, CS1 was as effective as the IFP control management (RE1). Between 2003 and 2007, with the treatments CS1, the scab severity on the fruits of the scab-susceptible cv 'Pinova' was reduced by at least 97% compared with water control and by 85% in 2008 (Figure V.4). On cv. 'Pinova', in both 2005 and 2006, the lime sulphur treatments (LS1) resulted in significantly lower scab damage on both the leaves and fruits compared with wettable sulphur treatments (RE1), although the same amount of elemental sulphur was applied. In 2006, the delayed lime sulphur treatment (SF1), was less effective than the 'during-infection' lime sulphur treatment (LS1), although the timing of the applications differed by no more than 0–144 DH. In most cases, for the high and medium scab-susceptible cultivars the lime sulphur treatment LS1 resulted in almost the same level of scab control as that achieved by the combined copper and sulphur treatments CS1 (Figure V.4).
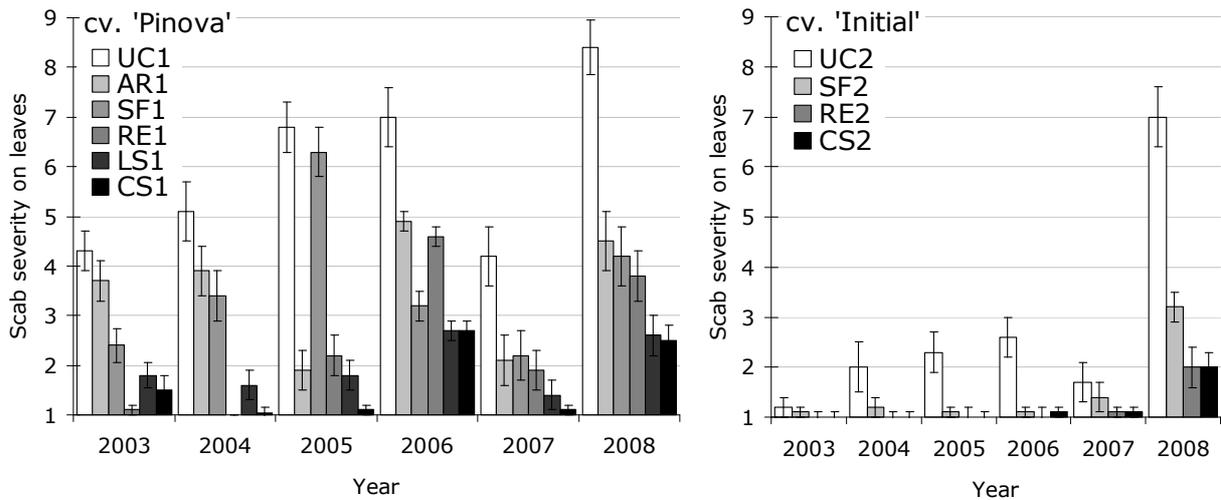
Figure V.3. Effects of spray treatments on overall scab severity on leaves (1−9 scale) assessed 60 days after flowering from 2003 to 2008, for cvs. 'Pinova' and 'Initial', the most susceptible cultivars in each orchard. Error bars denote standard error of the mean ($n = 6$).
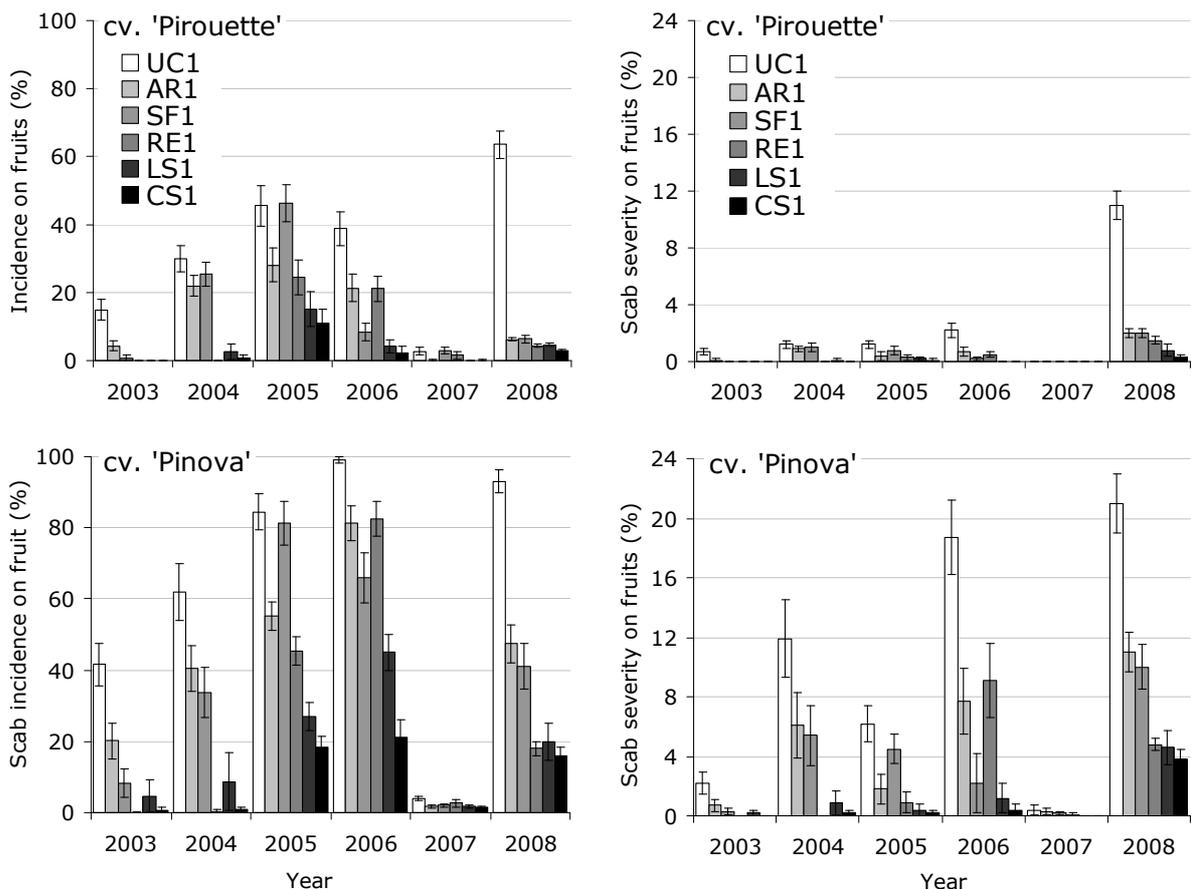
Figure V.4. Effects of spray treatments on scab incidence (left) and severity (right) of the fruit at harvest from 2003 to 2008, for cvs. 'Pirouette' (medium scab-susceptible) and 'Pinova' (high scab-susceptible). Fruit incidence: proportion of total harvested fruits with at least one spot. Fruit severity: mean scabbed area or mean proportion of fruit surface covered by scab. Error bars denote standard error of the mean ($n = 6$).

Each year the potassium bicarbonate treatments (AR1) significantly reduced apple scab severity on the leaves and fruits compared with water control (Figures V.3 and V.4). In most cases, AR1 was as effective as the wettable sulphur treatment (RE1) used in 2005 and 2006, using the same amount of active ingredients for both treatments. In 2005, the potassium silicate treatments (SF1) at 0.1%, using the 'during-infection' strategy, did not reduce scab severity on leaves, but did reduce it very slightly on fruits.

In 2007 the Quillaja saponaria extract treatments (SF1) and the grapefruit seed extract treatments (RE1) significantly reduced the scab severity on leaves, but the weather conditions were not conducive to the development of scab epidemics that year (Figure V.3). In 2008, both the Teawet TQ Liquid (SF1) and Prev-B2 (RE1) treatments used during and after flowering significantly reduced scab incidence and severity on fruits, confirming earlier greenhouse experiments (Jamar, 2007).

Scab control was more effective on the medium scab-susceptible cv. 'Pirouette' than on the high scab-susceptible cv. 'Pinova' (Figure V.4).

V.3.2. Yield, fruit number and size

In most cases, compared with the untreated control, all treatments significantly increased overall yield per tree (Figures V.5). In general, the lime sulphur treatments (LS1) did not affect mean yields per ha compared with wettable sulphur treatments (CS1). In CS plots, the cvs. 'Initial', 'Topaz' and 'Pinova' were the more productive cultivars, with an average mean yield per year of 16.8, 16.7 and 15.9 kg tree^{-1}, respectively (Figure 6). For cv. 'Pinova', with a very high scab rate, the yield values on CS1 plots were 2.6, 4.6 and 2.0 times higher than in the untreated control plots in 2005, 2006 and 2008, respectively. For cv. 'R. Capucins', with a very low scab rate, treatments also increased yield compared with the untreated control UC1 in those years with high scab pressure (2005, 2006 and 2008). This is due largely to the effects on fruit number per tree rather than on mean fruit weight (Table V.4 and Figure V.7). In most cases, compared with the untreated control, all treatments significantly increased the number of fruits per tree (Figure V.7).

In most cases, compared with the untreated control, the CS treatments significantly reduced the proportion of fruits smaller than 65 mm on the high scab-susceptible cultivars (Table V.4). The CS1 treatment significantly increased the mean cumulated yield of high scab-susceptible cultivars (cvs 'Pinova' and 'Initial') in comparison with the untreated control, although on the low scab-susceptible cultivars (cv. 'R. capucins') significant differences between treated and untreated plots were also registered (Table V.4 and Figure V.6).

None of the treatments adversely affected leaves in either year (no phytotoxicity, leaf size reduction or necrotic damage). All the scores ranged between 0 and 0.3 on the 0–5 scale used (data not shown). None of the treatments adversely affected fruit russet compared with the untreated control in any year, except for RE2 in either 2007 or 2008. For example, in 2007, the average fruit russet incidence (with >2% russet) was 16.2 % for the RE2 spray treatment compared with 1.2% for the CS2 spray treatment (data not shown).

Table V.4. Effects of spray treatments against scab on the mean cumulated yield and the mean proportion of fruit at a size of below 65 mm, from 2003 to 2008

Cultivars	Spray treatment	Mean cumulated yield (kg/tree)	Proportion of fruit < 65 mm (%) [a]					
			2003	2004	2005	2006	2007	2008
Pinova	UC1	56.1	12.2	22.1	15.3	68.6	42.8	44.6
	CS1	111.1* [b]	5.5*	3.0*	6.0*	26.6*	27.0*	27.8*
Pirouette	UC1	62.3	5.2	13.3	9.1	10.7	14.6	9.0
	CS1	83.4*	3.0	11.0	2.0*	2.7*	12.5	4.0*
R. Capucins	UC1	63.7	4.3	13.8	8.0	8.0	2.3	4.8
	CS1	88.2*	3.0	7.0	6.3	5.8	2.8	5.2
R. Hernaut	UC1	49.0	3.1	1.5	2.0	2.0	0.0	2.3
	CS1	56.6	4.0	1.0	3.1	0.0	0.0	1.0
Initial (*Vf*)	UC2	95.8	5.6	12.2	6.5	2.5	12.0	11.5
	CS2	117.9*	4.0	6.0*	1.0*	4.0	5.8*	2.5*
Topaz (*Vf*)	UC2	98.3	5.2	15.6	6.0	10.9	42.2	40.3
	CS2	116.7*	3.0	7.0	2.4	7.8	29.2*	19.0*
JN20/33/58 (*Vf*)	UC2	49.3	1.8	16.4	6.5	9.9	7.0	7.1
	CS2	56.5	0.0	7.2	7.0	7.1	14.0	5.3*
Zvatava (*Vf*)	UC2	55.9	2.9	18.7	9.1	30.2	61.8	46.2
	CS2	77.8*	1.0	8.8	8.0	23.0*	31.6*	44.8

[a] No hand or chemical thinning had been done during the growing season for any of the cultivars −
[b] Value followed by '*' are significantly different from the corresponding untreated control value (UC1 or UC2) according the Student-Neuwman-Keuls tests (P ≤ 0.05)
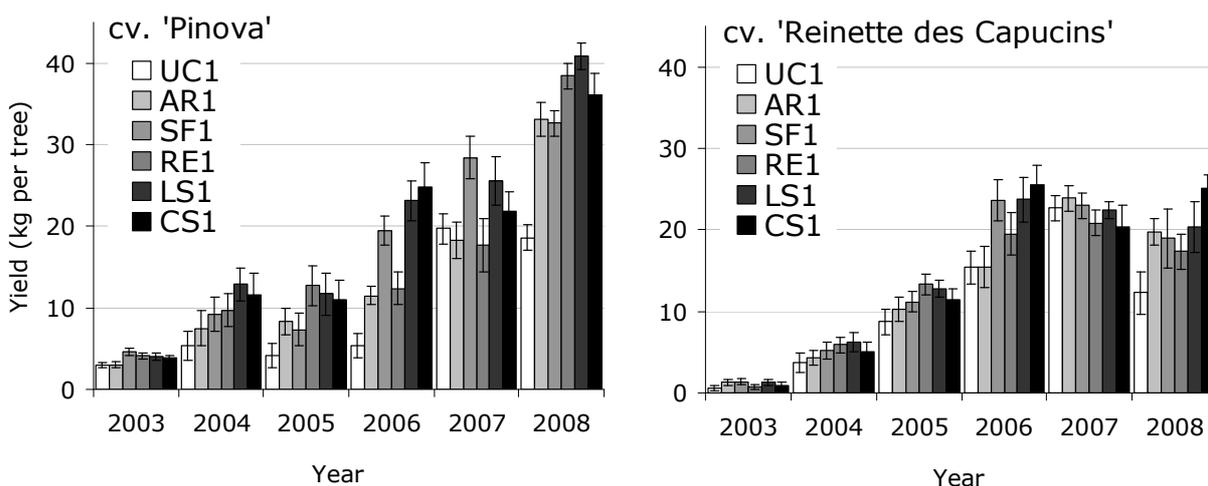

Figure V.5. Effects of spray treatments on overall yield for cv. 'Pinova' (high scab-susceptible) and cv. 'R. Capucins' (low scab-susceptible), from 2003 to 2008. Tree density was 1900 trees ha^{-1} in blocks or 1500 trees ha^{-1} in the overall orchards, including 20% of ecological zones. Error bars denote standard error of the mean (*n* = 6)
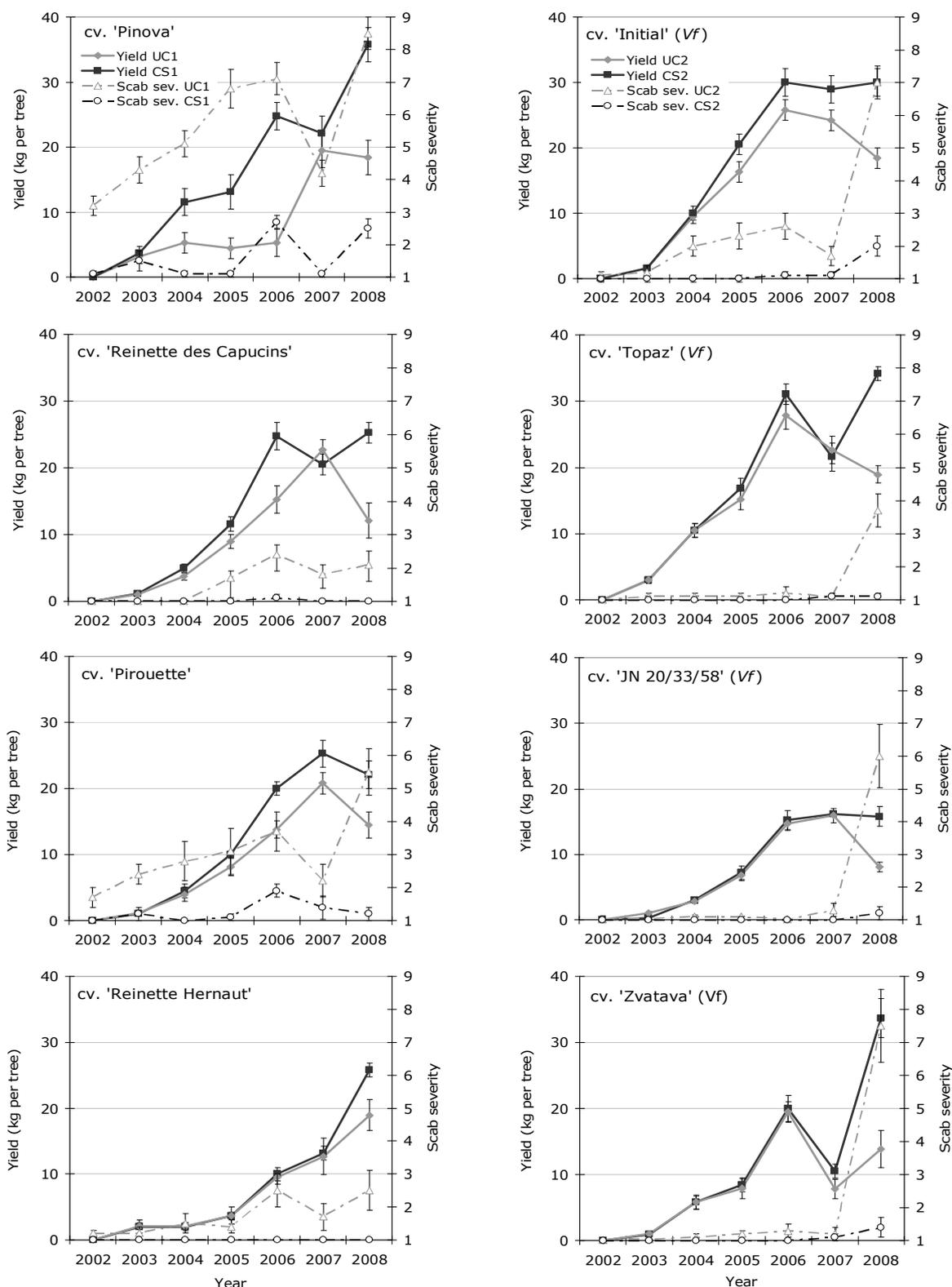
Figure V.6. Overall yield at harvest in relation to overall scab severity on leaves assessed 60 days after flowering (1−9 scale), subjected to CS spray treatments compared with the UC untreated controls from 2002 to 2008. Tree density was 1900 trees ha−1 in blocks or 1500 trees ha−1 in orchards, including 20% of ecological zones. Error bars denote standard error of the mean (*n* = 6). Overall yield obtained in 2009 for each cultivar are presented in Jamar et al. (2009).
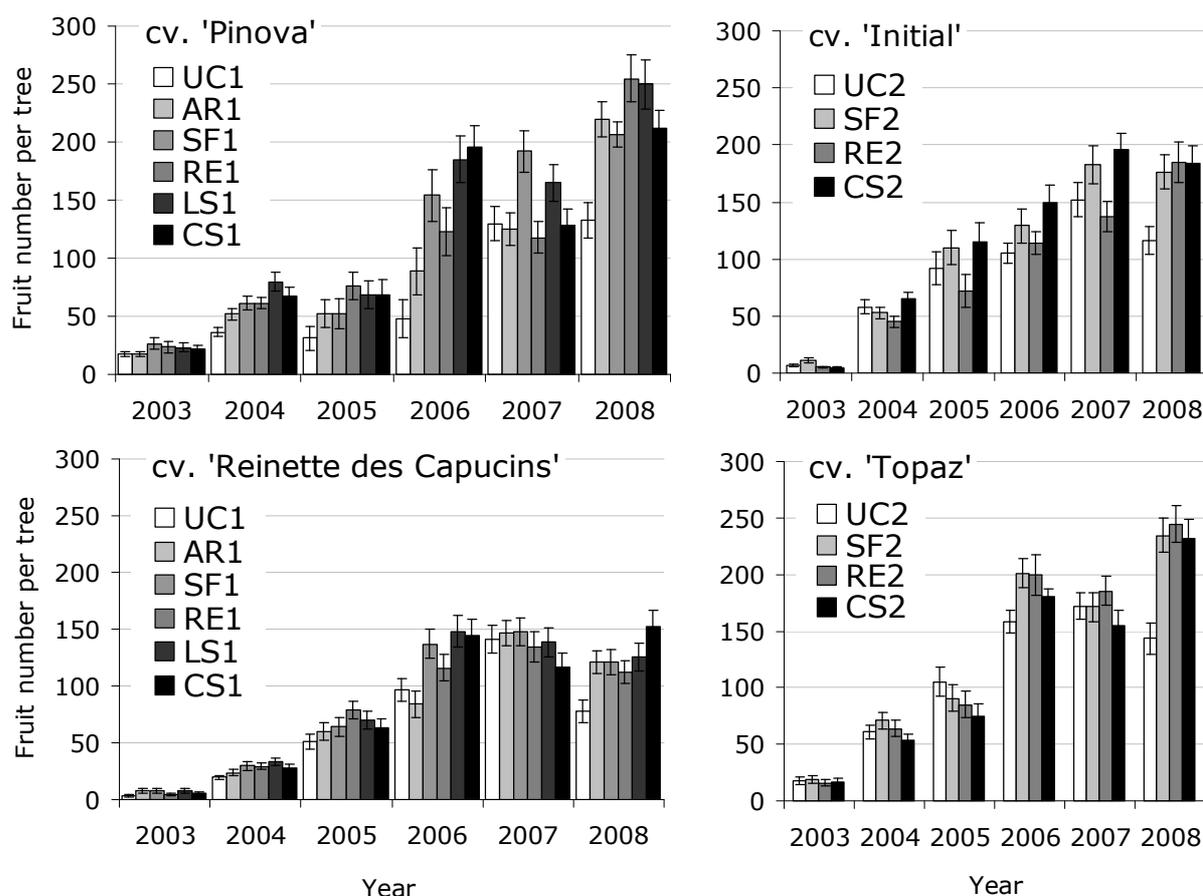
Figure V.7. Mean number of fruits per tree for each spray treatment from 2003 to 2008. Error bars denote standard error of the mean ($n = 6$) − Nombre moyen de fruit par arbre pour chaque schéma de traitement, de 2003 à 2008. Les barres d'erreur représentent l'erreur standard de la moyenne ($n = 6$).

V.3.3. Copper residues in soil and fruits

Copper residues on the standard and peel fruits at harvest were significant for cv. 'Pinova' in CS1 plots, treated with copper after flowering (Figure V.8). For cv. 'Topaz', no significant difference in copper residues between the UC2 untreated control and CS2 treatments was registered. Standard and peel fruit analysis did not show any sulphur residue at harvest for cvs. 'Pinova' and 'Topaz'. Sulphur and copper residues on the fruits at harvest from combined copper and sulphur treatments (CS1) were always far below the maximal residue level (LMR) permitted in apple by EC regulations (5 and 50 mg kg^{-1} for copper and sulphur, respectively).

Soil copper content in the experimental orchard was kept below 26.8 mg kg^{-1}. Any significant differences in copper soil content between CS and UC plots were registered in 2008, at the end of the experiment (Table V.5).

Table V.5. Copper content (mg kg−1)[a] of organic orchard soils at Gembloux, from 2002 to 2008

Spray treatments	2002	2004	2006	2008
UC1 + UC2	-	-	-	14.8 a[b]
CS1 + CS2	18.5	22.3	26.8	15.5 a

[a] EDTA and ammonium acetate extraction method.

[b] Values are the means of five replicates, and values followed by the same letter are not significantly different according the Student-Neuwman-Keuls tests (P ≤ 0.05)
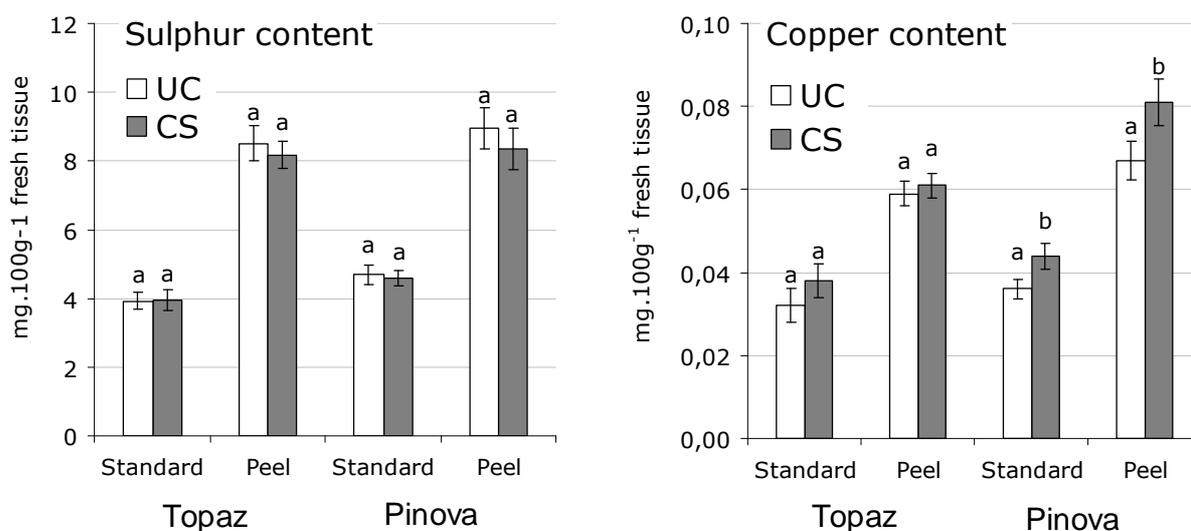

Figure V.8. Effects of the CS compared with UC spray treatments on the copper and sulphur fruit (standard) and peel contents at harvest in 2006. Mean dry weight content was 14.5 (± 0.5) and 20.6 (± 0.7) % for fruits and peels, respectively. Error bars denote standard error of the mean ($n = 6$). In the 'UC−CS' pairs, mean values with different letters are significantly different according the Student-Newman-Keuls tests ($P ≤ 0.05$)

V.4. Discussion

The results presented in this study show that the 'during-infection' spray strategy offers valuable advantages for effective apple scab control with a reduced amount of fungicide use such as sulphur, lime sulphur, copper, potassium bicarbonate and various plant extracts on high scab-susceptible cultivars, under conditions conducive to scab infections. The most efficient spray treatments used for apple scab control in this experiment (CS1 and CS2) never exceeded 40 kg of elemental sulphur and 2.1 kg of copper per ha per year, applied in a maximum of 12 treatments per season. These amounts of fungicides are less than 50% below the amounts usually used to control apple scab in organic production under humid climate conditions (Ellis et al., 1998; Holb et al., 2003; Palmer et al., 2003, Holb et al, 2005b). Up to 110 kg ha^{-1} year^{-1} of elemental sulphur combined with 8 kg ha^{-1} year^{-1} of copper were used for scab control in organic apple production in The Netherlands (Holb and

Heijne, 2001). In addition, the results of this study showed that (i) diluted copper is very effective under cold weather conditions during early primary scab infection periods, (ii) the lime sulphur treatment can be as effective as the combined wettable sulphur and copper treatment and (iii) the lime sulphur is more effective than wettable sulphur used alone, confirming the results of previous studies (Mills, 1947; Ellis et al., 1998; Holb et al., 2003).

A previous study (Jamar et al., 2010c) showed that the spray deposits at each sampling point of the tree canopy produced by the tunnel sprayer were not significantly different from the standard sprayer. Consequently, the performances obtained in the present study cannot be attributed to the specific characteristics of the tunnel sprayer.

The treatments were applied shortly after rainfall associated with primary scab infections, during the infection process, sometimes on drying leaves. At this stage, ascospores had been discharged, susceptible young tissues were present, and the minimum temperature and leaf-wetness conditions for infection according to the revised Mills criteria could be achieved, but penetration of the cuticle had not yet occurred (Figure V.1). Below 250 DH after rainfall, few ascopores reach the stadium of penetration, and a relevant portion will only reach this stage after 300 DH (Smereka et al., 1987; MacHardy, 1996). Sprays below 300 DH can be considered to be applied during the infection process (before infection), on germinating spores possibly already with appressoria but not yet with the primary stroma that allow the fungus to be protected by the plant cuticle. The poorer result registered with the delayed spray treatment (SF1 in 2006) showed that the timing of treatments must be close to the onset of infection, even with lime sulphur.

The 'during-infection' spray strategy has several important advantages compared with the preventive (before rainfall) spray strategy; these include (i) reduced washing effect from the rain, (ii) greater treatment effectiveness on germinating spores, (iii) avoidance of unnecessary treatments (Funt et al., 1990; Holb et al., 2003; Jamar et al., 2009) and (iv) in hot seasons, applying treatments during less sunny periods. However, there are potential problems with this strategy, such as (i) the need to be able to adapt the spray timing to within a few hours of the onset of rain, (ii) the risk of delaying spraying during an extended rainy period, (iii) the risk of spraying during windy weather and (iv) the risk of causing soil damage under wet conditions.

A low disease level at any given point in time during the growing season is needed when the management aim is not only to prevent infections on the fruits but also to keep inoculum pressure at a low level (Holb et al, 2005b). From the time the orchard was planted, until 2008, an increasing impact of the disease was observed, except in 2007 which was characterized by particularly poor weather conditions for scab. This suggests that some untreated and poorly treated plots led to heavy disease pressure during the following primary infection season. In order to limit the influence of the previous year, several sanitation practices such as autumn leaf-shredding and leaf-burying were carried out between two growing seasons (Holb, 2006), but theses practices did not suppress the scab inoculum sufficiently. In addition, the risk of early scab epidemics initiated by over-wintered conidia is high in organic orchards (Holb et al., 2005a) and this could explain the relatively low effectiveness of wettable sulphur and potassium bicarbonate used alone, since the primary 2006 scab season. This suggests that the results recorded were probably influenced by treatments applied in the previous year and shows the usefulness of presenting results covering a 6-consecutive-year study. As only two summer sprays were applied, some of the

scab damage observed on the harvested fruits might have arisen from secondary scab infection, especially in plots where primary scab control was partial (MacHardy, 1996; Holb et al., 2005b).

With regard to *Vf* scab-resistant cultivars, mainly in untreated control plots, significant and increasing scab damage was recorded on leaves and fruits of cv. 'Initial' from 2003 to 2008, as a result of the appearance of new scab races virulent to the *Vf* gene since the plantation year. The scab-inoculum pressure therefore increased from year to year in these orchards, which could explain the poorer treatment effectiveness at the end of the experiment, in 2008.

The efficiency of bicarbonate salts in controlling apple scab, as reported here and in previous studies (Schulze and Schönherr, 2003; Ilhan et al., 2006; Jamar and Lateur, 2007; Jamar et al., 2007; Jamar et al., 2008b), suggests that this compound could be introduced in apple disease management, although our results indicated that applications of Armicarb alone throughout the growing season were not effective enough against scab and that it needed to be supplemented by other active compounds. The fact that the bicarbonate salts are ubiquitous in nature and commonly present in human food, means that this simple compound is very appropriate for organic production systems. However, potassium bicarbonate acts as a contact fungicide and is not likely to be systemic or curative. Greenhouse experiments have shown that Armicarb is effective in controlling apple scab, but a long-lasting effect cannot be expected (Jamar et al., 2007). Bicarbonates are unstable compounds that are highly water-soluble and easily washed off the leaves by a small amount of precipitation. Therefore, they require frequent and well-targeted spray applications. Activity below 10°C is a prerequisite if copper is to be replaced and, so far, no data are available on the effectiveness of potassium bicarbonate under low temperature conditions.

Yucca schidigera extract was also effective against apple scab under greenhouse conditions (Jamar, 2007), but we decided not to experiment with it because of its very poor sustainable profile as it is based on harvesting wild plants that grow in desert areas with little vegetation and very little rainfall.

In our experimental conditions, silicon had a very poor effect on apple scab. However, Belanger et al. (1995) reported that there is cumulative evidence that increased silicon absorption offers protection against various fungal diseases. This suggests an eventual inadequate timing strategy for this kind of product in our experiments.

Although some authors have reported poorer leaf appearance with sulphur and copper treatments (Palmer et al., 2003; Holb et al., 2003), the amount of active substances used in our study to control apple scab did not induce any phytotoxic effects, plant damage or yield reduction. However, the treatment frequencies and fungicide doses in our experiment were very limited, particularly during the flowering period.

For the low scab-susceptible cultivar cv. 'Reinette Capucins', sprays based on sulphur and copper (CS1) led to a significant increase in the yield per tree, especially in the years with high scab pressure (2005, 2006 and 2008). These results contrast with earlier studies showing that sulphur applications reduced yield and fruit numbers (Mills, 1947; Holb et al., 2003; Palmer et al., 2003). Such positive effects on yield cannot be explained by the control of apple scab or other apple diseases such as powdery mildew (Podosphaera leucotricha),

because all years the infection levels in untreated plots were very low, even at a later stage. There are two possible explanations for this: (i) triggering defence responses and resistance mechanisms burn up energy in plants (Treutter, 2005), which is not necessary to use in treated plants (Doehlmann et al., 2008), (ii) the application of elemental sulphur to crops is increasingly advocated as a way of overcoming deficiency in this key nutrient, and sulphur deficiency has recently become a widespread nutrient disorder in crops, largely due to the reduced rate of fossil fuel burning (Schnug, 1998; Williams and Cooper, 2004). A chemical analysis of the leaves from cv. 'Reinette des Capucins', collected on 25 June 2006 and washed with acid solutions, showed that the leaf dry extracts from the SF1, LS1, RE1 and CS1 treatments contained 0.38% of sulphur, whereas the leaf dry extracts from the AR1 and UC1 control treatments contained 0.30% of sulphur (P<0.001).

Sulphur compounds can have harmful effects on useful phytoseiids (Kreiter et al., 1998). However, the absence of the phytophagous mites such as Panonychus ulmi and *Aculus schlechtendali* in the orchard from 2003 to 2008 might be associated with the very high density of the predator *Typhlodromus pyri* observed throughout the orchard in spite of various sulphur treatments (Jamar et al., 2008b). Some studies have reported predator mite populations with an acquired resistance to sulphur (Markoyiannaki-Printzioui et al., 2000) and bicarbonate salts (Beresford et al., 1996).

Earthworms are particularly sensitive to copper and zinc (Paoletti et al., 1998). Soil copper content in the experimental orchard was kept below 26.8 mg kg^{-1}, which is far below the estimated harmful level for earthworms. It was shown that copper does not have a negative impact on soil ecology or on earthworm populations at up to 36 mg kg^{-1} soil (e.g. van Rhee, 1976; Paoletti et al., 1998; Holb, 2008). Van Rhee (1976) found that earthworms were almost completely eradicated when the copper concentration was more than 80 mg kg^{-1} soil. Organic management significantly increases soil microbial activity and earthworm abundance in orchards (Jamar et al., 2008a; Jamar et al., 2010a, although (1) soil tillage operations for weed control can reduce earthworm abundance (Paoletti et al., 1998), (2) the presence of copper on leaves could reduce the earthworm consumption rates (Depta et al., 1999). Several authors have found that microbial and earthworm communities play an important role in leaf litter decomposition corresponding to a reduction in the amount of scab ascosporic inoculum in apple orchards (MacHardy, 1996; Paoletti et al., 1998).

V.5. Conclusion

This long-term study clearly shows that (i) the 'during-infection' spray strategy using reduced amounts of wettable sulphur and copper are very effective against primary scab infections; (ii) the use of lime sulphur significantly reduces or suppresses the copper used in a spray treatment based on a 'during-infection' spray strategy; (iii) the amount of copper for scab control could be reduced for medium and low scab-susceptible cultivars compared with high scab-susceptible cultivars; (iv) potassium bicarbonate significantly reduces apple scab and is, in some cases, as effective as wettable sulphur; (v) natural plant extracts (from orange peel, soapbark tree, tea seed, quinoa seed and grapefruit seed) significantly reduce apple scab; (vi) sulphur-based treatments increase yield even with a low scab-susceptible

cultivar; (vii) the fungicide doses and frequencies used in this study are not phytotoxic, do not adversely affect yield and do not leave undesirable residues on fruits and soils.

The study has shown that some new compounds, especially potassium bicarbonate and five new plant extracts, have an effect on *Venturia inaequalis* infections, and therefore have the potential, in combination with other compounds, to reduce copper treatments during both the primary and secondary scab seasons. Control did not, however, always reach the level desired by commercial fruit growers, and several practical issues need to be addressed before these materials can be considered as a useful alternative to copper.

As lime sulphur is not allowed in Belgium, copper is still needed for apple scab control in apple organic production in the country. Currently, lime sulphur appears to be the sole remaining option for replacing copper when temperatures are below 10°C in organic farming, and therefore scab management in Belgium would be compromised if there were new European or national regulations restricting or banning the use of copper-based compounds. Our 6-year study has demonstrated the potential of controlling apple scab with reduced and non-damaging amounts of inorganic fungicides, using clearly defined timing of treatments and a spray machine.

V.6. Acknowledgements

V.7. References

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Chapitre VI

Comparative performance of recycling tunnels and conventional sprayers using standard and drift-mitigating nozzles in dwarf apple orchards

FOREWORD

In the preceding chapters, the effectiveness of inorganic fungicides for the control of primary apple scab infections was studied in an experimental apple orchard including complex experimental designs. A prototype tunnel sprayer machine was used for treatment applications. Apart from environmental concerns, the benefit of tunnel sprayers would be to reduce plot size in treatment trials using such complex experimental designs following the EPPO guidelines. It could allow a smaller plot size to be used in multifactorial trials in which fully randomized or randomized block designs are recommended. However, the effectiveness of plant protection products applied with tunnel sprayers cannot be reliably assessed without a thorough investigation into spray performances. In particular, more information is needed about the spray distribution patterns on the tree canopy provided with this kind of sprayer.

Therefore, a set of three experiments was conducted in an apple orchard in order to check the performances of the tunnel sprayer used in the preceding studies. The results of this set of experiments are presented in this chapter.

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Running title: Performance of recycling tunnel sprayers in orchards

VI Chapter VI : Comparative performance of recycling tunnels and conventional sprayers using standard and drift-mitigating nozzles in dwarf apple orchards

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Abstract

The use of tunnel sprayers should be encouraged because they can potentially reduce pesticide input and drift in orchards. They could also allow smaller plot size in multifactorial trials in which fully randomized or randomized block designs are recommended. However, the effectiveness of plant protection products applied with tunnel sprayers cannot be reliably assessed without a thorough investigation into spray distribution in tree canopies. A set of three experiments was undertaken in an apple orchard to compare a new type of recycling tunnel sprayer with a standard axial fan sprayer, both of them fitted with either conventional hydraulic hollow cone nozzles (ATR) or drift-mitigating air induction cone nozzles (TVI). Its performance was assessed in terms of 1) spray deposit and coverage in the canopy, 2) sedimentation drift (spray drift to the ground) and 3) collection and recycling rate of the liquid sprayed in the tunnel. Artificial targets composed of cellulose papers and water-sensitive papers were used to evaluate the spray deposit and coverage at similar target positions for each treatment. A fluorescent dye was used as the spray tracer.

The study showed that, when using the ATR nozzles, the spray deposit, at each sampling point in the tree canopy, produced by the tunnel sprayer was not significantly different from that produced by the standard sprayer. The spray deposited on the top of the trees when using the TVI nozzles, however, was significantly less than with the standard sprayer. At the same spray deposit level, the spray cover on the canopy, estimated by image analysis, was relatively better with the standard sprayer than with the tunnel sprayer. At the same spray deposit level, the TVI nozzles resulted in significantly poorer spray cover of the canopy than the ATR nozzles. At low wind speeds, the sedimentation drift varied on average from 5.8 to 9.1% of the total sprayer output, irrespective of the type of sprayer or nozzle. The overall mean of the sedimentation drift was not significantly different for the two types of sprayers. The recovery system, which included a continuous recycling process in the tunnel sprayer, led to average savings of 28 and 32% of the applied spray mixtures for the ATR and TVI nozzles, respectively. The tunnel sprayer might therefore be suitable for small-scale apple orchards when fitted with traditional ATR nozzles rather than with air-induced TVI nozzles.

Keywords: air induction nozzle; droplet size; sedimentation drift; spray cover; spray deposition

VI.1. Introduction

The axial fan air-assisted sprayer fitted with hydraulic hollow cone nozzles is the predominant design of sprayers used in orchards. It produces a large radial spray plume, which could involve a significant risk of off-target contamination by spray drift and losses on the ground, a subject of increasing public concern. Several authors have reported losses in excess of 50% of the spray applied by axial fan sprayers (Cross et al., 2001). In apple orchards, spray losses on the ground can range from less than 2% to 39% of the total amount applied, and drift losses can account for 23-45%, depending mainly on leaf development and weather conditions (Vercruysse et al., 1999).

Other sprayer designs have been developed, including over-the-row tunnel spray systems. Although various studies have reported substantial savings in pesticides and a reduction in drift resulting from various types of tunnel spray systems (Peterson and Hogmire, 1995; Porskamp et al., 1994; Doruchowski and Holownicki, 2000; Planas et al., 2002; Ade et al., 2007), these sprayers are used only to a limited extent because of increased cost and reduced operational flexibility. In addition, few studies have looked at the spray distribution on the canopy and spray lost on the ground from a modern tunnel sprayer compared with a standard fan sprayer. The uniformity of deposition has been reported, in some cases, to be less satisfactory than that from conventional axial-fan sprayers (Porskamp et al., 1994; Planas et al., 2002). It has proved difficult to design a tunnel sprayer that distributes spray uniformly on the trees and significantly reduces losses on the ground (Molari et al., 2005). In general, the results from non-conventional orchard spray technologies are still debatable because little information is available, and what there is tends to be controversial.

Apart from environmental concerns, another benefit of tunnel sprayers would be to reduce plot size in treatment trials using complex experimental designs following the EPPO guidelines. In order to conduct reliable evaluation trials using the tunnel sprayers, more information is needed about the spray distributions on the tree canopy.

A new promising option for drift mitigation in orchards could be the use of air induction cone nozzles, which provide larger drop sizes. Coarser droplets reduce the air-borne drift losses by mixing less readily with the surrounding atmospheric boundary layer (Walklate, 1992; McArtney and Obermiller, 2008). Spray distribution can be improved, however, by applying greater numbers of finer droplets which are more easily carried by the forced airflow of the sprayer (Cross et al., 2001; Derksen et al., 2007). Finer droplets with a smaller diameter give a greater coverage for any given level of spray deposit. The net result of these counteracting effects has been investigated only to a limited extent in orchards. According to Cross et al. (2001), the coarse sprays produced slightly greater mean deposits and smaller spray losses, and were preferable from this point of view. Further work is needed to establish the effect of biological efficacy of these spray patterns, although it has been shown that the effectiveness of insecticides is inversely proportional to drop size, and the limited data for fungicides suggest similar conclusions (Chapple et al., 1997; McArtney and Obermiller, 2008).

The objectives of this study were to assess the tunnel sprayer and the drift-mitigating nozzle performances compared with reference treatments using standard technologies. The amount and macro-distribution of spray deposits on the canopy, together with spray losses on the ground (sedimentation drift), were measured in a modern apple orchard system in two experiments. In a third set of experiments, the recycling rate obtained with the tunnel sprayer was assessed.

VI.2. Materials and methods

VI.2.1.   Orchard and equipment

The study was conducted in an experimental dwarf apple orchard (cv. 'Pinova') planted in 2002 in Gembloux, Belgium (Jamar et al., 2008). Inter-row spacing was 3.5 m and intra-row spacing was 1.5 m. Orchard maintenance included a classical spindle shape training system. In 2008, the trees reached an average of 3.25 m high and 2.1 m wide.

Applications were performed with a standard axial fan air-assisted sprayer (Arbo AX 1000, Berthoud Agricole, 69220 Belleville sur Saône, France) and a recycling tunnel sprayer (Type 115, Munckhof, 5961 CV Horst, The Netherlands), including the so-called 'Closed Loop System Technology'. Both sprayers were fitted with two sets of six nozzles. For the air assistance system of the standard sprayer, the fan rotational speed was 1600 rpm (low gear position). With the air assistance system of the tunnel sprayer, the air is sucked from inside the tunnel, producing an under-pressure area which helps eliminate most of the forward or backward spray drift. Air-borne droplets are partly intercepted by the tunnel's special design features and partly sucked back in by six axial-flow fans for subsequent re-use. The recovered spray is sucked in at the bottom of the collector walls using a Venturi system and transported to the sprayer tank after filtering. The internal opening of the tunnel was set at 2.40 m wide for all experiments, so the distance between the nozzles and the centre of the row was kept constant at about 1.2 m. The air outlets were angled at 45° upwards. For each sprayer, two types of spray nozzle were tried: the classical hollow cone nozzle (yellow Albuz ATR 80) and the air induction cone nozzle (green Albuz TVI 80-015) manufactured by Céramique Techniques Desmarquest from Evreux in France. For all the experiments, the power take-off (PTO) speed was fixed at 560 rpm, with a travel speed of 6.6 km h^{-1}. The working pressures were held in position at 10.5 and 12 bars for the ATR and TVI nozzles, respectively (Table VI.1).

During each experiment, air temperature, relative humidity, wind velocity and wind direction were recorded within the orchard at 3.5 m above the ground, using an iMETOS® AG IMT300 weather recorder (Pessl Instruments GmbH., 8160 Weiz, Austria, 2007). The local weather conditions were electronically monitored at the time of each spray application.

Table VI.1 − Treatments

Treatment	1	2	3	4
Sprayer	Standard	Standard	Tunnel	Tunnel
Nozzle trademark and type [a]	Albuz ATR	Albuz TVI	Albuz ATR	Albuz TVI
Size	yellow	green	yellow	green
Number of nozzles	12	12	12	12
Pressure (bar)	10.5	12	10.5	12
Measured spray liq. flow rate (l min^{-1})	1.12	1.12	1.12	1.12
Forward speed (km h^{-1})	6.6	6.6	6.6	6.6
Spray volume [b] (l ha^{-1})	350	350	350	350
Volume median diameter VMD (μm)[c]	78	507	78	507
PTO speed (rpm)	560	560	560	560

[a] ATR = ceramic hollow cone nozzle, TVI = air induction cone nozzle
[b] Calculation based on 2857 m per ha
[c] D50 values at 10 bars measured by the Cemagref on Dantec calibration

VI.2.2.   Treatments

The experiment involved four treatments: (i) standard sprayer with ATR nozzles, (ii) standard sprayer with TVI nozzles, (iii) tunnel sprayer with ATR nozzles and (iv) tunnel sprayer with TVI nozzles. In order to avoid external sources of variability, all the working parameters were kept as constant as possible in all treatments. The sprayers were calibrated to apply a constant rate of 350 l ha^{-1}. The spray liquid consisted of a mixture of 2 g l^{-1} of the water-soluble dye (fluorescein-sodium tracer, C.I. 45 350, Merck, Germany) in water for the canopy distribution experiment and 9 g l^{-1} for the sedimentation drift experiment. A sample tank liquid was taken immediately before and after completing each spraying to determine the exact concentration of the tracer in the spray tank.

VI.2.3.   Spray deposit in the canopy

The first experiment was carried out at full-leaf development stage during summer 2008 and was repeated four times, on 25 July, 1 August, and 2 and 10 September under varying weather conditions. For each treatment, four sampling repetitions in space were carried out, obtaining a completely randomized experimental design. Four blocks were established in a 150 m-long x 100 m-wide orchard section. The blocks, each consisting of part of six 36 m-long rows, were separated from each other by 10 m. In each block a central row 36 m long was sprayed from both sides, following the normal procedure for applying plant protection products in orchards. In every sprayed row, a one-tree sample was established in which an artificial target composed of 20 absorbent cellulose papers (Whatman, 1004110, 110 mm ø, Schleicher & Schuell) and 20 water-sensitive papers (26 x 76 mm, 20301-1N, Teejet Spraying Systems Co) were fixed on a specific metallic structure inserted in the foliage in order to evaluate the spray distribution on similar target positions for each treatment (Figure VI.1). For each tree sample, the trees were divided into four zones according to their height (W: 0.0 to 0.20 m, X: 0.80 to 1.00 m; Y: 1.8 to 2.00 m; Z: 2.80 to 3.00 m) and into five

zones according to their depth within the crop (I: external left, II: external right; III: central; IV: central left and V: central right).

In order to assess the relative deposit in the canopy ($\mu g\ cm^{-2}$) from each of the 20 sampling positions, the absorbent paper was collected immediately after spraying and put into a 125 ml plastic container and stored in a dark cool box in the field. In the laboratory the samples were cut into small square pieces (approx. 2 x 2 cm). The fluorescent dye was extracted by soaking and agitating each filter paper for 15 min in a constant volume of 0.5 l distilled water. The concentration of the dye tracer in the extraction water solution was determined using a UV-2101PC Shimadzu spectrophotometer working at a maximum absorbance wavelength of 492 nm, and the quantification was performed using the external standard calibration.

Water-sensitive papers were used to quantify the relative percentage spray cover, the number of droplets per cm^2 and the average droplet diameters resulting from the different spray techniques. The papers were clipped to a vertical wire-mesh support, matching the same 20 sampling positions in the canopy described earlier. The images of each water-sensitive paper were digitised using a Fujifilm FinePix S1Pro (6 million pixels SuperCCD, 1 pixel = 66 µm) and stored on a PC. The percentage spray cover, the number of droplets per cm^2 and the average droplet diameters on each water-sensitive paper were estimated by image analysis using Image-Pro Plus software version 6.1 (MediaCybernetics, East-West Hwy, USA).
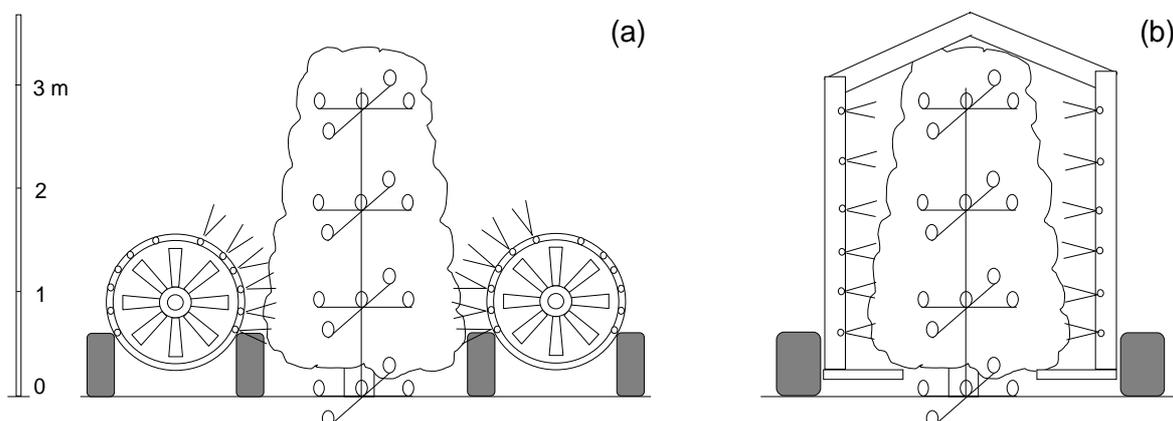

Figure VI.1 : Schematic representation of one-tree sample including 20 artificial collectors used in the spray deposit experiment, in relation with the nozzle positions of the standard sprayer (a) and the tunnel sprayer (b).

VI.2.4.   Sedimentation drift

A second experiment, including the same four treatments described earlier, was performed to determine the spray losses on the ground on the downwind side of the experimental apple orchard with one boundary-row application. Four plots, separated from each other by 10 m, were established on a boundary row in a 150 m-long x 100 m-wide orchard section. The plots, each consisting of 36 m-long rows, were sprayed from both sides following the normal procedure for applying treatments in orchards. One sample location was established in each plot. A sample location consisted of eight cellulose collectors (110 mm ø large) placed on metallic boards on the ground and six cellulose strip collectors in the target space (an open space, free of vegetation) in the row up to 3.2 m high. The ground collectors were placed outside the orchard up to 10.5 m at a right angle to the rows. For the four treatments, four sampling repetitions in space were carried out based on a fully randomized experimental design and the experiment was repeated three times on 25 July, 1 August and 2 September 2008 under varying weather conditions. The procedure described above was used to collect and assess spray deposits from cellulose papers in the laboratory.

VI.2.5.   Spray recycling

A third experiment was performed to assess the recycling spray rate of the tunnel sprayer. In the same orchard as the one described in 2.1 (a 7-year-old apple orchard), the experiment consisted of spraying 0.5 ha with water at a spray rate of 350 l ha^{-1} and to assess the spray mixture recycling rate. Both the applied and recycled volumes were easily measured because the experimental tunnel sprayer was fitted with an individual tank receiving the recycled mixture. The experiment was repeated three times before flowering and three times after flowering, using either ATR or TVI nozzles as described earlier. The working speed of the sprayer was 6.6 km h^{-1}. The working pressure was 10.5 bars for ATR nozzles and 12 bars for TVI nozzles in order to obtain the same flow rate for both nozzles.

VI.2.6.   Data analysis

The measured deposits were normalized for differences in dye concentration in the spray mixture. Before statistical analyses, transformation of the variables had been applied to reflect normality and variance equality. For spray deposits (μg cm^{-2}) and average droplet diameters (μm), the log transformation was applied, and for the spray cover (%) and the number of droplets per cm^2 the angular and square root transformations, respectively, were carried out. All data analyses were performed using SAS software version 9.1 (SAS Institute, Cary, North Carolina, USA). The Student-Newman-Keuls multiple range tests, with a 95% confidence level, were performed to investigate the differences between the deposition levels obtained with the tested sprayers and nozzles.

VI.3. Results and Discussion

VI.3.1. Spray deposit in the canopy

All the treatments were conducted under constant climatic conditions and very low wind speeds (below 2 m s^{-1}), which therefore did not have any significant influence on the results.

The first experiment showed that for the classical hollow cone nozzle (ATR), the application with the tunnel sprayer produced an amount of spray deposit comparable with the standard sprayer at each level of the canopy (Table VI.2). With regard to the air induction nozzle (TVI), the tunnel sprayer produced a significantly lower spray deposit in the treetop than the standard sprayer. The greater spray deposit was obtained with the standard sprayer combined with the TVI nozzles, but this modality of treatments included a lower spray cover than the ATR nozzle option. For both sprayers and both nozzles, notably lower spray deposits and spray covers were registered in the high part of the tree compared with the middle and low parts of the tree, but these differences were much more pronounced with the combination of the TVI nozzles and the tunnel sprayer. In addition, compared with the standard sprayer, the tunnel sprayer produced significantly greater deposits on sampling beneath the row of trees, whatever the nozzle types (Table VI.2). With the TVI nozzle, the tunnel sprayer greatly increased the deposit beneath the row of trees compared with the standard sprayer (Fig. VI.2).

This suggests that the air-assistance design of the tunnel sprayer used should be adapted to the kind of nozzle and the orchard structure. It seems that the design of the tunnel sprayer tested was not well adapted to trees that were 3.25 m high, but was better adapted to smaller trees. Previous studies have demonstrated that the tunnel sprayers performed better than conventional sprayers on dwarf trees 2.7 m high, in terms of in-canopy spray deposit (Peterson and Hogmire, 1995; Hogmire and Peterson, 1997; Holownicki et al., 1997a), leaf coverage or distribution uniformity (Cross and Berrie, 1993; Holownicki et al., 1997a). Others studies have reported that the uniformity of the canopy spray deposition was worse than that obtained with the axial flow sprayer in a 2.80 m-high apple orchard (Planas et al., 2002) and in a 3.25 m-high apple orchard (Mostade et al., 2008). The authors attributed this to poor adjustment of the tunnel to the crop size or to inadequate air-jet design. In medium-sized trees, however, the tunnel sprayer provided a similar level of apple scab or powdery mildew control (Cross and Berrie, 1993; Holownicki et al., 1997b; Panneton et al., 2001) compared with the standard sprayer. In addition, apple scab control was provided by a low annual amount of active ingredient using a tunnel sprayer for treatment applications in 5- and 6-year-old apple orchards 3 m-high composed of high scab-susceptible cultivars (Jamar et al., 2008).

The percentage of spray cover was significantly higher with the standard sprayer than with the tunnel sprayer, for both nozzles (Table VI.2). The stain diameter obtained with the tunnel sprayer for either nozzle was greater and the number of stains was fewer compared with the standard sprayer. As shown by Sierra et al. (2006), Derksen et al. (2007) and McArtney and Obermiller (2008), several factors, including carrier rate, turbulence and formulation as well as droplet size, can affect spray coverage. The number of stains per cm^2 was significantly lower with the TVI, in line with increased stain diameters. In most cases,

the TVI nozzles provided significantly higher spray deposits and lower spray cover in the canopy than the ATR nozzles, for both sprayers. There was evidence of an inverse relationship between spray cover rate and average droplet diameter, and a direct relationship between spray cover and number of droplets per cm^2. If differences in the micro- and macro-distribution occur with nozzle types, the consequences for biological effectiveness need to be determined. For some pesticides, at least theoretically, a coarser pattern of spray deposition on the leaf surface could be less effective biologically. Further investigation of the effects of spray quality adjustment on biological effectiveness is required. According to Allen et al. (1978), finer sprays are considerably more effective. Such sprays would therefore be preferable because the dose rate could be reduced, although finer sprays could lead to greater air-borne drift and spray losses.

Table VI.2 − Distribution of normalized spray deposits on artificial targets positioned beneath the tree canopy and in different sampling zones of the tree canopy

Nozzle	Sprayer	Beneath the tree canopy	Inside the tree canopy[a]			
		Zone W	Zone X	Zone Y	Zone Z	Mean X,Y,Z
Spray deposit (µg cm^{-2})[b]						
ATR	Standard	0.33 a[c]	0.79 ab	0.70 a	0.50 b	0.66 a
	Tunnel	0.61 b	0.77 a	0.84 ab	0.46 ba	0.69 a
TVI	Standard	0.37 a	1.03 c	0.94 b	0.79 c	0.92 b
	Tunnel	1.48 c	0.98 bc	0.82 ab	0.36 a	0.72 a
	P-value	***	**	*	***	***
Spray cover (%)						
ATR	Standard	18.1 a	46.9 b	51.2 c	36.5 c	44.9 c
	Tunnel	32.2 b	36.8 ab	45.6 cb	19.7 b	34.1 b
TVI	Standard	13.5 a	35.4 ab	35.9 b	29.8 c	33.7 b
	Tunnel	47.1 c	24.8 a	23.9 a	7.4 a	18.7 a
	P-value	***	**	***	***	***
Number of stains per cm^2						
ATR	Standard	294 d	231 c	227 d	273 d	244 d
	Tunnel	203 c	195 c	193 c	225 c	204 c
TVI	Standard	57 a	107 b	90 b	95 b	97 b
	Tunnel	79 b	60 a	69 a	47 a	59 a
	P-value	***	***	***	***	***
Average stain diameters (µm)						
ATR	Standard	187 a	189 a	179 a	173 a	180 a
	Tunnel	213 a	199 a	189 a	180 a	190 a
TVI	Standard	335 b	260 b	279 b	290 b	277 b
	Tunnel	312 b	410 c	364 c	409 c	395 c
	P-value	***	***	***	***	***

[a] Sampling zones according to height (zone W: from 0.0 to 0.2 m; zone X: from 0.8 to 1.0 m; zone Y: from 1.8 to 2.0 m; zone Z: from 2.8 to 3.0 m).

[b] Normalized fluorescein concentration in the sprayers was 1177.6 µg ml^{-1}

[c] In each experiment, values within columns followed by different letters are significantly different according to the Student-Newman-Keuls multiple range tests (*, **, *** significant at P≤0.05, 0.01, 0.001, respectively). Because of the back-transformation of the variable data sets, no SED (Standard Errors of Differences) values are available.
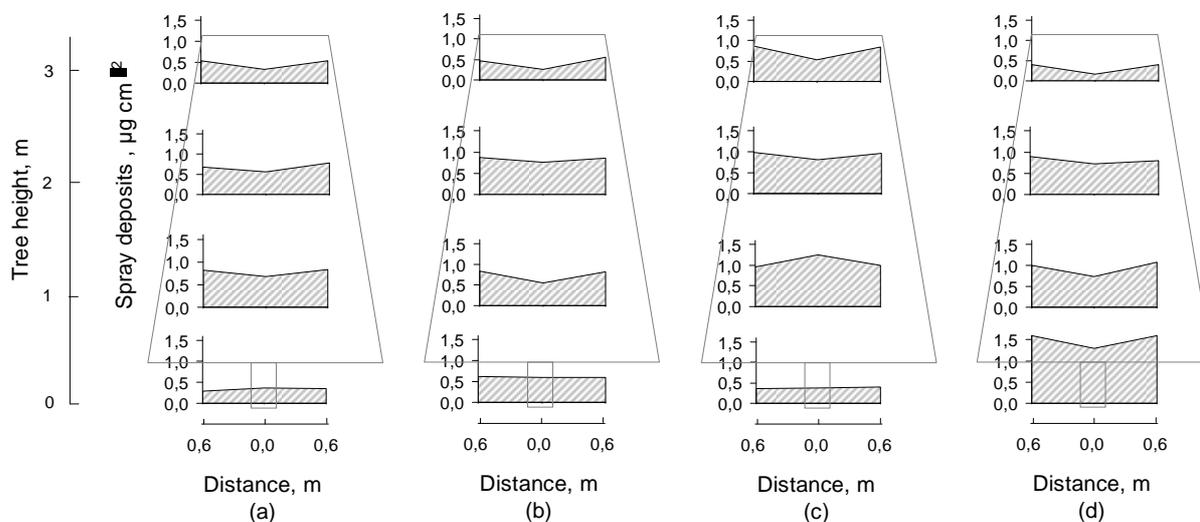
Fig. VI.2[1] : Profile of spray deposition on the soil and in the canopy on sampling lines normal to the track measured with the standard sprayer/ATR nozzles (a), the tunnel sprayer/ATR nozzles (b), the standard sprayer/TVI nozzles (c), and the tunnel sprayer/TVI nozzles (d), in apple orchard.

In most cases, a slightly lower spray deposit was registered in the centre of the canopy compared with the external part of the canopy for both sprayers and nozzles (Table VI.3), but these differences were not significant. This means that a relatively good degree of penetration was obtained with all modalities of treatments.

Table VI.3 − Distribution of normalized spray deposits in the centre and on the external part of the canopy

Nozzle	Sprayer	Spray deposit (μg cm−2)[a]			Spray cover (%)		
		Centre	Extern	Ratio C/E	Centre	Extern	Ratio C/E
ATR	Standard	0.56 a[b]	0.69 ab	0.81	34.3 dc	47.5 d	0.72
	Tunnel	0.56 a	0.72 ab	0.77	28.4 bc	35.5 dc	0.80
TVI	Standard	0.87 b	0.93 b	0.94	29.0 bc	34.8 dc	0.83
	Tunnel	0.60 a	0.75 ab	0.80	14.3 a	19.8 ab	0.72
	P value	***			***		

[a] Normalized fluorescein concentration in the sprayers was 1177.6 μg ml−1
[b] In each experiment, values followed by different letters are significantly different according to the Student-Newman-Keuls multiple range tests (*** significant at P≤0.001).

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[1] The figure VI.2 has been added to the thesis after acceptation of the manuscript "Comparative performance of recycling tunnels and conventional sprayers using standard and drift-mitigating nozzles in dwarf apple orchards" by the *Crop Protection* editors. Therefore, the corresponding illustration is not present in the original publication.

VI.3.2. Sedimentation drift

In the second experiment, the overall mean of the sedimentation drift was similar for all treatments, whatever the sprayer or nozzle. For all treatments, the average sedimentation drifts ranged from 5.8 to 9.1% of the total amount of the tracer sprayed (Table VI.4). This finding seems to be linked to the high canopy density at the full-leaf development stage, the high tree row volume value and the low wind speeds. Accordingly to Vercruysse et al. (1999) and Cross et al. (2001), spray losses on the ground are generally greatest in small tree orchards with poor foliage densities, and lowest in the large tree orchards with full foliage development.

The sedimentation drift distribution, however, was quite different depending on the sprayers and the nozzles. In comparison with the standard sprayer, the tunnel sprayer produced significantly greater sedimentation drift on the ground samplings beneath the row of trees and smaller sedimentation drift on the ground samplings downwind of the sprayer (2.5–10.5 m from the treated row of trees) (Table VI.4). Therefore, spray losses on the ground measured during the tunnel sprayer applications were restricted mainly to beneath the crop rows, confirming previous findings (Porskamp et al., 1994; Doruchowski and Holownicki, 2000; Planas et al., 2002). With the TVI nozzle, the tunnel sprayer greatly increased the sedimentation drift beneath the row of trees compared with the standard sprayer (Table VI.4). By contrast, with the standard sprayer the sedimentation drift beneath the tree rows was not significantly influenced by nozzle type. This suggests that the airflow characteristics of the tunnel sprayer are not well adapted to coarse droplet size spectrums, composed of heavier drops. New simulative methods have been developed to forecast appropriate air fluxes at the design stage. For example, Molari et al., (2005), use 'computational fluid dynamics' studies for checking sprayer performance and building an improved prototype of recycling tunnel sprayers with reduced losses on the ground and improved spray distribution on the canopy.

In contrast, for the application with the standard sprayer, the sedimentation drifts were significantly greater on sampling downwind of the sprayer compared with the tunnel sprayer. The TVI treatments increased the ground deposit downwind of the sprayer compared with the ATR treatments (Table VI.4). If there are differences in sedimentation drifts, the correlation with air-borne drift needs to be determined. In our experiments only the spray losses on the ground were evaluated, although during spray applications the spray that was discharged and not deposited on the canopy was lost either to the ground as fallout or to the air as air-borne drift. Cross et al. (2001) reported that the fine spray qualities resulted in more spray being lost as air-borne drift than with coarser spray, although the differences were not always significant.

Table VI.4 – Normalized sedimentation drift

Mean sedimentation drift (µg cm^{-2})$^{a\,b}$

Nozzle	Sprayer	Distance from the treated row of trees (m)						
		0.0	*1.25*	*2.5*	*4.5*	*6.5*	*8.5*	*10.5*
ATR	Standard	0.64 a^c	0.62 a	0.46 b	0.38 b	0.28 b	0.19 a	0.15 a
	Tunnel	1.60 b	1.07 a	0.33 a	0.16 a	0.16 a	0.13 a	0.13 a
TVI	Standard	0.69 a	0.91 a	0.95 c	0.69 b	0.45 b	0.17 a	0.16 a
	Tunnel	4.40 c	0.88 a	0.18 a	0.14 a	0.14 a	0.14 a	0.14 a
	P value	***	ns	***	***	***	ns	ns

Weighted mean sedimentation drift (µg cm^{-2})

Nozzle	Sprayer	Space from the treated row of trees (m)		
		0 – 2.5	*2.5 – 10.5*	*0 – 10.5*
ATR	Standard	0.59 a	0.29 b	0.36 a
	Tunnel	1.02 b	0.17 a	0.37 a
TVI	Standard	0.80 a	0.47 c	0.56 a
	Tunnel	1.59 c	0.15 a	0.48 a
	P value	**	**	ns

[a] Normalized fluorescein concentration in the sprayers was 5285.8 µg ml^{-1}
[b] Mean target space (free of vegetation) deposits were not significantly different among treatments and the average value was 3.14 µg cm−2 .
[c] Values within columns followed by different letters are significantly different according to the Student-Newman-Keuls multiple range tests (ns: nonsignificant, **, ***: significant at $P{\leq}0.01$, 0.001, respectively).

VI.4. Spray recycling

For the spray recycling experiment, the weather conditions at each assessment date are shown in Table VI.5. The recovery system, which included a continuous recycling process in the tunnel sprayer, led to an average of 30% being saved from the applied spray mixtures when spraying under moderate wind speed (≤ 2.5 m s^{-1}) in a 7-year-old apple orchard (Table VI.5). The level of spray saved depended greatly on the tree growth stages. The measured spray savings due to the recycling system varied from 22 to 38%, depending on leaf development stage and nozzle type, which accords with previous studies using various tunnel sprayer designs (Cross and Berrie, 1993; Holownicki et al., 1997b). It has been found that the recycling rate increases with higher spray volume rates and with decreasing driving speed and size of trees, and many researchers have reported a decrease in the recycling rate as the season progressed, following the leaf development stage (Doruchowski and Holownicki, 2000).

Table VI.5 − Recycling rate achieved with the tunnel sprayer using ATR and TVI nozzles during the 2008 season, in a 7-year-old apple orchard (applied volume was $350\,l\,ha^{-1}$)

Date	GS[a]	T[b]	RH[b]	Wind speed	Wind direction[b]	Recycled rate	
						ATR	TVI
		°C	%	m s^{-1}	°	%	%
10 April	D	13	60	2.1	75	36	38
18 April	E	15	55	1.6	89	35	36
25 April	F	12	58	2.4	55	31	35
16 May	H	15	74	1.9	120	24	32
13 June	I	17	71	1.6	102	23	26
04 July	J	18	68	2.3	42	22	24

[a] GS = Tree growth stages according to the Fleckinger-growth stage scale (F = full bloom)
[b] T = temperature, RH = relative humidity; Wind direction in relation to the sprayer track.

VI.5. Conclusions

This study showed that, under low wind speed, in comparison with the standard axial fan sprayer, the tunnel sprayer produced an equivalent spray deposit in all areas of the trees showing comparable vertical and horizontal macro-distribution uniformity, with the hydraulic hollow cone (ATR) nozzles, a nozzle that produces small droplets. With the air-induction (TVI) nozzles, however, a nozzle that produces large droplets, the tunnel sprayer produced a significantly lower spray deposit and a lower spray cover, especially in the top of the canopy, compared with the standard sprayer. At the same spray deposit level, the tunnel sprayer provided relatively lower spray cover on the canopy than the standard sprayer, whatever the nozzle type. At the same spray deposit level, the ATR nozzles provided significantly greater spray cover on the canopy than the TVI nozzles for both sprayers. The greatest spray deposit was obtained with the combination 'standard sprayer - TVI nozzles', although the spray cover rate was not the highest in this case.

The average sedimentation drifts ranged from 5.8 to 9.1% of the total amount of the tracer sprayed, whatever the sprayer or nozzle. In comparison with the standard sprayers, the tunnel sprayer produced a statistically comparable overall mean sedimentation drift, although the distribution of the sedimentation drift was quite different between the two sprayers. The tunnel sprayer limits the sedimentation drift beneath the tree rows and could, therefore, be used successfully in experimental design with reduced plot sizes. The TVI nozzles did not significantly improve the overall ground losses compared with the ATR nozzles, for both sprayers.

The spray mixture recycling rate in the tunnel sprayer varied from 38 to 22% over the growing season, showing high environmental sustainability compared with traditional machines.

These experiments indicate that the tunnel sprayer could be suitable for use with traditional hydraulic nozzles (ATR), but not with the air-induced nozzle (TVI), to make

applications on dwarf apple orchards up to 2.8 m high. The tunnel sprayer should be adapted to improve the performances and the spray distributions on the treetop, particularly in the presence of higher trees and drift-mitigating nozzles producing coarser droplets. Further studies are needed to clarify (i) differences in biological effectiveness between tunnel and standard sprayers and (ii) differences in canopy distribution and ground losses under higher wind speeds and less favourable climatic situations.

VI.6. Acknowledgements

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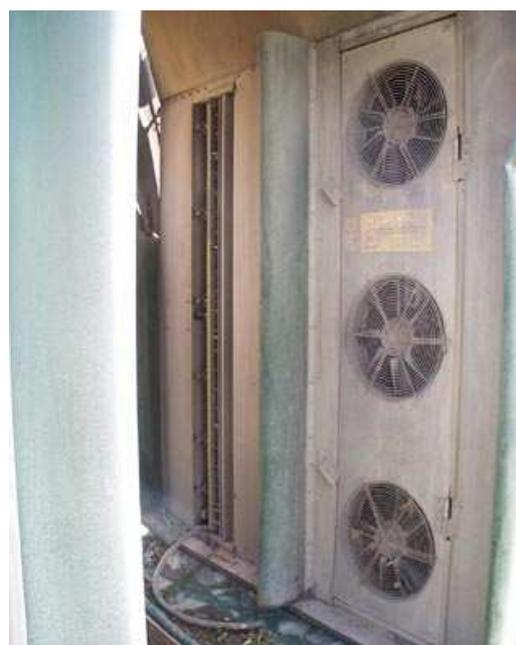
Figure VI.3. The recycling experimental tunnel sprayer (Munckhof, 5961 CV Horst, The Netherlands), including the so-called 'Closed Loop System Technology', used for treatment applications in the experimental orchard. For the air assistance system of the tunnel sprayer, the air is sucked from inside the tunnel, producing an under-pressure area which helps eliminate most of the forward or backward spray drift. Air-borne droplets are partly intercepted by the tunnel's special design features and partly sucked back in by six axial-flow fans for subsequent re-use. The recovered spray is sucked in at the bottom of the collector walls using a Venturi system and transported to the sprayer tank after filtering. The spray mixture recycling rate in the tunnel sprayer varied from 38 to 22% over the growing season, showing high environmental sustainability compared with traditional machines.

Chapter VII

Effect of organic farming practices on five orchard soil bio-indicators

Foreword

In the preceding chapters, innovative strategies for the control of apple scab were studied over six growing seasons, under organic management system. The straight effects of such practices on yield and quality were studied. Chapter V report high yield and quality of apple provided by the organic orchard. Management techniques such as cover crops, mechanical tillage for weed control and organic fertilizers play an important role in maintaining and enhancing soil productivity and are central to organic practices. These techniques are believed to encourage soil fauna and flora, improve soil formation and structure and create more stable systems. In turn, nutrient and energy cycling is increased and the retentive abilities of the soil for nutrients and water are enhanced, compensating for the non-use of mineral fertilizers (FAO, 2010). The experimental orchard offered the ideal opportunity to evaluate the effects of seven years of organic fruit production systems, on several soil biological and chemical properties in comparison with conventional practices applied next to the organic orchard. Even if the experimental design applied in this soil biological study is not based on a strict multisite replication system, experiments described in this part contribute strongly for better understanding of howmuch soil management practices could influence some soil bio-indicators. Also, orchard performances observed in the preceding chapters may be correlated to some aspects presented in this chapter. For these reasons, it was decided to integrate this soil study in the present Doctoral thesis.

This chapter has been published in a slightly modified version as:

VII Chapter VII : Effect of organic farming practices on five orchard soil bio-indicators.

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Abstract

The goal of the study was to compare the effects of seven years of conventional and organic fruit production systems on soil biological and mineral properties. The experiment was conducted at Gembloux, Belgium on two adjacent experimental orchards, both planted in 2002 on a flat site with a common cultivation history. The first orchard was managed following organic guidelines and the second one was under conventional management over seven growing seasons. Soil management practices in the organic orchard included additions of composted cattle manure and organic fertilizers and the use of mechanical tillage for weed control. Conventional soil management practices included adding synthetic fertilizers and using herbicides for weed control. Both orchards received different kinds of fungicides. In year 2 (2003), the soil methane oxidation process was measured in order to compare the orchards' soil biological activities. In year 5 (2006), the overall soil microbial activity was assessed by measuring, during five short-term experiments from May to October, the basal respiration (BAS), the substrate-induced respiration (SIR) and the *in situ* soil CO_2 efflux (CDE). Closed-dynamic-chamber systems were used to analyse the soil CO_2 efflux *in situ* in the orchards. In year 5 and 7 (2006 and 2008), earthworm abundance was assessed together with chemical soil parameters. No significant difference in the methane oxidation rate was observed between the two orchard management systems in year 2. In year 5, however, the BAS, SIR and CDE values were higher in the organic orchard than in the conventional one on most sampling dates. Total earthworm abundance was strongly improved by organic practices. Soil mineral analysis and soil pH values did not show important differences between the two orchard management systems.

Key words: biomass, earthworms, microbial respiration, organic fruit production, soil CO2 efflux, soil quality

VII.1.   Introduction

Intensive fruit production in Belgium has increased in recent decades in order to meet market demands, but concerns about the negative effect of these new fruit production practices on environmental factors have also increased. Although studies have found that alternative management practices might improve soil quality compared with conventional management practices, few studies have specifically compared the effects of conventional and organic management on soil quality in orchards (Glover et al., 2000; Mäder et al., 2002). Given the ecological benefits of soil biodiversity (Brussaard et al., 2007), soil organisms are key for estimation of agro-ecosystems sustainability (Cenci and Sena, 2006). It is therefore important to encourage agricultural practices that increase the abundance and diversity of soil organisms by enhancing habitat conditions or resource availability (Altieri, 1999). Sustained agricultural productivity might depend on the selection of management practices that enhance the soil biological activities involved in the fixation of atmospheric N, recycling carbon and nutrients, reducing soil pathogens, decomposing leaf litter, etc. Soil microbial activities lead to the liberation of nutrients available for plants and play an important role in biogeochemical cycling and stabilizing soil structure (Cenci and Sena, 2006; Brussaard et al., 2007). Management techniques such as cover crops and organic fertilizers play an important role in maintaining and enhancing soil productivity and are central to organic practices. These techniques are believed to encourage soil fauna and flora, improve soil formation and structure and create more stable systems. In turn, nutrient and energy cycling is increased and the retentive abilities of the soil for nutrients and water are enhanced, compensating for the non-use of mineral fertilizers (FAO, 2010). The objective of this study was to evaluate the effects of seven years of conventional and organic fruit production systems on several soil biological and chemical properties.

To estimate soil microbial activity, the methods can be divided into those intended for measuring respiration (i) in the field and (ii) in the laboratory (Bloem et al., 2006). Measuring soil respiration in the field is usually accomplished by covering a specific soil surface with a gas-tight chamber. During incubation for a specific time, under ambient climate conditions, changes in gas composition (CO_2 or O_2) are monitored. Field measurements are needed to assess the general microbial activity under natural conditions (Bloem et al., 2006).

Measurement of soil respiration in the laboratory is accomplished by measuring basal respiration (BAS) and substrate-induced respiration (SIR) (ISO 16072, 2002; Bloem et al., 2006; Dilly, 2006). BAS is the steady rate of respiration in soil, which originates from the turnover of organic matter (predominantly native carbon). It can therefore constitute an integrated index of the potential of the soil biota to degrade introduced organic substances under given environmental conditions. The SIR method is based on the principle that, under standardized conditions, the metabolism of glucose added in excess is limited by the amount of active aerobic micro-organisms in the soil. Thus, only glucose-responsive and active organisms are measured. It detects predominantly bacterial biomass, but the method has been calibrated to determine the total soil microbial biomass. BAS and SIR have been used in the laboratory for monitoring soil quality and for ecotoxicological risk assessment of soils contaminated with heavy metals or organic contaminants (Bloem et al., 2006).

Because of their interaction with soil, earthworm populations are greatly affected by agricultural practices, such as soil tillage, crop residues and the use of fertilizers and pesticides (Edwards and Bohlen, 1996; Paoletti et al., 1998; Peres et al., 2006; Holb et al., 2006, Jamar et al., 2009). So, earthworms may be used as bio-indicators of soil management because they are easy to rear and classify and are very sensitive to both chemical and physical soil parameters (Paolletti et al., 1998).

In summary, soil biological properties such as microbial biomass or activity, as well as earthworm abundance or diversity, have been reported to be major soil bio-indicators which can be used for soil quality evaluation (Paolletti et al., 1998; Glover et al., 2000; Bloem et al., 2006, Pompili et al., 2006; Peres et al., 2006; Brussaard et al., 2007).

VII.2.   Material and Methods

The experiment was conducted at Gembloux, Belgium on two adjacent 1-ha orchards. One was an organically-managed apple orchard and the other a conventionally-managed cherry orchard. They were both planted in 2002, on dwarfing rootstocks, on a flat site with a common cultivation history.

Soil management practices in the organic orchard included additions of 20 tons ha^{-1} of composted cattle manure in 2002 and 2005, 800 kg ha^{-1} year^{-1} of organic fertilizers (5.5/2.5/2.5), 1000 kg ha^{-1} year^{-1} of hydrated lime for pH management and the use of mechanical tillage for weed control, involving the 'Swiss-Sandwich-System' the first three years. Conventional soil management practices included applying 850 kg ha^{-1} year^{-1} synthetic fertilizers (7/9/16) and using herbicides for weed control (paraquat and diquat at 2 kg ha^{-1} three times per year, plus propyzamide at 1.5 kg ha^{-1} in years 5 and 6).

Disease control included an annual application of copper (3 kg ha^{-1}) and sulphur (40 kg ha^{-1}) in the organic orchard (Jamar et al., 2010). In the conventional orchard, disease control included an annual mean application of copper (10 kg ha^{-1}) and thiram (6 kg ha^{-1}). In addition, in the conventional orchard, tolylfluanide (1.25 kg ha^{-1}) and fenhexamide (0.5 kg ha^{-1}) were applied in year 5 and captan (1.2 kg ha^{-1}) was applied twice during the study. For pest control, full doses of granulosis virus and *Bacillus thuringensis* were applied twice a year in the organic orchard, and cyclofurine (0.015 l ha^{-1}), pyrimicarb (0.345 l ha^{-1}) and teletox (0.24 l ha^{-1}) were applied 4, 4 and 5 times during the study, respectively, in the conventional orchard.

In year 2 (2003), methane oxidation, an important soil ecological process, was measured in order to compare the orchards' soil biological activities (Seghers et al., 2003). Methanotrophs form a unique group of methyllotrophic bacteria that use methane as their sole source of carbon and energy (Hanson and Hanson, 1996). They can be divided into two distinct physiological groups. The 'Type I' methanotrophs are methylotrophs belonging to the gamma-proteobacteria and using the ribulose monophosphate pathway for formaldehyde assimilation. The 'Type II' methanotrophs belong to the alpha-proteobacteria and use the serine pathway for formaldehyde assimilation (Hanson and Hanson, 1996). The methanotrophic community structure was evaluated by group-specific Denaturing Gradient

Gel Electrophoresis (DGGE) analysis and based on methods reported by Peacock et al. (2001). The performance of the methane oxidizing bacteria was evaluated using a methane oxidation test described by Seghers et al. (2003). Each orchard system was sampled nine times.

In year 5 (2006), the overall microbial activity was assessed by measuring the BAS through O_2 consumption rate and the SIR through O_2 consumption rate after a glucose addition (ISO 16072, 2002; Bloem et al., 2006). Five assessments were performed during the growing season, from May to October. Each assessment included six replicates per orchard. For each replicate, 25 individual soil samples (0-15 cm) were taken with an auger in the intra-row parts and then mixed to make one composite soil sample that was analysed. Closed-dynamic-chamber systems were used to analyse *in situ* carbon dioxide efflux (CDE) due to soil respiration in the orchards (Perrin et al., 2004). The spatial variability of the soil efflux was measured over five short periods between May and October, based on a score of 60 points (30 points per orchard).

Earthworm abundance as a useful bio-indicator of agro-ecosystem sustainability was assessed together with chemical soil parameters in 2006 and 2008. All species were grouped according to the ecological classes of Bouché reported also by Paoletti et al. (1998): "Endogées", species inhabiting soil horizons, feeding on soil organic matter and "Aneciques", species living in burrows, feeding on surface litter or "Epigées", species living on surface litter. Both assessments included four and eight samples per orchard in 2006 and 2008, respectively. Earthworm abundance estimations were performed after rainy periods, using the mustard extract method described by Chan and Munro (2001).

Analysis of variance (ANOVA) was conducted to examine the relationships between individual biological measurements and orchard management systems, using SAS software. Unless noted otherwise, only results significant at p< 0.05 are discussed.

VII.3. Results

The PCR amplification reaction from bacterial DNA extraction indicated the presence of methane oxidizing bacteria of Type I within all soil samples, but the absence of methane oxidizing bacteria of Type II. No significant difference in methane oxidation rate was observed between the two orchard management systems in year 2 (2003) (data not shown). However, the DGGE image and the cluster analysis of the soil samples showed a distinguishable methanotrophic community structure in the organic soil samples compared with the conventional soil samples. This effect could be traced by molecular fingerprinting of the methanotrophic community. Various bands (phylotypes) were present from the organic samples, but were not visible from the conventional samples.

In year 5 (2006), however, apparent differences were detected for microbial activity in orchards subjected to different agricultural practices. The BAS, SIR and CDE values tended to be higher in the organic than in the conventional orchard on most sampling dates (Fig. VII.1). The CDE values were generally higher in the organic orchard than in the conventional orchard on all sampling dates (Fig. VII.1). The variability of CDE values was

Basal respiration

Substrate-induced respiration
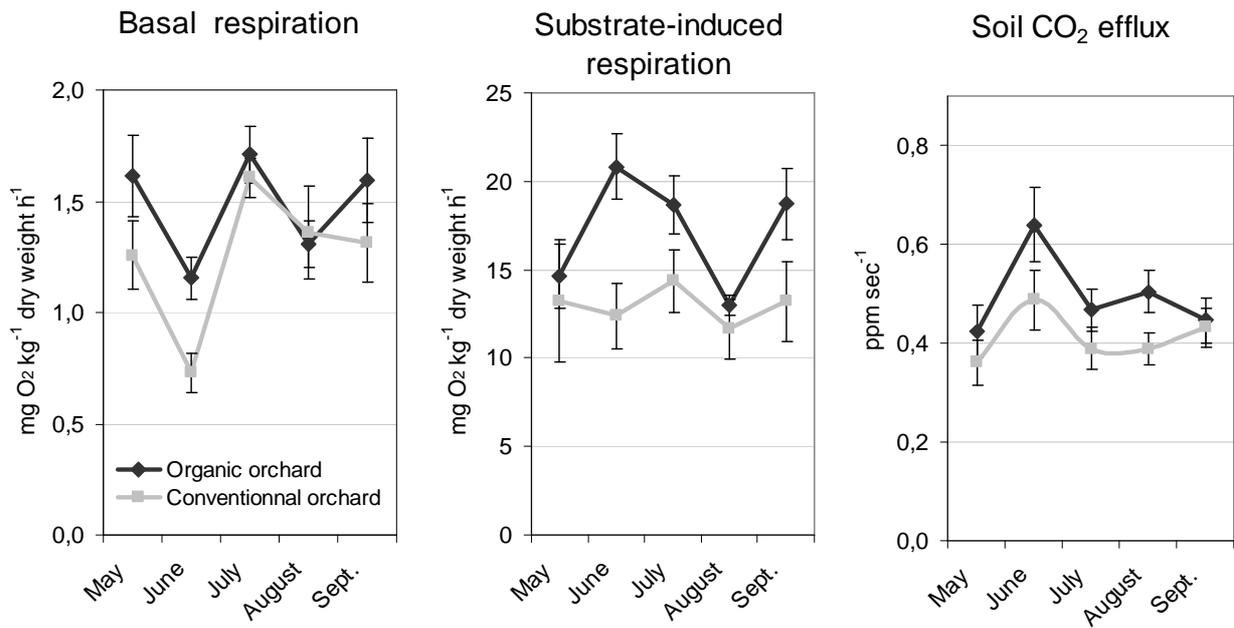
Soil CO$_2$ efflux

Figure VII.1. Basal respiration and substrate-induced respiration in soil samples and *in situ* soil CO$_2$ efflux, from two adjacent conventional and organic orchard soils in Gembloux, Belgium in 2006. Error bars indicate confidence interval of the mean (α= 0.05).

Epigéiques + Anékiques

Endogéiques

	2006	2008
■ Organic	174	272
□ Conv.	109	82

|	2006	2008
■ Organic	130	228
□ Conv.	73	39
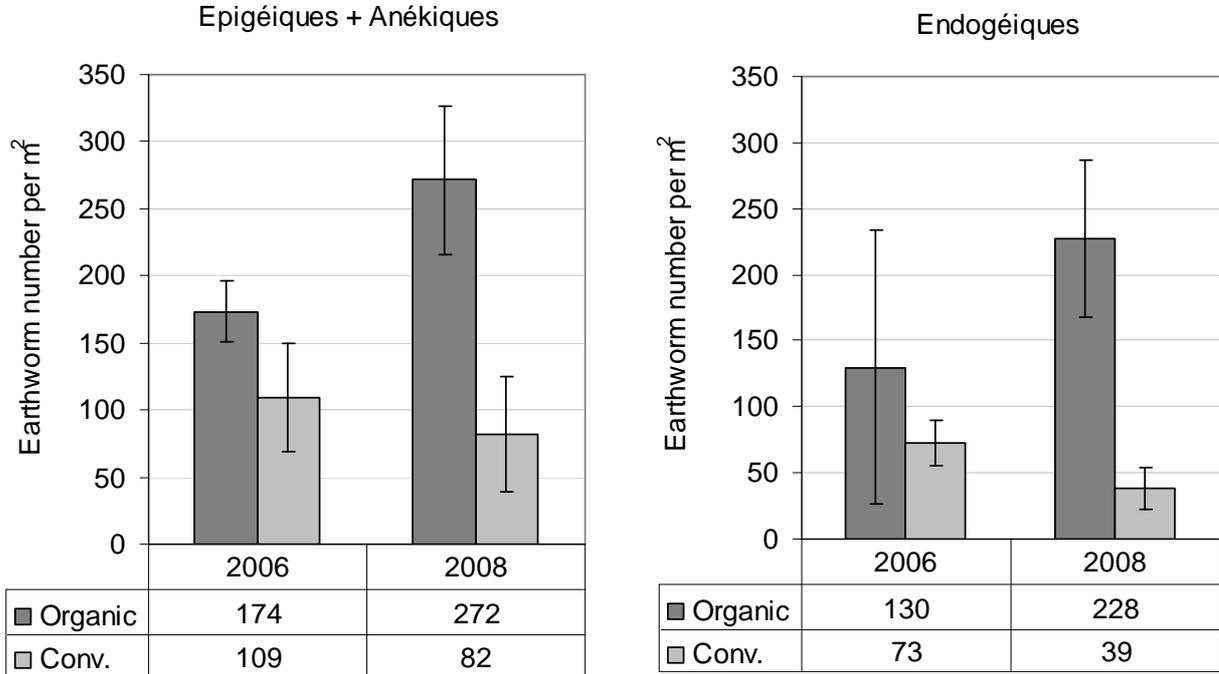
Figure VII.2. Mean number of earthworms per m^2 of orchard floor in conventional and organic management systems at Gembloux, Belgium in 2006 and 2008. Error bars indicate confidence interval of the mean (α= 0.05 and *n* = 4 and 8 in 2006 and 2008 respectively).

high in spite of similar measures of soil temperature and relative soil water content for both orchard samples (Jamar et al., 2008).

Total earthworm abundance was generally lower in the orchard managed with conventional practices (Fig. VII.2). The earthworm populations within soil varied between the two management systems, with the mean numbers in the organic plots always higher, whatever the ecological classes. Soil mineral analysis and soil pH values were similar within the two orchard management systems, except for the nitrogen, boron and copper elements in 2006 and 2008, as well as phosphorus and potassium elements in 2008 (Table VII.1 and VII.2). In both systems, soil analysis showed zinc deficiencies.

Table VII.1 − Major element contents of conventional and organic orchard soils at Gembloux, Belgium in 2006 and 2008

Year	Orch.	pH (KCl)	C %	N %	P[a]	K	Mg	Ca	CEC meq/100g
2006	conv.	6.3(0.2)[b]	1.15(0.1)	0.13(0.01)	26(9)	33(2)	17(2)	174(15)	10.6(0.5)
	org.	6.5(0.1)	1.05(0.1)	0.11(0.02)	23(2)	31(4)	26(9)	151(36)	9.8(0.4)
2008	conv.	6.7(0.2)	1.34(0.1)	0.13(0.02)	8(1)	34(2)	17(1)	199(9)	10.5(0.6)
	org.	6.0(0.2)	1.04(0.1)	0.10(0.01)	3(1)	10(2)	15(1)	153(8)	9.8(0.5)
Norm. range[c]		6.5-7.0	1.0-1.5	−	7-10	13-20	9-16	175-385	−

[a] For P, K, Mg, Ca, values are expressed in mg $100g^{-1}$.
[b] Values in brackets are the standard deviations of the means (n = 4 and 8 in 2006 and 2008 respectively).
[c] Normal range in this kind of soil (heavy loam) in Belgium.

Table VII.2 − Minor element contents of conventional and organic orchard soils at Gembloux, Belgium in 2006 and 2008

Year	Orch.	Fe[a]	Mn	Cu	Zn	Na	B	S
2006	conv.	260(33)[b]	229(20)	39.9(4)	6.2(1.1)	18(4)	0.42(0.2)	163(5)
	org.	242(28)	209(18)	26.8(5)	5.3(0.6)	15(3)	0.47(0.1)	163(7)
2008	conv.	265(31)	235(9)	24.0(3)	6.0(0.9)	30(7)	0.40(0.2)	160(6)
	org.	245(21)	215(16)	15.5(1)	5.3(0.4)	26(5)	0.43(0.1)	166(5)
Norm. rangec		120-180	80-110	7-11	9-13	31-61	0.5-0.9	100-500

[a] All elements are expressed in mg kg^{-1}.
[b] Values in brackets are the standard deviations of the means (n = 4 and 8 in 2006 and 2008 respectively).
[c] Normal range in this kind of soil (heavy loam) in Belgium.

VII.4.   Discussion

The organic management system applied over 2 years influenced the community structure of the methanotrophic bacteria. It seems, however, that the differences observed in the methanotrophic community structure were not reflected in the mean methane oxidation after 2 years of organic management.

The soil biological properties analysed in this study showed that the conventional management system applied over 5 years could affect non-target soil microbial activity compared with an organic management system in orchards. On the base of the present trials, the organically-managed soils exhibited greater microbiological activity than the conventionally-managed soils.

Being a very complex concept, a global evaluation of soil fertility is almost impossible and this is why fertility factors are generally reported in three distinct categories: physical, chemical and biological. The complex interaction of these three aspects makes up agronomic or integral fertility of soil, from which productivity depends (Pompili et al., 2006). The microbial fraction represents a really important component in soil fertility whose absence could make soil a simple mechanical support for plants. Measurements of microbial activity are actually included as indicators in many national and international monitoring programs on soil quality (Pompili et al., 2006). Soil is a living medium hosting an extensive biodiversity which can consist of more than 1000 species within one gram of soil. Respiration is probably the process that is most closely associated with life (Bloem et al., 2006). Soil respiration is attributed to a wide range of micro-organisms, such as fungi, bacteria, protozoa and algae. The soil fauna also make a significant contribution. Generally, the microbial contribution to the total release of CO_2 (excluding root respiration) is thought to be about 90%, compared with 10% released by the fauna. Although fungal biomass often dominates microbial biomass, the fungi:bacteria relationship with regard to respiration can vary considerably due to, for example, type of ecosystem or soil management (Bloem et al., 2006). But plant roots also contribute between 12% and 30% to the total release of CO_2 through respiration in the field (Bloem et al., 2006). Hence, field-based methods (measuring CDE) give the sum of respiration of all organisms (including roots), whereas laboratory methods (assessing BAS and SIR) give only the microbial respiration. Field methods are implemented under uncontrolled conditions and therefore often result in large spatial and temporal variations in gas fluxes.

Organic management greatly increased earthworm abundance in the present study, although soil tillage operations for weed control in orchards can reduce earthworm abundance (Paoletti et al., 1998). Earthworms are particularly sensitive to copper and zinc (Paoletti et al., 1998). Several studies reported negative effects on earthworm abundance following long term copper applications in perennial crops (Edwards and Bohlen, 1996; Paoletti et al., 1998). Van Rhee (1976) found that earthworms are almost completely eradicated from orchards if the soil copper concentration is >80µg/g. According to Paoletti et al. (1998), 'Endogées' are more sensitive to copper than 'Anecique' and 'Epigées' species, and disappear when the copper concentration is higher than 175 ppm. Although soil copper content, in our experiment, was significantly higher in the conventional orchard, under both systems soil copper and zinc content seemed to be kept below the estimated harmful level for earthworms.

In temperate regions, the earthworms, in terms of biomass, constitute the principal component of the total faunal biomass. They have a large influence on soil physical, chemical and biological properties and thus are considered as important agent for promoting soil fertility in agro-ecosystems (Peres et al., 2006). In particular, the common earthworm (*Lumbricus terrestris*) plays an important role in the leaf litter decomposition deposited on the orchard floor and can consume fruiting bodies of different fungi including *V. inaequalis* and thus significantly reducing primary scab inoculum (Niklas and Kennel, 1981; MacHardy, 1996; Paoletti et al., 1998; Glover et al., 2000).

Even if the orchard management system did not greatly influence soil chemical parameters in our study, positive effects on microbial and earthworm communities were observed in the organic orchard. These positive effects on soil bio-indicators might favourably shape the performance of important soil functions (Brussaard et al., 2007). But further studies are needed to better understand the effects of individual soil management practices on soil biodiversity functions. Only additional experiments will help to clarify this issue and to assess the real effect of the two types of farming practices on soil fertility and sustainable crop production. On the base of the present findings, organic management system seems the best farming approach to maintain soil quality with regard to biological indicators.

VII.5.   Acknowledgements

VII.6.   References

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Chapter VIII

Conclusions and prospects

VIII Chapter VIII: Conclusions and prospects

The fungal parasite *Venturia inaequalis* (Cooke) G. Wint, the cause of apple scab, is not life threatening to apple trees, but it is a continual threat to the apple market and apple industry. Apple scab is a major problem in apple-growing regions worldwide, particularly in temperate regions. The disease can affect 100% of the yield if no control measures are applied. In France, under reasoned chemical protection, which is used on the largest part of the French fruit growing area, apple orchards receive between 13 and 20 treatments only for the scab control (Sauphanor *et al.,* 2009). This state is similar in Belgium.

Most commercial apple cultivars are very susceptible to scab. The fungal *V. inaequalis*, is not an obligate parasite, but it is almost one. It also has a saprophytic, sexual phase on infected fallen leaves, when the host (*Malus x domestica*) is dormant, that is critical to its survival and to its success as a parasite. For scab control, organic apple production in Europe currently depends to a great extent on the use of copper fungicides.

The research detailed in this thesis has clearly defined measures for reducing the use of fungicides and, in particular, copper fungicides in organic apple production, under north-west European climatic conditions. Special emphasis was put on primary scab infection control in spring, looking for the best (i) timing of treatments, (ii) alternative anti-fungal products and (iii) technical option for treatment applications.

Why a research focused on organic fruit farming system?

In France and Belgium, as in most European countries, conventionnal fruit tree production consumes more than 25% pesticides used in agriculture whereas it only represents about 1% of the total agricultural surface area (Demeyere and de Turck, 2002; Codron *et al*., 2003). Apple orchards are the most intensively sprayed fruit crop, receiving e.g. in France an average of 37 treatments per year (Sauphanor *et al.,* 2009).

According to the report on pesticide residue monitoring in food of plant origin in Belgium (AFSCA, 2007), 6.9% of fruits and vegetables in the Belgian market exceeded the maximum residue levels set in the legislation and samples with detectable residues exceeded 55% in 2007. The risks from exposure to chemical mixtures, better known as the "cocktail effect" are today not assessed or managed (Kortenkamp and Faust, 2009).

Several recent studies have reported the negative impact of synthetic pesticides on the environment and on human health (Cenci and Sena, 2006; Brussaard *et al*., 2007; Lewis *et al*., 2009; Andersen, 2008; Benachour and Seralini, 2008; Bjørling-Poulsen *et al*., 2009; Costello *et al*., 2009; Orsi *et al*., 2009; Thompson *et al*., 2008; Veillerette, 2010; Wigle *et al*., 2009; Winchester *et al*., 2009; see also general introduction)

Chemical control under conventional fruit production system could become deadlocked. In France, for example, in a control apple orchard managed strictly in compliance with the

current French phytosanitary regulations, almost all the Granny Smith, Galaxy, Golden Smothee and Royal Gala variety fruit was unmarketable (Anon., 2008).

In the European context, demand for certified organically farmed fruits is rising sharply. Over the past decade, public concern about pesticide residues on fruit and in the environment coming from intensive fruit production has generated much interest in organic apple production.

According to the Codex Alimentarius Commission and all existing national regulations, "organic agriculture is a holistic production management system that avoids use of synthetic fertilizers, pesticides and genetically modified organisms, minimizes pollution of air, soil and water, and optimizes the health and productivity of interdependent communities of plants, animals and people."

FAO describes in a paper, 'Organic Agriculture and Food Security', presented at an International Conference on Organic Agriculture and Food Security (3-5 May 2007, Rome), recent models that suggest that organic agriculture has the potential to secure a global food supply, just as conventional agriculture does today, but with reduced environmental impact (FAO, 2007).

A recent Dutch study assumed that organic fruit farming performs better than conventional farming in the areas of environment, bio-diversity, and nature development (Spruijt-Verkerke *et al.,* 2004). According to the same study, the negative impact of conventionnal fruit farming on the environment is 20 times higher than organic fruit farming system.

A new model for optimal spray timing

The evidence presented in this work suggests a model for an efficient timing of treatments in apple orchards for primary scab control. This model consists of spraying only during the infection process in spring, with each potential primary infection period identified via a scab warning model (Figure VIII.1). This 'during-infection' spray strategy was tried out, for the first time, over six growing seasons, from 2003 to 2008, in a well-maintained experimental apple orchard, planted in 2002 at Gembloux in Belgium (Figure II.2 and II.3 in chapter II), following a multifactorial split-plot design based on six randomized blocks.

In order to determine the potential infection periods in the field, a computer-based weather recorder connected to a scab warning model was needed. The scab warning system calculated the infection periods based on hourly recorded meteorological data, the modified Mills table (MacHardy and Gadoury, 1989), the simulation of ascospore release and the effect of previously used sprays. In addition, in order to anticipate infection periods, the short-term local weather forecasts were registered every 6 hours by the scab warning model, for infection risk extrapolations.

In this strategy, treatments are applied during the infection process, after ascospore inoculation and before hyphal penetration which occurs at about 300 degree-hours (DH) of leaf wetness. In practice, treatments are applied between 50 and 300 degree-hours (DH) of leaf wetness, after the onset of contaminant rains (rains associated with infections). This

means that the treatments are applied just before or at the beginning of the infection risk periods detected by the scab warning system. At the starting point of the infections identified by the scab warning model, the ascospores have already been discharged (according to the scab warning model), susceptible young tissues are present, and the minimum temperature and leaf-wetness conditions for infection according to the revised Mills criteria are achieved, but penetration of the cuticle has not yet occurred (Figure VIII.1). Below 250 DH after rainfall, few ascopores reach the stadium of penetration, and a relevant portion will reach this stage only after 300 DH (Smereka *et al.*, 1987; MacHardy, 1996). Sprays below 300 DH can be applied during the infection process (before penetration), on germinating spores possibly already with appressoria but not yet with the primary stroma that allow the fungus to be protected by the plant cuticle. The poorer result registered with the delayed spray treatments showed that the timing of treatments should be close to the onset of infection.
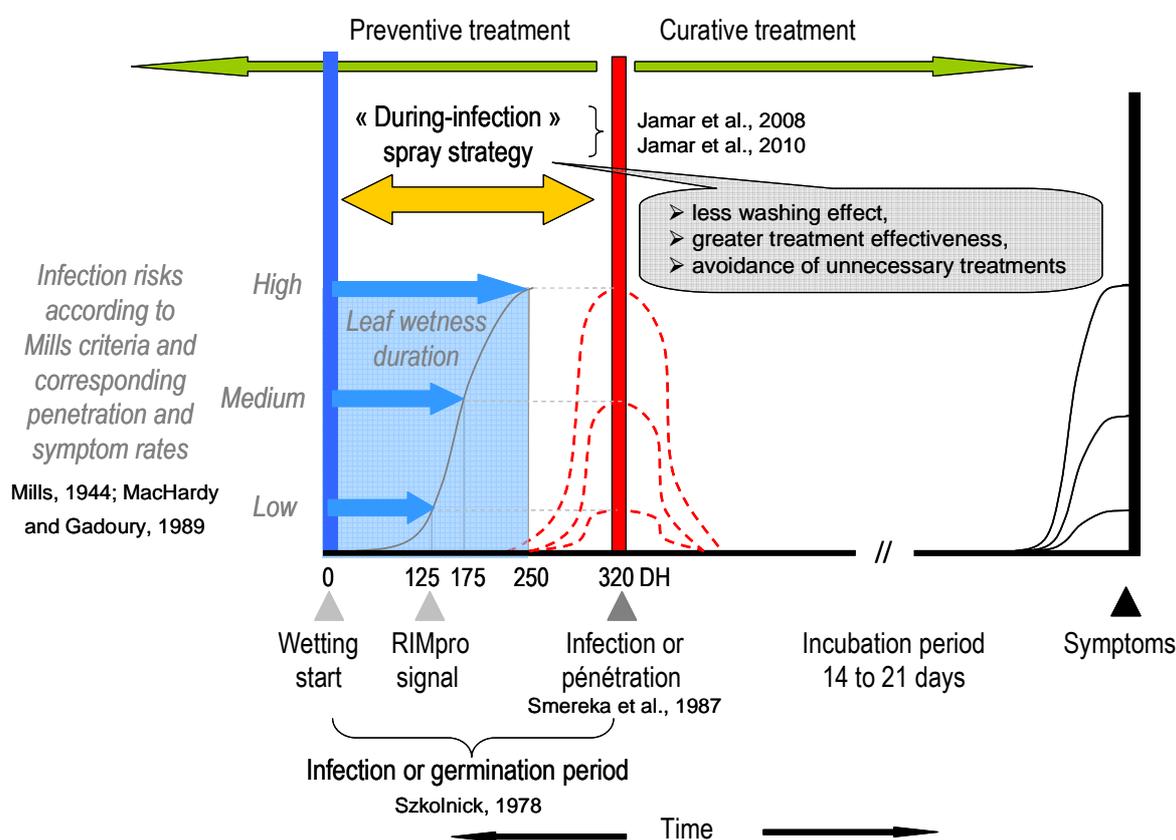

Figure VIII.1. Schematic illustration of the 'during-infection' spray timing (treatment applied 0−300 degree-hours [DH] after the wetting start) in relation to minimum leaf wetness duration, infection risks according to revised Mills criteria, fungus (*V. inaequalis*) activity and the approximate RIMpro infection starting point.

The results presented in this study show that the 'during-infection' spray strategy offers valuable advantages for effective apple scab control with a reduced amount of fungicide use, such as sulphur and copper, on high scab-susceptible cultivars under conditions conducive to scab infections. The most efficient spray treatments used for apple scab control in our experiments never exceeded 40 kg of elemental sulphur and 2.1 kg of copper per ha per year, applied in a maximum of 12 treatments per season. These amounts of fungicides are less than 50% below the amounts usually used to control apple scab in organic production under humid climate conditions (Holb *et al*., 2003; Holb *et al*., 2005).

The 'during-infection' spray strategy has several important advantages compared with the preventive (before rainfall) spray strategy; these include (i) reduced washing effect from the rain, (ii) greater treatment effectiveness on germinating spores, (iii) avoidance of unnecessary treatments (Holb *et al*., 2003) and (iv) the ability, in hot seasons, to apply treatments during less sunny periods. However, there are potential problems with this strategy, such as (i) the need to be able to adapt the spray timing to within a few hours of the onset of rain, which could mean adapting the spraying techniques in large fruit farms, (ii) the risk of delaying spraying during an extended rainy period, (iii) the risk of causing soil damage under wet conditions and (iv) the risk of spraying during windy weather that favours spraying drifts.

However, this technology, including the 'during-infection' spray approach, has not been widely adopted by organic growers, probably because of (i) the lack of an accurate local warning system, including weather forecast management at individual orchard level, (ii) the lack of adapted spraying techniques that allow spraying within a few hours of the onset of rain, mainly on large fruit farms, (iii) the lack of a balanced protection programme based on prophylactic or defensive practices limiting infection risks and (iv) the lack of expertise and knowledge leading to a full understanding of these concepts and methods.

Scab-resistance durability

Based on the apple scab symptoms present in the untreated plots of our experimental organic orchard, as well as those present in other local untreated orchards including cvs. 'Gala' and 'Golden Delicious', the following susceptibility ranking for the cultivars could be proposed for 2008: high to very high scab-susceptible cultivars for cvs. 'Pinova', 'Initial', 'Zvatava' and 'JN 20/33/58'; medium scab-susceptible cultivars for cvs. 'Pirouette' and 'Topaz'; and low scab-susceptible cultivars for cvs. 'Reinette Hernaut' and 'Reinette des Capucins'. In our experiments, only the polygenic resistance cultivars showed long-term scab resistance.

Very few scab infections were recorded on the untreated monogenic *Vf* scab-resistant cultivars up to 2007, except for the cv. 'Initial' that had already presented scab symptoms since 2003, as a result of the appearance of new scab races (strains) virulent to the *Vf* gene, almost since the plantation year. But in 2008 and 2009, scab infections were very severe on all *Vf* scab-resistant cultivars, indicating that the *Vf* major gene protection had been completely broken down by new virulent races. In 2008, cv. 'Topaz' was the only cultivar that still expressed good residual resistance after the *Vf* gene breakdown. Under our

experimental conditions, the treatments did not completely prevent the propagation of these new virulent scab races from 2006 onwards, showing the great virulence of these scab races.

The scab-inoculum pressure increased from year to year in the experimental orchard, which could have resulted in the presence of untreated control plots randomly distributed in the orchard since 2002. This could partly explain the poorer treatment effectiveness observed under a heavy scab epidemic in 2008 at the end of the experiment.

Before human beings interfered with the natural ecosystem, sexual reproduction, along with horizontal and ontogenic resistance systems, was how apple trees kept the infection rate low enough to survive and produce reasonable yields (i.e., for a seedling to grow and produce fruit). Outbreeding (self-incompatibility) is the host strategy to diversify, as much as possible, the alleles that reduce parasite fitness. Currently, apple cultivars are propagated vegetatively, and commercial orchards are planted mainly as monoculture; therefore, the sexual process that produces seedlings with new combinations of resistance genes, some of which may have greater resistance to the local pathogen population, is circumvented. In contrast, *V. inaequalis* reproduces sexually each year, and each year it has multiple opportunities to infect and reinfect the same cultivar with new genotypes, some of which may have improved parasitic and biological fitness factors. This situation clearly favours change in the parasite, potentially at the expense of the host.

Plant defence against pathogen

With regard to the very low scab-susceptible cultivar cv. 'Reinette des Capucins', which contains polygenic resistance traits, sprays based on sulphur and copper led to a significant increase in yield per tree, especially in the years with high scab pressure (2005, 2006 and 2008) (Chapter V and VI). Such positive effects on yield cannot be explained by the control of apple scab or other apple diseases such as powdery mildew (*Podosphaera leucotricha*), because in all years the infection levels in untreated plots were very low, even at a later stage. There are two possible explanations for this: (i) triggering defence responses and resistance mechanisms burns up energy in plants (Treutter, 2005) which is not necessary to use in treated plants (Doehlmann *et al.*, 2008), (ii) the application of elemental sulphur to crops is increasingly advocated as a way of overcoming deficiency in this key nutrient, and sulphur deficiency has recently become a widespread nutrient disorder in crops, largely due to the reduced rate of fossil fuel burning (Williams and Cooper, 2004).

Replacement of copper for scab control

The effectiveness of 60 products against apple scab was studied on seedlings under controlled conditions (Table III.1). The trials included natural mineral, organic and biological compounds from which anti-fungal activity against scab was, in many cases, never studied. The aim of these preliminary experiments was to prepare further field experiments conduced in a modern apple orchard system, including an optimum ecological balance.

Preliminary *in vitro* experiments with sodium, ammonium and potassium bicarbonate, as well as sodium and potassium silicate used at 1%, reduced colony growth of *V. inaequalis* by 99, 98, 89, 90 and 92%, respectively, which clearly demonstrated the direct anti-fungal properties of various bicarbonate salts (Chapter III).

Greenhouse experiments conducted on seedlings under controlled conditions showed that, among the 60 products used, several alternative products were particularly interesting as copper replacements for scab control: Armicarb®100 (US potassium bicarbonate formulation) (Chapter III), Quiponin (*Quillaja saponaria* extract), lime sulphur (calcium polysulfide), Prev-B2 (orange peel extract), Citripur (grapefruit seed extract), Norponin (*Yucca schidigerra* extract), Teawet TQ (*Camellia oleifera* and *Chenopodium quinoa* seed extract). All products, at 1% concentration, controlled apple scab, with more than 98% effectiveness under protective applications. In addition, the lime sulphur and Prev-B2 showed fairly good curative activity and were effective until 432 DH after scab artificial inoculation. The lime sulphur was far less washed away by the rain compared with other sulphur formulations (Jamar, 2007). As one of the oldest fungicides, however, its use has not been allowed in Belgium since 1970.

In another set of experiments, on grafted potted plants, it was demonstrated that the efficiencies of various fungicide compounds was greater, in most cases, on partially scab-resistant cultivars compared with susceptible cultivars. The applied active ingredient doses could be favourably adapted to the degree of cultivar resistance (Jamar, 2007).

The long-term field study clearly showed that: (i) the 'during-infection' spray strategy allows a reduction in the use of copper fungicides for primary scab control; (ii) the use of lime sulphur can significantly reduce or suppress the copper used in a spray treatment based on a 'during-infection' spray strategy; (iii) the amount of copper used for scab control could be significantly reduced for medium and low scab-susceptible cultivars compared with high scab-susceptible cultivars; (iv) potassium bicarbonate significantly reduces apple scab and is, in some cases, as effective as wettable sulphur; (v) natural plant extracts (from orange peel, soapbark tree, tea seed, quinoa seed and grapefruit seed) significantly reduce apple scab; and (vi) the fungicide doses and frequencies used in this study are not phytotoxic, do not adversely affect yield and do not leave undesirable residues on fruits and soils (Chapter IV and V). In addition, the results of this study showed that: (i) diluted copper is very effective under cold weather conditions during early primary scab infection periods; (ii) the lime sulphur treatment can be as effective as the combined wettable sulphur and copper treatment; and (iii) the lime sulphur is more effective than wettable sulphur used alone, confirming the results of previous studies (Holb *et al*., 2003).

The study showed that some new compounds, especially potassium bicarbonate and five new plant extracts, have an effect on *V. inaequalis* infection and therefore have the potential, in combination with other compounds, to reduce copper treatments during both the primary and secondary scab seasons. Finally, this work highlighted the fact that our experimental control did not always reach the external fruit quality level desired by the commercial market, and several practical issues need to be addressed before these products can be considered as a useful alternative to copper. The potential and limitations of potassium bicarbonate used to control apple scab in the field were discussed at length.

Impact of treatments on the predatory mite population

The absence of the phytophagous mite *P. ulmi* and the very low density of *A. schlechtendali* during all the growing seasons in the experimental organic orchard could be associated with the very high density of the predator *T. pyri* observed throughout the orchard (Chapter V and VI). The slight and temporary reduction of *T. pyri* on treated plots in June might be correlated with periods with higher treatment frequencies. The treatments might have had harmful effects on *T. pyri,* but the reduction of *T. pyri* during these treatment periods might also be due to the decrease in prey availability. Some studies have reported that sulphur compounds can have harmful effects on phytoseiids. However, other studies have reported predator mite population tolerance of treatments with sulphur compounds, probably due to the development of tolerant strains with an acquired resistance to sulphur. Similarly, another study showed that bicarbonate salts did not reduce predator mite numbers or disrupt biological mite control.

Using the tunnel sprayer for treatment applications

The use of tunnel sprayers should be encouraged because they can potentially reduce pesticide input and drift in orchards. Apart from environmental concerns, the benefit of tunnel sprayers would be to reduce plot size in treatment trials using complex experimental designs following the EPPO guidelines. They could allow a smaller plot size to be used in multifactorial trials in which fully randomized or randomized block designs are recommended.

In our experiments, the effectiveness of inorganic fungicides for the control of primary apple scab infections was studied in apple orchards including complex experimental designs. A tunnel sprayer machine was used for treatment applications. However, the effectiveness of plant protection products applied with tunnel sprayers could not be reliably assessed without a thorough investigation into spray distribution in tree canopies.

The study showed that, under low wind speed conditions, in comparison with the standard axial fan sprayer, the tunnel sprayer produced an equivalent spray deposit in all areas of the trees showing comparable vertical and horizontal macro-distribution uniformity, with the hydraulic hollow cone (ATR) nozzles, which produce small droplets. However, with the air-induction (TVI) nozzles, which produce large droplets, the tunnel sprayer produced a significantly lower spray deposit and a lower spray cover, especially in the top of the canopy, compared with the standard sprayer (Chapter VI).

At the same spray deposit level, the spray cover in the canopy, estimated by image analysis, was relatively better with the standard sprayer than with the tunnel sprayer. At low wind speeds, the sedimentation drift varied on average from 5.8 to 9.1% of the total sprayer output, irrespective of the type of sprayer or nozzle. The overall mean of the sedimentation drift was not significantly different for the two types of sprayers. The recovery system, which included a continuous recycling process in the tunnel sprayer, led to average savings of 28 and 32% of the applied spray mixtures for the ATR and TVI nozzles, respectively. The tunnel sprayer might therefore be suitable for small-scale apple orchards when fitted with traditional ATR nozzles rather than with air-induced TVI nozzles.

In conclusion, the canopy spray distribution provided with the tunnel sprayer was not significantly better than with the standard sprayer (when using conventional hydraulic hollow cone nozzles). Consequently, the general performance obtained in plant protection product experiments within the framework of this thesis cannot be attributed to the specific characteristics of the tunnel sprayer.

Impact of organic farming on some soil bio-indicators

The orchard management system did not greatly influence soil chemical parameters in our study. However, positive effects on microbial and earthworm communities were observed in the organic orchard in comparison with the conventional one. These positive impacts on soil bio-indicators might have a favourable effect on the performance of important soil functions (Brussaard *et al*., 2007). Given the ecological benefits of soil biodiversity, soil organisms are key for assessing the sustainability of agro-ecosystems (Cenci and Sena, 2006).

In temperate regions, earthworms in terms of biomass constitute the principal component of the total faunal biomass. They have an important influence on soil physical, chemical and biological properties and thus are considered as an important agent for promoting soil fertility in agro-ecosystems (Peres *et al*., 2006). In particular, the common earthworm (*Lumbricus terrestris*) plays an important role in the leaf litter decomposition deposited on the orchard floor and could consume fruiting bodies of various fungi, including *V. inaequalis,* and thus significantly reduce primary scab inoculum (Niklas and Kennel, 1981; MacHardy, 1996; Paoletti *et al*., 1998; Glover *et al*., 2000).

This study has shown that the evolution of earthworm abundance was positive from 2006 to 2008 in the organic orchard. Organic management greatly increased earthworm abundance in comparison with conventional management, although soil tillage operations for weed control in organic orchards can reduce earthworm abundance (Paoletti *et al*., 1998).

Management techniques such as cover crops and organic fertilizers play an important role in maintaining and enhancing soil productivity and are central to organic practices. These encourage soil fauna and flora, thus improving soil formation and structure and creating more stable systems. In turn, nutrient and energy cycling is increased and the retentive abilities of the soil for nutrients and water are enhanced, compensating for the non-use of mineral fertilizers (FAO, 2010). Soil microbial activities lead to the liberation of nutrients available for plants and play an important role in biogeochemical cycling and in stabilizing soil structure (Cenci and Sena, 2006; Brussaard *et al*., 2007). Although studies have found that alternative management practices might improve soil quality compared with conventional management practices, few studies have specifically compared the effects of conventional and organic management on soil quality in orchards (Glover *et al*., 2000; Mäder *et al*., 2002).

Further studies are needed to better understand the impact of individual soil management practices on soil biodiversity functions in orchards. Only additional experiments will help to clarify the real impact of the two types of farming practices on soil

fertility and sustainable crop production. On the basis of the present findings, organic management systems seem the best farming approach for maintaining soil quality with regard to biological indicators.

Fertlization, vegetative growth and scab-susceptibility

In our experiments, annual nitrogen fertilization was maintained from 50 to 60 U ha^{-1}, only from organic sources, in order to maintain a balance between vegetative growth and fruiting (Jamar *et al.*, 2010). Trees were grown vigorously until the canopy fills the allotted space. Thereafter, lower nitrogen fertilizations were applied to keep the trees calm with a balance between vegetative growth and fruiting. The fertilization rates were kept compatible with the pruning system, tree training, tree spacing, soil feature, rootstock and cultivars. The Swiss 'sandwich' system, involving a clover strip below the tree rows, was successfully adopted during the first 3 years (Figure II.2).

The successful management of apple trees in all apple-planting system depends on maintaining a balance between vegetative growth and fruiting. If vigour is too low, excessive fruiting results, fruit size declines, biennial bearing increases and trees fail to fill their allotted space soon enough to make the orchard profitable. If vegetative vigour is excessive, the flowering and fruiting are reduced and containment of the tree to the allotted space becomes problematic. Pruning and tree-training strategies are the primary management methods, along with fertilization strategies that are used to achieve this balance between vegetative growth and cropping throughout the orchard's life (Robinson, 2003).

Many mature high-density orchards receive excessive nitrogen fertilizer rates, which cause severe canopy management problems. 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 *Venturia 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.

Further investigations are needed to meet the optimal balance for micro and macronutrient status in environmentally friendly apple orchards and to find the rootstock-cultivar combinations with high physiological efficiency adapted to low-input fertilization level. Improved nutrient-uptake abilities, tolerance to weed competition, sufficient anchorage without staking, should be important tree's traits from the point of view of the organic fruit growers (Weibel and Häseli, 2003). In a study carried out in Hungary, Holb *et al.* (2009) show that the lower nutrient content of soil and also the generally poorer uptake of N, P, and K nutrients generally found in organic apple orchards could result in higher production risk in the organic apple orchards compared to that in integrated ones.

Sufficient quantities of mineral nutrients can contribute to the resistance against pests and diseases in plants. For example, a potassium deficiency leading to high N/K value in apple trees could increase scab susceptibility (Chaboussou, 1980).

Orchard biodiversity and pest management

During this study, mainly focused on the effects of scab control strategies, it was imperative to keep all other pests and diseases below a damage threshold, in order to limit interference on the yield and the productivity. The codling moth (*Cydia pomonella*) and the rosy apple aphid (*Dysaphis plantaginea)* are the biggest pests in apple orchard throughout the world, severe infestations can often been seen in organic apple orchards.

In our experimental orchard planted in 2002, the codling moth was easy controlled by the confusion method, allowed in organic fruit production. Very little rosy apple aphid damage was observed throughout the orchard's life; even if none insecticidal treatments were applied during this period. The control of the rosy apple aphid seems to be achieved by the abundance of predatory fauna present in the orchard, likely related with the presence of 20% of ecological zones situated inside the orchard, between experimental blocks (Figure II.2). These ecological zones are notably composed of *Corylus avellana, Sambucus nigra, Sambucus aurea and Sambucus laciniata* and twenty flower species. Besides, optimal nutrient and mineral balance in plants could affect the performance of the aphids as suggested below.

Recent studies have shown that plant resistance to insect pests is linked to optimal physical, chemical, and, perhaps most importantly, biological properties of soil. Several researchers have reported lower numbers of pest insects on crops grown with organic compared with synthetic sources of fertilizer. Subsequent experiments supported the mineral balance hypothesis suggesting that the organic matter and microbial activity associated with organically managed soils provide a buffering capability to maintain optimal nutrient and mineral balance in plants, which in turn affects the performance of phytophagous insects (Zehnder *et al.,* 2007).

The preservation and promotion of biodiversity within orchards and their boundaries are considered today as a favourable way to control pests in apple orchards. The key role of natural enemies has been demonstrated for psyllids, mites, aphids, leafminers in apple and pear orchards (Boller *et al*., 2004). However, a recent literature review (Simon et al., 2010), show that the effects of the manipulation of plant diversity and habitats on the control of pests by arthropod and bird communities in apple orchards were mostly positive (55% cases), or null (30%), but also negative in some cases (15%). This finding reveals the difficulties of identifying selected plants assemblages for the control of key pests and choosing the optimal orchard design for organic or low-input system.

The structure of the arthropod community differs among high- and low-intensity pest management regimes, and the natural control of some apple pests may altered under intensive management regimes: the beneficial arthropod complex is thus no longer present for ecosystem services (Suckling *et al.,* 1999; Simon *et al.,* 2007; Jamar *et al.,* 2010). Enhanced ecosystem services for pest control permitted by a reduction in pesticide exposition such as in organic or low-input orchards illustrate the mutual benefits between conservation biological control and a reduced pesticides use (Brown, 2001; Jamar *et al*., 2008; Simon *et al*., 2009).

Because birds occupy a high or top position in the food-web, they constitute bio-indicators which are used to assess the effect of cultural practices in the environment. Several studies show that bird communities are more abundant and diversified in organic apple orchards, or to a lesser extent in IPM orchards, than in conventional intensive ones where the number of insectivore species is also lower (Bouvier *et al*., 2005). Interest of birds for pest control is seldom demonstrated but Mols et al. (2005), showed that all species of Tits may reduce fruit damage caused by codling moth larvae, as they feed their brood with Lepidoptera caterpillars.

The reduction use of pesticides or use of more specific target pesticides could lead to the resurgence of secondary pests as Apple blossom weevil (*Anthonomus pomorum*) and Apple Sawfly (*Hoplocampa testudinea*) (Watteau *et al.,* 2011). Further researches are therefore needed to identify the processes involved on different scales for biological control; orchard systems should be re-designed to optimize ecosystem services provided by biodiversity and low-input cultural practices. Also, cropping systems based on carefully designed species mixtures reveal many potential advantages such as (1) higher overall productivity, (2) better control of pests and diseases, (3) enhanced ecological services and (4) greater economic profitability (Malézieux *et al*, 2009).

Prospects

The results of this work identified some low scab-susceptible cultivars as well as moderate scab-susceptible cultivars, for which efficient apple scab control using a reduced amount of fungicides is promising under organic farming systems. However, most of these cultivars present major problems with regard with other agronomic characters. Most growers adopting organic farming systems today expect to be able to use new cultivars with durable resistance to scab. Current research requirements include breeding for strengthening the host's natural (horizontal) resistance. The strategies to achieve durable resistance are to (i) assemble several quantitative, minor-effect resistance loci, (ii) combine horizontal and vertical resistance genes in a single cultivar and eventually (iii) pyramid several exotic major resistance genes. The rationale is that the pathogen would have to mutate several avirulence genes to virulence genes before it could successfully infect a cultivar with such pyramid genes.

Although there is no evidence that modern orchards are under greater disease pressure (more vulnerable to scab) than orchards 100 years ago, the severe outbreak of scab that follows a missed application or incorrect application rate of fungicide does indicate the very high potential for a scab epidemic that exists in orchards today. In the early 1900s, three fungicides sprays, probably not as efficiently applied as today, provided acceptable control of scab, whereas today missing one spray during the peak ascospore season in a schedule, which might include 10-18 sprays, can result in an unacceptable level of scabbed fruit (MacHardy *et al*., 2001). However, there is no evidence that *V. inaequalis*, considered as a population entity, has become more aggressive. Scab lesions are not larger today than they were 100 years ago, and the pathogen is still restricted to the subcuticular space until leaf fall, with little noticeable damage to underlying cells. According to MacHardy *et al.* (2001),

the major effect of modern apple production practices on scab epidemiology results from an increase in the proportion of successful infections (an increase in infection efficiency, caused by an increase in the proportion of compatible ascospores in an orchard or region). The general increase in the infection efficiencies in the actual apple production system seems to be attributed to:

- the genetic uniformity in modern orchards planted in high density with one or very few cultivars, mainly with poor scab-resistance characteristics
- the sexual reproduction of *V. inaequalis*, which leads, each year, to new genotypes with improved parasitic and biological suitability
- the herbicide-treated strips within tree rows and repeated mowing provide an unobstructed pathway for discharged ascospores to reach the tree canopy,
- the semi-dwarfing and dwarfing rootstocks and the new pruning techniques which put the tree canopy close to the inoculum source.

We do believe that by increasing host genetic diversity in orchards through cultivar selection based on our understanding of pathogen race evolution in orchards could lead to improve scab management.

The present work has proposed a general timing of treatments ('during-infection' strategy) in apple orchards leading to very efficient primary scab control. However, poorer treatment effectiveness was observed at the end of the experiment, in 2008 (Chapter V). A quantitative assessment of the primary inoculum showed that the scab-inoculum pressure increased from year to year in the experimental orchard, which could result in the presence of untreated control plots randomly distributed in the orchard since 2002 and the concomitant absence of efficient sanitation practices during those years. A low disease level at any given point in time during the growing season is needed when the management aim is not only to prevent infection on the fruits but also to keep inoculum pressure at a low level (Holb *et al.*, 2005).

Until now, no study has linked the combination of an accurate scab fungicide schedule, such as the 'during-infection' spray strategy, with well-developed sanitation practices that reduce the inoculum dose available to cause infection. Sanitation practices that reduce the population density of *V. inaequalis* indirectly by their action on the leaf litter might remove the leaf litter, render it unsuitable for the saprophytic and sexual activities of *V. inaequalis* that occurs in dead leaves, or prevent discharged ascospores from escaping during spring into the orchard air (Jamar *et al.*, 2011). Examples include (i) physically removing fallen leaves from the orchard using machinery, (ii) raking and then ploughing the leaf litter into the soil, (iii) raking and then burning the leaf litter (iv) shredding the leaf litter with a flail mower (Figure VIII.2, demonstration day in the frame of the Interreg IV TransBioFruit program[1]), (v) treating the leaf litter with chemicals that hasten decomposition (by making it

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[1] The TransBioFruit project (Increasing Cross-border Expertise in Organic Fruit Production) is implemented within the context of the Interreg IV France-Wallonia-Flanders program (2008-2012), with the support of the European Regional Development Fund (ERDF), the Regional Nord Pas-de-Calais Council, North Departmental Council, Pas-de-Calais Departmental Council and the Regional Government of Wallonia. The overall objective of this project is to develop a network of producers and support organizations in the two regions to stimulate and develop expertise, experience and innovation in addressing organic fruit production issues in the cross-border area. The Walloon Agricultural Research Centre (CRA-W) is involved mainly in the activities focused on developing

more vulnerable to attack or by increasing the population density of decomposers), (vi) treating the leaf litter with microorganisms that decompose the leaves or grow on the leaf surface and physically prevent discharged ascospores from escaping into the orchard air and (vii) utilizing earthworms to bury leaves. Practices that act directly on *V. inaequalis* may restrict or prevent pseudothecial production and maturation or prevent ascospore maturation and discharge. Examples include (i) treating scabbed leaves with chemicals or plant extracts that suppress the development of pseudothecia and ascospores and (ii) treating scabbed leaves with antagonistic (e.g., hyperparasitic) microorganisms (MacHardy, 1996).

Other innovative systems aimed at reducing leaf litter and therefore scab inoculum, such as artificial rains during dry periods in spring, integration of sheep or bird rearing in orchards, would be original ways of investigation. Some fruit growers in Europe have already started with the introduction of sheep into orchards. This practice offers also a novel way of reducing the use of herbicides known to contaminate ground and surface water in many countries (Geddes and Kohl, 2009).

On the basis of the present findings, organic management systems seem a very good farming approach for maintaining soil quality with regard to biological indicators. This positive impact on soil bio-indicators might have a favourable effect on the performance of important soil functions. But further studies are needed to better understand the impact of individual soil management practices on soil biodiversity functions. Only additional experiments will help to clarify this issue.

All treatments used in this study were applied at low spray rate of 300 l of water per ha. Whether higher spray rates for treatment applications could improve the effectiveness of treatments, which are based mainly on contact fungicides, still has to be investigated. Despite emerging data about the tunnel sprayer performances, further studies are needed to clarify (i) differences in biological effectiveness between tunnel and standard sprayers and (ii) differences in canopy distribution and ground losses under higher wind speeds and less favourable climatic conditions.

In conclusion, combining multiple factors such as planting cultivars with more durable resistance to scab, planting mixed cultivars in higher functional diversity orchards, and increasing the emphasis on biologically and culturally based practices aimed at reducing initial inoculum, coupled with an accurate spray strategy in spring as defined in the present study, are the most likely approaches for substantial further reductions in fungicides and for sustainable fruit production that is acceptable to consumers and fully compliant with organic crop production standards.

This research should lead to innovations involving a large number of parameters that could apply to fruit growers practising Integrated Fruit Production as well as to those practising Organic Production.

This 6-years study has demonstrated the potential of controlling apple scab with reduced and non-damaging amounts of inorganic fungicides, compliant with European regulations for organic production. Therefore, the present study is in full agreement with the

direct and indirect innovative prophylactic protection methods and strategies against orchard pests and diseases.

current European and National sustainable development policies. The products used to protect crops against weeds, parasites and pests have over the years revealed the risks for the environment and human health they bring with them. As respond to the outcome of the new European Action Programmes regarding natural resource preservations and public health, all member states have draw up and adopted National Action Plans for reducing the use of pesticides (e.g., in Belgium, the *Program for Reduction of Pesticides and Biocides* (PRPB); in France, '*Grenelle environment' laws*, '*Objective Lands 2020*', '*Plan Ecophyto 2018*', '*Organic Agriculture: horizon 2012*'). These plans define the possible ways forward to a reinvented agriculture that reconciles economic performance with ecological effectiveness. They will involve dialogue, research, innovation and the structuring of new supply chains, making our farming techniques an integral part of natural cycles.

In just a few decades our horizon has changed. Global warming, exhaustion of our most vital resources and damage to biodiversity have erupted from the domain of purely scientific observation into the social and economic realms. These new global realities can no longer be ignored by the agricultural sectors, industries, trade union bodies and citizens. In Europe, political support for agriculture has just begun to respond to these new demands. The role of the public action is, however, essential if we are to meet the growing and changing demand for food, to preserve the environment and contribute to sustainable growth. Being the first to face the growing fragility of our damaged environment, farmers have to develop practices that are not only imaginative and effective, but also ecologically sustainable and productive. They should demonstrate that agriculture sector can put scarce resources to good use, preserve the rich variety of ecosystems, make production secure with fewer inputs, loosen the energy constraint and combat the disruption of the climate. We have now the opportunity to rethink the tools we use to cope with the world's new challenges; the opportunity to adapt our agricultural policy for today, tomorrow and the future. This thesis is a tangible and concrete contribution to the new agricultural challenges of the century.

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Figure VIII.2 − Demonstration day organised at Gembloux on 26 November 2009 by the Interreg IV TransBioFruit program. Sanitation practices that reduce the population density of *V. inaequalis* directly or indirectly by their action on the leaf litter, rending the leaf litter unsuitable for the saprophytic and sexual activities of *V. inaequalis* that occur in dead leaves, and therefore preventing discharged ascospores from escaping during spring into the orchard air. Examples include physically removing fallen leaves from the orchard by machinery (A and C), raking and then ploughing the leaf litter into the soil (E), raking the leaf litter (D), raking and then shredding the leaf litter with a flail mower (B), leaving untreated leaves on the orchard soil (F) (Jamar *et al.*, 2010b; Jamar *et al.*, 2011).

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