

Primary scab control using a “during-infection” spray timing and the effect on fruit quality and yield in organic apple production

Laurent Jamar, Marc Cavelier, Marc Lateur

Centre wallon de Recherches agronomiques. Département Sciences du Vivant. Rue de Liroux, 4. B-5030 Gembloux (Belgique). E-mail : jamar@cra.wallonie.be

Reçu le 10 septembre 2009, accepté le 2 février 2010.

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 (cv. ‘Pirouette’) and two old cultivars expressing low to very low scab susceptibility (cvs. ‘Reinette Hernaut’ and ‘Reinette des Capucins’). Apart from these cultivars, four monogenic *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: a specific spray timing involving spraying during the infection processes, before fungal penetration, determined by the RIMpro software warning system, and 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 ($\leq 40 \text{ kg}\cdot\text{ha}^{-1}$ per year) combined with low rates of copper ($\leq 2.1 \text{ kg}\cdot\text{ha}^{-1}$ per year) 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, plant extract, potassium bicarbonate, scab resistance, *Venturia inaequalis*.

Protection contre la tavelure ciblée sur les infections primaires et effets sur la qualité des fruits et les rendements en production biologique de pomme. La production européenne de pommes en agriculture biologique dépend fortement des fongicides à base de cuivre pour la protection contre la tavelure (*Venturia inaequalis*). L’objectif de cette étude d’une durée de six années, menée de 2003 à 2008, était de rechercher les moyens pour réduire l’usage de fongicides à base de cuivre en production biologique de pommes. Un verger expérimental implanté en Belgique, selon un dispositif expérimental en split-plot, a été mis en place afin d’étudier l’efficacité d’une stratégie de pulvérisation « durant-infection » comparant les applications de soufre (avec ou sans cuivre), de bouillie sulfocalcique, de bicarbonate de potassium, d’un extrait de silice et de cinq extraits naturels de plante (pelure d’orange, bois de Panama, graines de thé ou de quinoa et pépins de pamplemousse) pour la lutte contre les infections primaires de tavelure. Les effets des traitements ont été étudiés sur une série de quatre variétés (cvs) de pommes présentant un gradient de niveaux de résistance partielle à la tavelure : une variété très sensible (cv. ‘Pinova’), une moyennement sensible (cv. ‘Pirouette’) et deux anciennes variétés peu à très peu sensibles (cvs. ‘Reinette Hernaut’ et ‘Reinette des Capucins’). Par ailleurs, une série de quatre variétés résistantes possédant le gène *Vf* ont également été intégrées dans le verger (cvs. ‘Initial’, ‘Topaz’, ‘Zvatava’ et ‘JN 20/33/58’). Dans le but de réduire de façon optimale les quantités de fongicides à appliquer, les stratégies suivantes ont été expérimentées : un positionnement des traitements impliquant des pulvérisations

pendant les processus d'infection, déterminée à l'aide du système d'avertissement du logiciel RIMpro et un pulvérisateur tunnel pour l'application des traitements. Suivant les années, un total annuel de 8 à 12 applications ont été réalisées. Dans des conditions extérieures très favorables au développement de tavelure, chaque année, de faibles quantités de soufre élémentaire (< 40 kg·ha⁻¹ par an) combinée à de faibles quantités de cuivre (< 2,1 kg·ha⁻¹ par an) ont donné la meilleure protection contre la tavelure. En comparaison du témoin non traité, ces traitements ont permis de réduire la sévérité de tavelure sur les fruits de 85 % à 100 % selon les variétés et les années. Dans la plupart des cas, le schéma de traitement à base de bouillie sulfocalcique, excluant tout usage du cuivre, a donné quasi les mêmes résultats que le schéma combinant le soufre mouillable et le cuivre. Le soufre, le bicarbonate de potassium et tous les extraits de plante ont réduit significativement la sévérité de tavelure sur les feuilles et les fruits. Tous les traitements ont augmenté le rendement en pomme aussi bien sur les variétés très sensibles et modérément sensibles que sur les variétés très peu sensibles à la tavelure. Dans les conditions expérimentales définies, aucun des traitements appliqués n'a provoqué de la phytotoxicité, ni n'a augmenté la rugosité sur fruit et finalement, aucun résidu indésirable n'a été détecté sur les fruits à la récolte et dans le sol. Le potentiel et les limites de la pulvérisation « durant-infection », comme moyen de protection contre la tavelure du pommier en culture biologique, sont discutées.

Mots-clés. Cuivre, gestion des maladies, bouillie sulfocalcique, extrait de plante, bicarbonate de potassium, résistance variétale, *Venturia inaequalis*.

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 et al., 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 fungicides used for scab control in an organic apple orchard, using compounds with poor curative properties (Jamar et al., 2008b; 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 compounds with curative properties and 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 adverse effects on the environment or human health known) 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 et al., 1996; Schulze et al., 2003; Ilhan et al., 2006; Jamar et al., 2007a; 2007b). Various natural plant extracts containing triterpenoid saponins, polyphenols and specific flavonoids have been reported to possess antifungal properties (Köhl et al., 2007; Bahraminejad et al., 2008; Bengtsson 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.

2. MATERIALS AND METHODS

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 (‘Pinova’), one medium scab-susceptible cultivar (‘Pirouette’), one low scab-susceptible cultivar (‘Reinette Hernaut’) and one very low scab-susceptible cultivar (‘Reinette des 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 1,900 trees per ha in blocks or 1,500 trees per ha in orchards, including 20% of ecological zones.

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 1,000 kg·ha⁻¹ of organic fertilizers (5% N) and 2,000 kg·ha⁻¹ of hydrated lime for pH enhancement (Table 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, Dordrecht, The Netherlands) connected to a RIMpro scab warning system (Trapman et al., 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 et al., 1989), the simulation of ascospores 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.

2.2. Treatments

Each year, the experiment was conducted on 1,440 trees and involved 10 experimental spray treatments each year. Each treatment was applied to

Table 1. Organic fertilizers and amendments applied in the experimental orchard — *Fertilisants organiques et amendements appliqués dans le verger expérimental.*

Compound	2002	2003	2004	2005	2006	2007	2008	Mean
Compost 0.5% N (t·ha ⁻¹)	30.0	-	-	25.0	-	-	-	7.9
Lin-waste 5/2/2 (t·ha ⁻¹)	0.5	1.0	1.0	-	1.0	0.8	1.0	0.7
Patentkali (t·ha ⁻¹)	2.0	-	0.3	-	-	-	-	0.3
Natural phosphate 50% (t·ha ⁻¹)	1.0	-	0.3	-	-	-	-	0.2
Hydrated lime 50% (t·ha ⁻¹)	2.0	1.0	2.0	2.0	2.0	2.0	2.0	1.8
Nitrogen unit (u.N·ha ⁻¹) ^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 — *Estimation de la disponibilité de l'azote fourni par le compost : 30 % l'année 1, 20 % l'année 2, 15 % l'année 3 et par le tourteau de lin : 50 % l'année 1 et 50 % l'année 2*; ^b Number of foliar treatments — *Nombre de traitements foliaires.*

144 trees (36 trees per cultivar). The treatments were randomized 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.

The applied products and active ingredients application rate are described in the **table 2**.

In the first orchard, the six spray treatments were (**Table 3**):

- untreated control (UC1);
- potassium bicarbonate-based compounds (AR1);
- depending on the year, sulphur from wettable sulphur, silicon, delayed lime sulphur, soapbark, tea seed or quinoa seed (SF1);
- depending on the year, products used in Integrated Fruit Production (IFP), sulphur, grapefruit seed or copper combined with orange peel (RE1);

- sulphur from lime sulphur (LS1);
- sulphur from wettable sulphur combined with copper from the hydroxide form (CS1).

In the second orchard, there were four spray treatments (**Table 3**):

- untreated control (UC2);
- sulphur from wettable sulphur (SF2);
- depending on the year, products used in IFP, a combination of copper and wettable sulphur (RE2);
- other combinations of copper and wettable sulphur (CS2).

Each spray treatment was maintained on the same plots from 2003 to 2008.

All treatments were applied at a low spray rate of 300 l·ha⁻¹. 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 ascospores inoculation and before hypha penetration (**Figure 1**). In practice, the treatment timings, defined as the number

Table 2. Treatments and active ingredients application rates — *Traitements et taux d'application des substances actives.*

Code	Trademark (Manufacturer)	Active ingredient a.i. (%)	a.i. application rate (%) ^a
arm	Armicarb®100 (Helena Chem. Co., US)	Potassium bicarbonate (85%)	1.6 (0.8) ^b
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 and <i>Equisetum arvense</i> (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 Internat., US)	Soapbark tree, <i>Quillaja saponaria</i> (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 and quinoa seed extract (100%)	1.0 (1.0)

^a All treatments were applied at a low spray rate (300 l·ha⁻¹) — *Tous les traitements ont été appliqués avec un faible volume d'eau (300 l·ha⁻¹)*; ^b Application rate apart from flowering (values in brackets are application rate during flowering) — *Taux d'application en dehors de la période de floraison (les valeurs entre parenthèses indiquent le taux d'application pendant la période de floraison).*

Table 3. Spray treatments applied from 2003 to 2008 in the two experimental orchards — *Schémas de traitement appliqués de 2003 à 2008 dans les deux vergers expérimentaux.*

Orchard 1	2003	2004	2005	2006	2007	2008
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 treatments ^b	3 + 3 + 6	3 + 2 + 5	4 + 1 + 3	4 + 1 + 5	0 + 0 + 8	3 + 1 + 5
Orchard 2	2003	2004	2005	2006	2007	2008
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 treatments ^b	3 + 3 + 5	3 + 2 + 4	4 + 1 + 3	4 + 1 + 5	0 + 0 + 8	3 + 1 + 5

Orchard 1 — *verger 1*: cvs. ‘Pinova’, ‘Pirouette’, ‘Reinette Hernaut’, ‘Reinette des Capucins’; Orchard 2 — *verger 2*: cvs. ‘Initial’, ‘Topaz’, ‘Zvatava’, ‘JN 20/33/58’; ^a for treatments explanations, see **table 2** — *pour l’explication des traitements, voir le tableau 2*; the ‘ipm’ (Integrated Production Management) includes dodine, captane, kresoxym méthyl, dithianon, pyriméthanyl and difénoconazole at recommended doses — *l’« ipm » (système de production intégrée) implique l’usage de la dodine, du captane, du kresoxym méthyl, du dithianon, du pyriméthanyl et du difénoconazole, aux doses recommandées*; cop: includes treatments only before flowering — *implique des traitements seulement avant la floraison*; cop⁺: includes additional 0.04% copper treatments from flowering — *implique des traitements supplémentaires de cuivre à 0,04 % à partir de la floraison*; 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 — *implique des traitements supplémentaires de cuivre à 0,04 % pendant la floraison jusqu’au stade de basculement des fruits et à 0,08 % par la suite*. Prv, tea: include treatments only after flowering, and all others include treatments before and after flowering — *impliquent des traitements seulement après la floraison et tous les autres impliquent des traitements avant, pendant et après la floraison*. In ‘cop-sul’, ‘cop⁺-sul’ and ‘cop⁺⁺-sul’ treatments, ‘sul’ was associated with ‘cop’ if the temperature rose above 10°C — *Dans les traitements ‘cop-sul’, ‘cop⁺-sul’ and ‘cop⁺⁺-sul’, ‘sul’ a été associé avec le ‘cop’ si la température était supérieure à 10 °C*; ^b number of treatments applied during the primary scab infection seasons, from March to June (first, second and third value = 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) — *Nombre de traitements appliqués pendant la période d’infection primaire, du mois de mars au mois de juin (première, deuxième et troisième valeur = avant, pendant et après la floraison, respectivement)*. De 2004 à 2008, tous les schémas de traitements impliquent deux applications supplémentaires d’été à 2 % de soufre (pour lutter contre la tavelure) et de 2002 à 2006, deux applications supplémentaires après la récolte à 0,2 % de cuivre (pour lutter contre le chancre *Nectria galligena*).

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 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 by the Environmental Protection Agency (EPA) and can be used in organic farming systems in the USA. 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., 2007b).

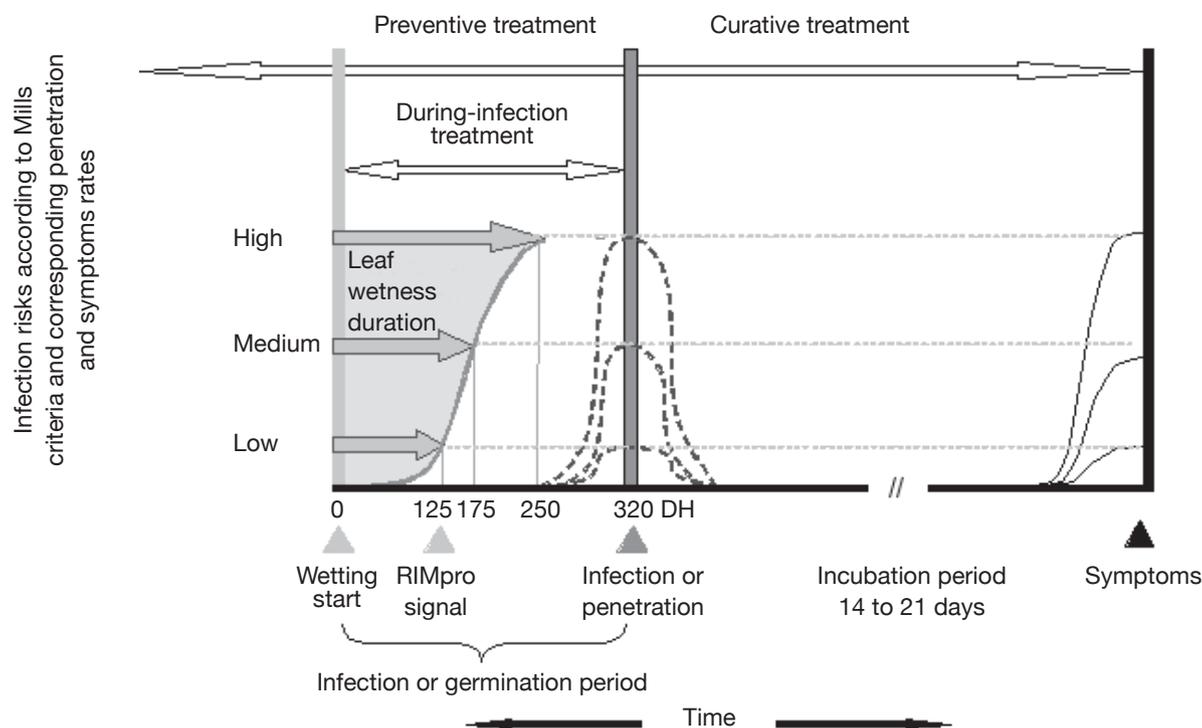


Figure 1. Schematic illustration of the “during-infection” spray timing [treatment applied 0-320 degree-hours (DH) after the wetting start] in relation to leaf wetness duration, infection risks according to Mills criteria, fungus (*Venturia inaequalis*) activity and the approximate RIMpro infection starting-point — *Représentation schématique de la stratégie de traitement « durant-infection »* [traitements appliqués de 0 à 320 degrés-heures (DH) après le début de la pluie] en relation avec la durée d’humectation du feuillage, les risques d’infections selon les critères de Mills, l’activité du champignon (*Venturia inaequalis*) et le point de départ approximatif du signal RIMpro.

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 ($\leq 15 \text{ km}\cdot\text{h}^{-1}$) 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.

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 = $\leq 1\%$ infected leaves with at least one lesion; 3 = $\leq 5\%$ infected leaves with at least one lesion; 4 = 5-50% infected leaves with at least one lesion; 5 = $\geq 50\%$ leaves with lesions and with $\leq 5\%$ 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 et al., 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%; 6 = $\geq 50\%$ 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.

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 EPPPO/OEPP standards based on a scale of 1 to 4 whereby: 1 = no russet; 2 = < 10%; 3 = 10-30%; 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 EPPPO/OEPP standards.

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.

2.6. Data analysis

Year factors were analyzed 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 analyzed 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 $P = 0.05$.

3. RESULTS

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 et al., 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 des 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 2**). Under our conditions, the treatments did not completely prevent the propagation of these new virulent scab races between 2006 and 2008 (**Figures 3 and 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 3 and 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 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"

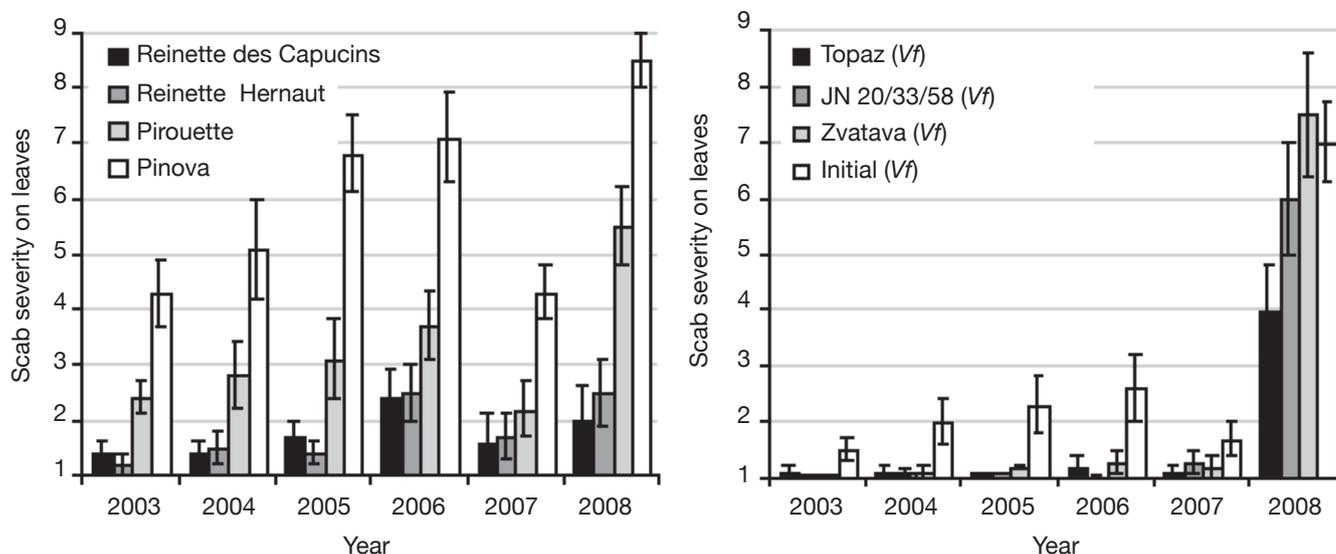


Figure 2. Evolution of overall scab severity on leaves (1-9 scale: 1 = no scab, 9 = maximum scab infection) assessed in untreated control plots 60 days after flowering, for each cultivar from 2003 to 2008 — *Évolution de la sévérité globale de la tavelure sur les feuilles (échelle de 1 à 9 : 1 = pas de symptôme de tavelure, 9 = infection maximum de tavelure) évaluée sur les parcelles « Témoin non traité », 60 jours après la floraison pour chaque variété de 2003 à 2008.*

Error bars denote standard error of the mean (n = 6) — *Les barres d'erreur représentent l'erreur standard de la moyenne (n = 6).*

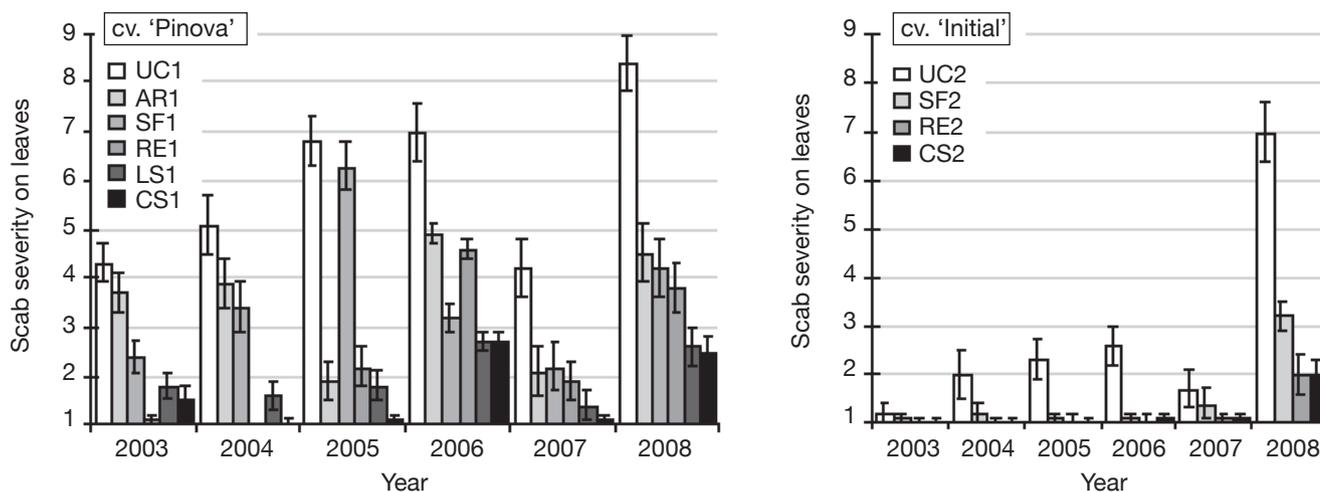


Figure 3. Effects of spray treatments on overall scab severity on leaves (1-9 scale) assessed 60 days after flowering from 2003 to 2008 for cvs. 'Pinova' and 'Initial', the most susceptible cultivars in each orchard — *Effets des schémas de traitement sur la sévérité globale de la tavelure (échelle de 1 à 9) évalués 60 jours après la floraison de 2003 à 2008 sur les variétés 'Pinova' et 'Initial', les plus sensibles de chaque verger.*

For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3*; Error bars denote standard error of the mean (n = 6) — *Les barres d'erreur représentent l'erreur standard de la moyenne (n = 6).*

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 4**).

Each year the potassium bicarbonate treatments (AR1) significantly reduced apple scab severity on the leaves and fruits compared with water control (**Figures 3 and 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

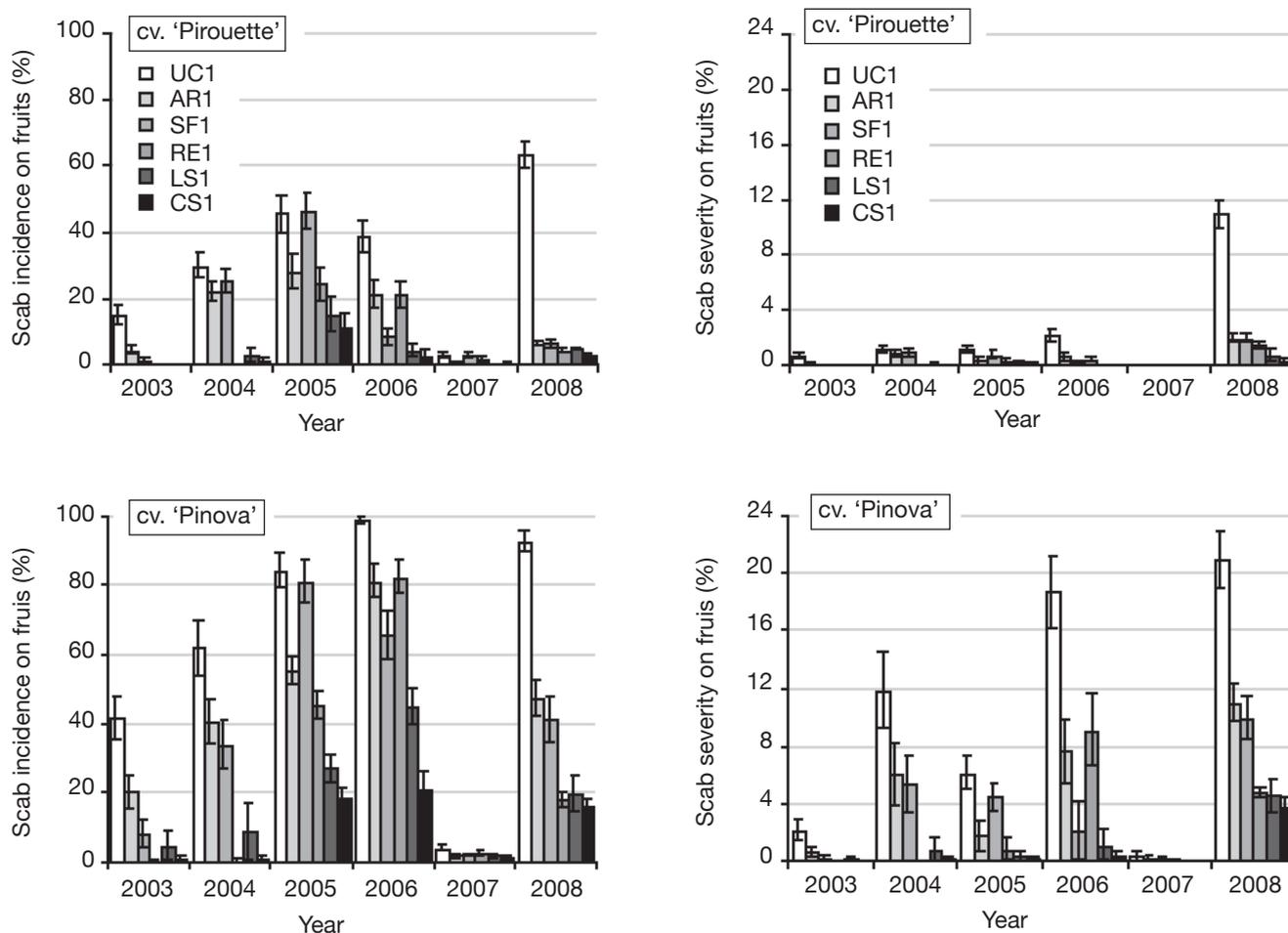


Figure 4. Effects of spray treatments on scab incidence (left) and severity (right) of the fruit at harvest from 2003 to 2008 for cvs. ‘Pirouette’ (medium scab-susceptible) and ‘Pinova’ (high scab-susceptible) — *Effets des schémas de traitement sur l’incidence et sur la sévérité de la tavelure sur les fruits récoltés, des variétés ‘Pirouette’ (modérément sensible) et ‘Pinova’ (très sensible) de 2003 à 2008.*

Fruit incidence: proportion of total harvested fruits with at least one spot — *Incidence sur les fruits : la proportion des fruits récoltés avec au moins une tache*; Fruit severity: mean scabbed area or mean proportion of fruit surface covered by scab — *Sévérité sur les fruits : surface moyenne tachée de tavelure ou proportion moyenne de la surface des fruits couverte de tavelure*; For treatments explanations, see p 426 and **table 3** — *Pour l’explication des traitements, voir p 426 et tableau 3*; Error bars denote standard error of the mean ($n = 6$) — *Les barres d’erreur représentent l’erreur standard de la moyenne ($n = 6$).*

(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 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 4**).

3.2. Yield, fruit number and size

In most cases, compared with the untreated control, all treatments significantly increased overall yield per tree (**Figure 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 per tree, 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. ‘Reinette des Capucins’, with a very low scab rate, treatments

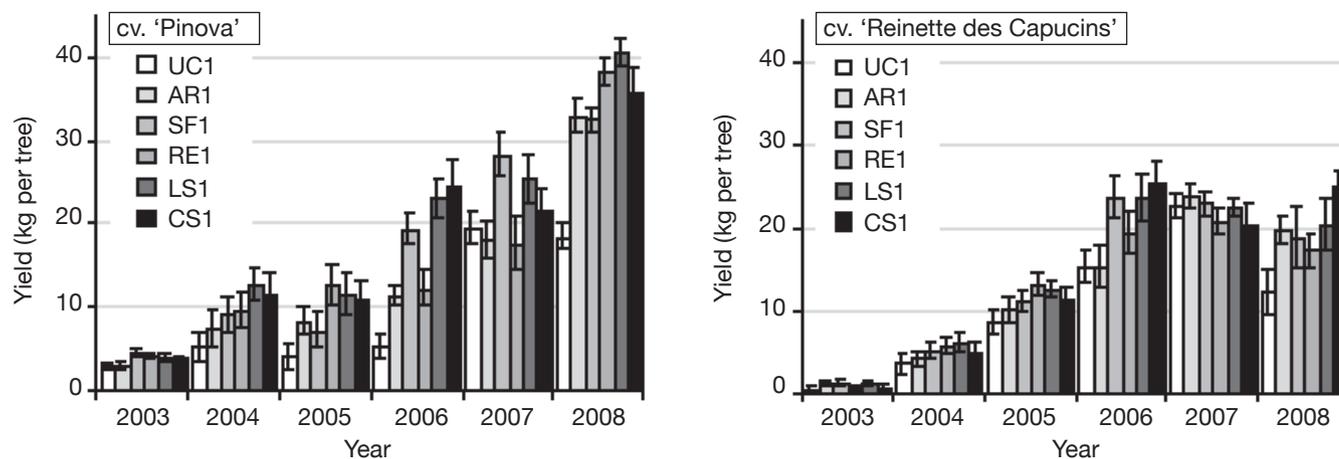


Figure 5. Effects of spray treatments on overall yield for cv. 'Pinova' (high scab-susceptible) and cv. 'Reinette des Capucins' (low scab-susceptible) from 2003 to 2008. — *Effets des schémas de traitement sur les rendements globaux pour la variété 'Pinova' (très sensible à la tavelure) et la variété 'Reinette des Capucins' (très faiblement sensible à la tavelure) de 2003 à 2008.*

For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3*; Tree density was 1,900 trees per ha in blocks or 1,500 trees per ha in the overall orchards, including 20% of ecological zones — *La densité de plantation était de 1900 arbres par ha dans les blocs expérimentaux ou, si l'on considère les 20 % de zone de compensation écologique, 1500 arbres par ha dans l'ensemble des vergers*; Error bars denote standard error of the mean ($n = 6$) — *Les barres d'erreur représentent l'erreur standard de la moyenne ($n = 6$).*

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 4** and **Figure 7**). In most cases, compared with the untreated control, all treatments significantly increased the number of fruits per tree (**Figure 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 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. 'Reinette des Capucins') significant differences between treated and untreated plots were also registered (**Table 4** and **Figure 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).

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 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 $\text{mg}\cdot\text{kg}^{-1}$ for copper and sulphur, respectively).

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

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

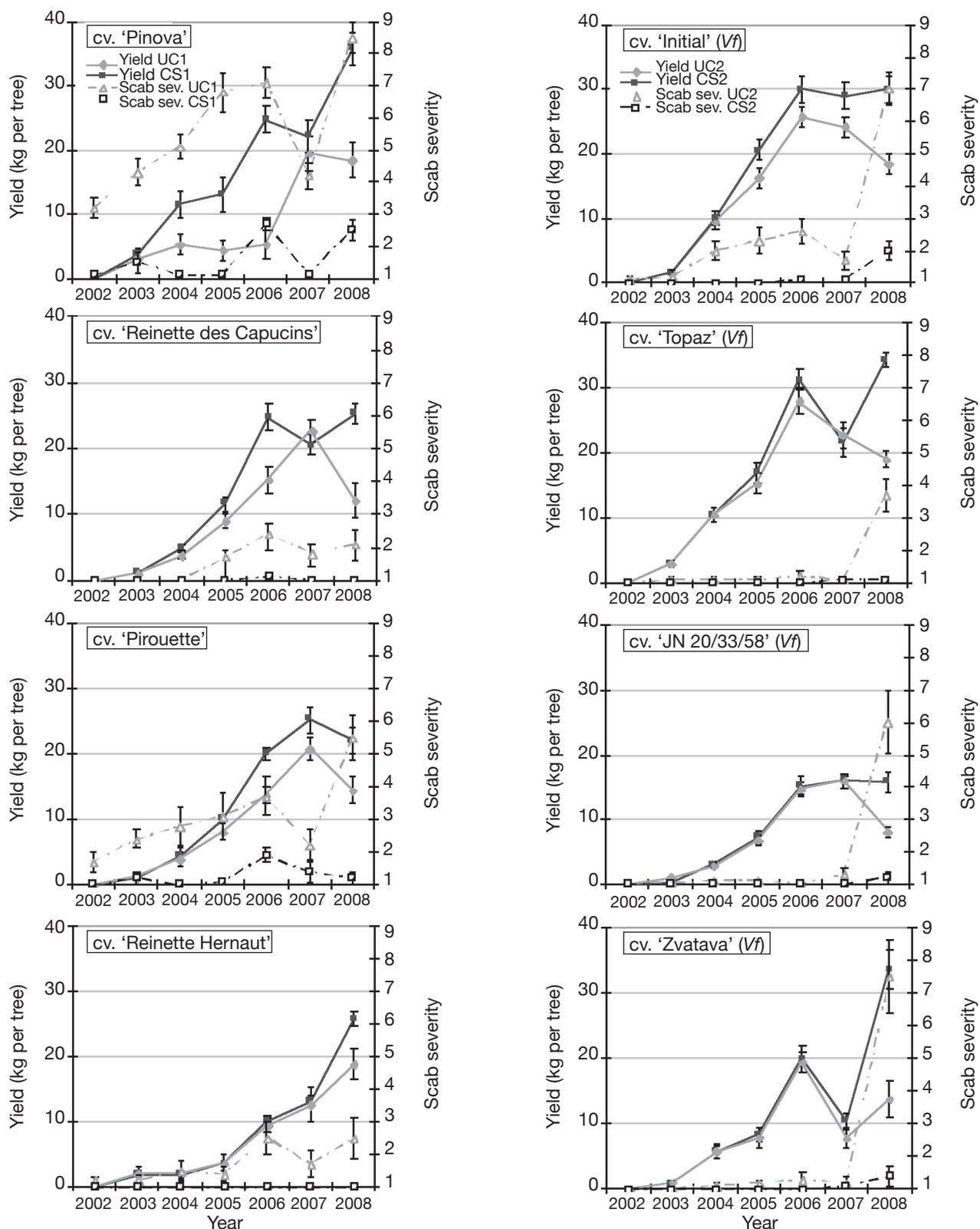


Figure 6. Overall yield at harvest in relation to overall scab severity on leaves assessed 60 days after flowering (1-9 scale), subjected to CS spray treatments compared with the UC untreated controls from 2002 to 2008 — *Poids total de fruits récoltés en relation avec la sévérité globale de la tavelure sur les feuilles (échelle de 1 à 9) évaluée 60 jours après la floraison, obtenu avec le schéma de traitement « CS » et le schéma témoin non traité « UC » de 2002 à 2008.*

Tree density was 1,900 trees per ha in blocks or 1,500 trees per ha in orchards, including 20% of ecological zones — *La densité de plantation était de 1900 arbres par ha dans les blocs expérimentaux ou, si l'on considère les 20 % de zone de compensation écologique, 1500 arbres par ha dans l'ensemble des vergers*; Error bars denote standard error of the mean (n = 6) — *Les barres d'erreur représentent l'erreur standard de la moyenne (n = 6).*

Table 4. Effects of spray treatments against scab on the mean cumulated yield and the mean proportion of fruits at a size of below 65 mm, from 2003 to 2008 — *Effets des schémas de traitement de protection contre la tavelure sur les rendements cumulés moyens et sur la proportion moyenne de fruits avec un calibre inférieur à 65 mm, de 2003 à 2008.*

Cultivar	Spray treatment ^a	Mean cumulated yield (kg per tree)	Proportion of fruits < 65 mm (%) ^b					
			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* ^c	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*
Reinette des 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
Reinette 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*
JN 20/33/58 (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 For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3.* ^b No hand or chemical thinning had been done during the growing season for any of the cultivars — *Aucun éclaircissage manuel ou chimique n'a été effectué durant la saison de croissance pour aucune variété;* ^c value followed by '*' are significantly different from the corresponding untreated control value (UC1 or UC2) according the Student-Newman-Keuls tests ($P \leq 0.05$) — *Les valeurs suivies de '*' sont significativement différentes des valeurs correspondantes « témoin non traité » (UC1 ou UC2) au sens de Student-Newman-Keuls ($P \leq 0,05$).*

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

Table 5. Copper content ($\text{mg}\cdot\text{kg}^{-1}$)^a of organic orchard soils at Gembloux from 2002 to 2008 — *Teneur en cuivre ($\text{mg}\cdot\text{kg}^{-1}$)^a du sol du verger biologique à Gembloux de 2002 à 2008.*

Spray treatments ^b	2002	2004	2006	2008
UC1 + UC2	-	-	-	14.8 ^c
CS1 + CS2	18.5	22.3	26.8	15.5

^a EDTA and ammonium acetate extraction method — *EDTA et méthode d'extraction de l'acétate d'ammonium;* ^b For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3;* ^c values are the means of five replicates, and values followed by the same letter are not significantly different according the Student-Newman-Keuls tests ($P \leq 0.05$) — *les valeurs sont des moyennes de cinq répétitions et les valeurs suivies par les mêmes lettres ne sont pas significativement différentes au sens de Student-Newman-Keuls ($P \leq 0,05$).*

(Ellis et al., 1998; Holb et al., 2003; Palmer et al., 2003; Holb et al., 2005b). Up to 110 $\text{kg}\cdot\text{ha}^{-1}$ per year of elemental sulphur combined with 8 $\text{kg}\cdot\text{ha}^{-1}$ per year of copper were used for scab control in organic apple production in The Netherlands (Holb et al., 2001). In addition, the results of this study showed that:

- diluted copper is very effective under cold weather conditions during early primary scab infection periods;
- the lime sulphur treatment can be as effective as the combined wettable sulphur and copper treatment;
- 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

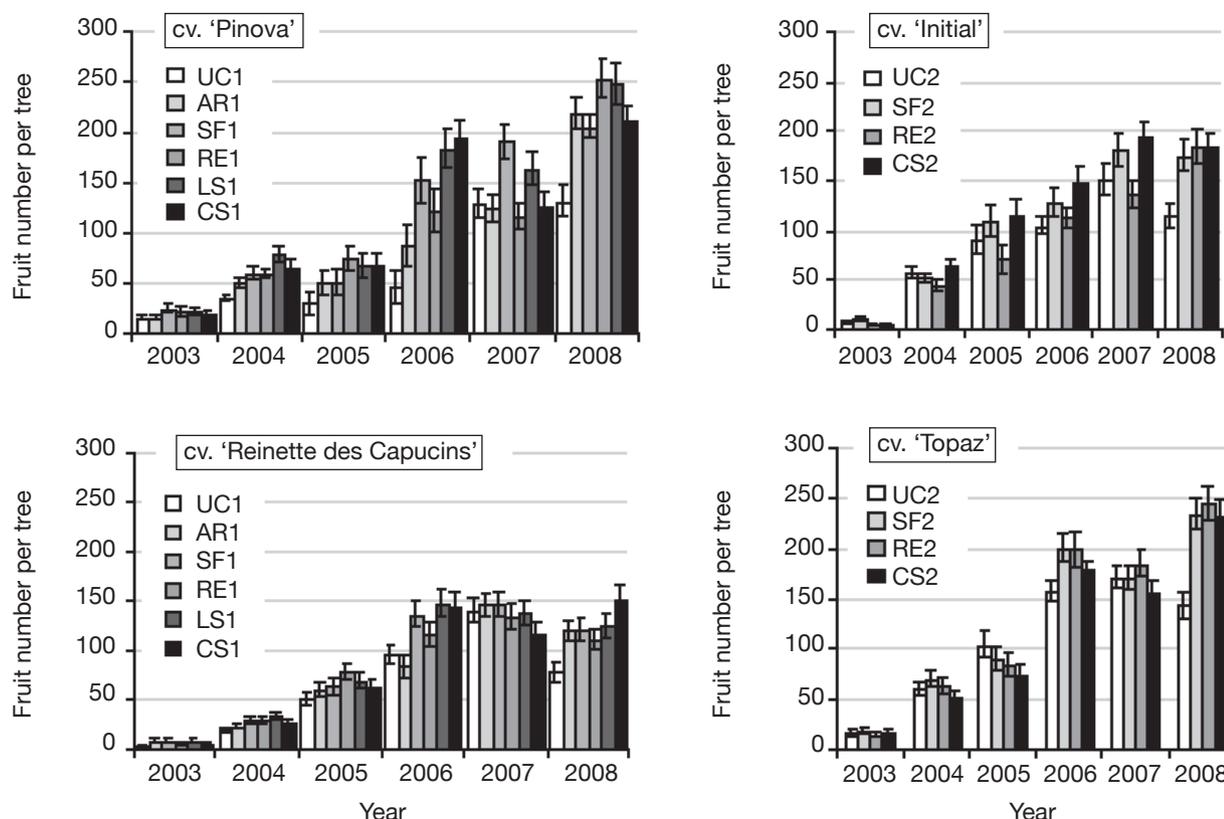


Figure 7. Mean number of fruits per tree for each spray treatment from 2003 to 2008 — *Nombre moyen de fruits par arbre pour chaque schéma de traitement de 2003 à 2008.*

For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3*; Error bars denote standard error of the mean (n = 6) — *Les barres d'erreur représentent l'erreur standard de la moyenne (n = 6).*

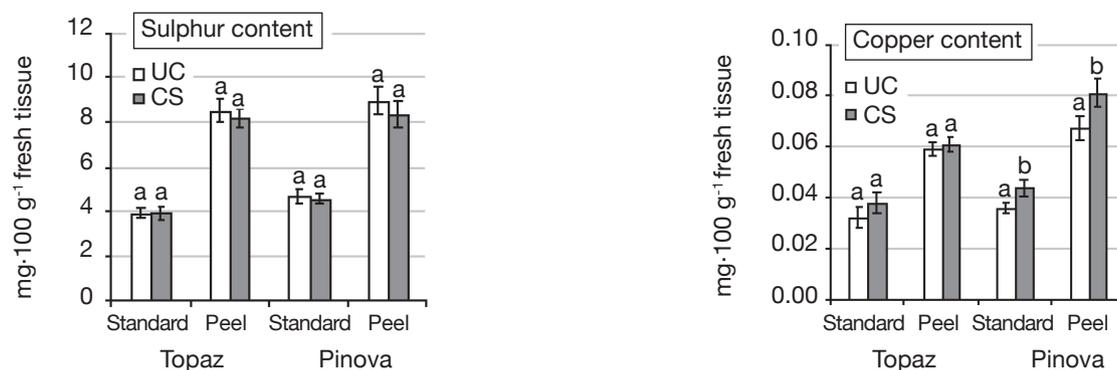


Figure 8. Effects of the CS compared with UC spray treatments on the copper and sulphur fruit (standard) and peel contents at harvest in 2006. Mean dry weight content was 14.5% (± 0.5) and 20.6% (± 0.7) for fruits and peels, respectively — *Comparaison de l'effet du schéma de traitement « CS » avec l'effet du schéma témoin non traité « UC » sur la teneur en cuivre et soufre dans les fruits (Standard) et dans les pelures des fruits (Peel) à la récolte en 2006. Le poids sec moyen était de 14,5 % ($\pm 0,5$) et 20,6 % ($\pm 0,7$) pour, respectivement, les fruits et les pelures.*

For treatments explanations, see p 426 and **table 3** — *Pour l'explication des traitements, voir p 426 et tableau 3*; Error bars denote standard error of the mean (n = 6) — *Les barres d'erreur représentent l'erreur standard de la moyenne (n = 6)*; In the 'UC-CS' pairs, mean values with different letters are significantly different according the Student-Newman-Keuls tests ($P \leq 0.05$) — *Au sein des paires « UC-CS », les valeurs moyennes suivies par des lettres différentes sont significativement différentes au sens de Student-Newman-Keuls ($P \leq 0,05$).*

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 1**). Below 250 DH after rainfall, few ascospores 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:

- reduced washing effect from the rain;
- greater treatment effectiveness on germinating spores;
- avoidance of unnecessary treatments (Funt et al., 1990; Holb et al., 2003; Jamar et al., 2009);
- in hot seasons, applying treatments during less sunny periods.

However, there are potential problems with this strategy, such as:

- the need to be able to adapt the spray timing to within a few hours of the onset of rain;
- the risk of delaying spraying during an extended rainy period;
- the risk of spraying during windy weather;
- 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 these 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 et al., 2003; Ilhan et al., 2006; Jamar et al., 2007a; 2007b; 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., 2007b). 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 (Holb et al., 2003; Palmer 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 des 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:

- triggering defence responses and resistance mechanisms burn up energy in plants which is not necessary to use in treated plants (Doehlmann et al., 2008);
- 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 et al., 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 $26.8 \text{ mg}\cdot\text{kg}^{-1}$, 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 $36 \text{ mg}\cdot\text{kg}^{-1}$ soil (e.g. van Rhee, 1976; Holb, 2008; Paoletti et al., 1998). Van Rhee (1976) found that earthworms were almost completely eradicated when the copper concentration was more than $80 \text{ mg}\cdot\text{kg}^{-1}$ soil. Organic management significantly increases soil microbial activity and earthworm abundance in orchards (Jamar et al., 2008a; 2010a), although:

- soil tillage operations for weed control can reduce earthworm abundance (Paoletti et al., 1998);
- 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 ascospore inoculum in apple orchards (MacHardy, 1996; Paoletti et al., 1998).

5. CONCLUSION

This long-term study clearly shows that:

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

Acknowledgements

This research is funded by the Ministry of the Walloon Regional Government, General Department of Agriculture, Research Direction, project RW D31-1144. The authors would like to thank Dr R. Oger (CRA-W, Gembloux) for his valuable help in the statistical analysis, Ir P. Creemers (PCF-KOG, St Truiden) for stimulating discussions during this study and B. Pahaut for his excellent co-operation in this research.

Bibliography

- Bahraminejad S., Asenstorfer R.E., Riley I.T. & Schultz C.J., 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *J. Phytopathol.*, **156**, 1-7.
- Belanger R.R., Bowen P.A., Ehret D. & Menzies J.G., 1995. Soluble silicon: its role in crop and disease management of greenhouse crops. *Plant Dis.*, **79**, 329-336.
- Bengtsson M. et al., 2009. Comparative studies on the effects of a yucca extract and acibenzolar-S-methyl (ASM) on inhibition of *Venturia inaequalis* in apple leaves. *Eur. J. Plant Pathol.*, **124**, 187-198.
- Beresford R.M. et al., 1996. Slaked lime, baking soda and mineral oil for black spot and powdery mildew control in apples. In: *Proceedings of the 49th New Zealand plant protection conference, August 1996, Quality Hotel Rutherford, Nelson, New Zealand*, 106-113.
- Croxall H.E., Gwynne D.C. & Jenkins J.E.E., 1952. The rapid assessment of apple scab on fruit. *Plant Pathol.*, **2**, 89-92.
- Depta B., Koscielniak A. & Rozen A., 1999. Food selection as a mechanism of heavy metal resistance in earthworms. *Pedobiologia*, **43**, 608-614.
- Doehlemann G. et al., 2008. Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph *Ustilago maydis*. *Plant J.*, **56**, 181-195.
- Ellis M.A., Ferree D.C., Funt R.C. & Madden L.V., 1998. Effects of an apple scab-resistant cultivar on use patterns of inorganic and organic fungicides and economics of disease control. *Plant Dis.*, **82**, 428-433.
- EC, 2008. Council Regulation (EC) 889/2008 on organic production and labelling of organic products with regard to organic production, labelling and control. *Off. J. Eur. Union*, **L 250**, 18.09.2008, 1-83.
- Funt R.C., Ellis M.A. & Madden L.V., 1990. Economic analysis of protectant and disease-forecast-based fungicide spray programs for control of apple scab and grape black rot in Ohio. *Plant Dis.*, **74**, 638-642.
- Gessler C. et al., 2006. *Venturia inaequalis* resistance in apple. *Crit. Rev. Plant Sci.*, **25**, 473-503.
- Hindorf H., Rövekamp I.F. & Henseler K., 2000. Decision aids for apple scab warning services (*Venturia inaequalis*) in Germany. *OEPP/EPPO Bull.*, **30**, 59-64.
- Holb I.J. & Heijne B., 2001. Evaluating primary scab control in organic apple production. *Gartenbauwissenschaft*, **66**, 254-261.
- Holb I.J., De Jong P.F. & Heijne B., 2003. Efficacy and phytotoxicity of lime sulphur in organic apple production. *Ann. Appl. Biol.*, **142**, 225-233.
- Holb I.J., Heijne B. & Jeger M.J., 2005a. The widespread occurrence of overwintered conidial inoculum of *Venturia inaequalis* on shoots and buds in organic and integrated apple orchards across the Netherlands. *Eur. J. Plant Pathol.*, **111**, 157-168.
- Holb I.J. et al., 2005b. Analysis of summer epidemic progress of apple scab at different apple production systems in the Netherlands and Hungary. *Phytopathology*, **95**, 1001-1020.
- Holb I.J., 2006. Effect of six sanitation treatments on leaf litter density, ascospore production of *Venturia inaequalis* and scab incidence in integrated and organic apple orchards. *Eur. J. Plant Pathol.*, **115**, 293-307.
- Holb I.J., 2008. Timing of first and final sprays against apple scab combined with leaf removal and pruning in organic apple production. *Crop Prot.*, **27**, 814-822.
- Horst R.K., Kawamoto S.O. & Porter L.L., 1992. Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of roses. *Plant Dis.*, **76**, 247-251.
- Ilhan K., Arslan U. & Karabulut O.A., 2006. The effect of sodium bicarbonate alone or in combination with a reduced dose of tebuconazole on the control of apple scab. *Crop Prot.*, **25**, 963-967.
- Jamar L., 2007. *Recherche de stratégies innovantes de lutte contre la tavelure du pommier (Venturia inaequalis) applicable dans le mode de Production Fruitière Biologique*. Travail de fin d'études en vue de l'obtention du Diplôme d'Études Approfondies en Sciences Agronomiques et Ingénierie Biologique : Faculté universitaire des Sciences agronomiques de Gembloux (Belgique).
- Jamar L. & Lateur M., 2007. Strategies to reduce copper use in organic apple production, ISHS. *Acta Hort.*, **737**, 113-120.

- Jamar L., Lefrancq B. & Lateur M., 2007. Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment. *J. Plant Dis. Prot.*, **115**, 221-227.
- Jamar L. et al., 2008a. Soil microbial activity and earthworm abundance in orchards under conventional and organic growth management systems. In: *Proceedings of 13th International conference Eco-fruit, 18-20 February 2008, Weinsberg, Germany*, 167-171.
- Jamar L., Lefrancq B., Fassotte C. & Lateur M., 2008b. A “during-infection” spray strategy using sulphur compounds, copper, silicon and a new formulation of potassium bicarbonate for primary scab control in organic apple production. *Eur. J. Plant Pathol.*, **122**, 481-492.
- Jamar L., Oste S., Tournant L. & Lateur M., 2009. Protection contre la tavelure du pommier ciblée sur les infections primaires en production biologique. In: *Actes des Journées techniques nationales fruits et légumes biologiques, 8-9 décembre 2009, ITAB-GRAB, Paris*, 49-54.
- Jamar L. et al., 2010a. Effect of organic farming practices on five orchard soil bio-indicators. *Acta Hort.*, in press.
- Jamar L., Pahaut B. & Lateur M., 2010b. A low input strategy for apple scab control in organic apple production. *Acta Hort.*, in press.
- Jamar L. et al., 2010c. Comparative performance of recycling tunnel and conventional sprayers using standard and drift mitigation nozzles in dwarf apple orchards. *Crop Prot.*, **29**, 561-566.
- Köhl J. et al., 2007. *Replacement of copper fungicides in organic production of grapevine and apple in Europe. Publishable final activity report*, 1-69, http://www.repco.nl/Documents/Publ%20Executive%20Summaries/REPCO%20Final_executive_summary.pdf, (15.01.10).
- Kreiter S., Sentenac G., Barthes D. & Auger P., 1998. Toxicity of four fungicides to the predacious mite *Typhlodromus pyri* (Acari: Phytoseiidae). *J. Econ. Entomol.*, **91**, 802-811.
- Lateur M., Wagemans C. & Populer C., 1999. Evaluation of fruit tree genetic resources as sources of polygenic scab resistance in apple breeding. ISHS. *Acta Hort.*, **484**, 35-42.
- Lateur M. & Blazek J., 2002. Evaluation descriptors for *Malus*. In: *Report of a working group on Malus/Pyrus. Second meeting, May 2002, Dresden-Pillnitz, Germany*, 76-82. Roma: International Plant Genetic Resources Institute.
- Lateur M., 2002. Perspectives de lutte contre les maladies des arbres fruitiers à pépins au moyen de substances naturelles inductrices d'une résistance systémique. *Biotechnol. Agron. Soc. Environ.*, **6**, 67-77.
- Lefrancq B. & Lateur M., 2009. Monitoring and occurrence of new races of *Venturia inaequalis* on apple in Belgium. *Commun. Appl. Biol. Sci.*, **74**(3), 623-631.
- MacHardy W.E. & Gadoury D.M., 1989. A revision of Mills's criteria for predicting apple scab infection periods. *Phytopathology*, **79**, 304-310.
- MacHardy W.E., 1996. *Apple scab, biology, epidemiology and management*. St Paul, MI, USA: APS Press.
- MacHardy W.E., Gadoury D.M. & Gessler C., 2001. Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. *Plant Dis.*, **85**, 1036-1051.
- Markoyiannaki-Printzioui D., Papaioannou-Souliotis P., Zeginis G. & Giatropoulos C., 2000. Observations on acarofauna in four apple orchards of Central Greece. I. Incidence of pedoclimatic conditions and agricultural techniques on phytoseiid mites (Acari: Phytoseiidae). *Acarologia*, **41**, 109-126.
- Mills W.D., 1944. Efficient use of sulphur dusts and sprays during rain to control apple scab. *Cornell Ext. Bull.*, **630**.
- Mills W.D., 1947. Effects of sprays of lime sulphur and of elemental sulphur on apple in relation to yield. *Cornell Exp. Stn.*, **273**.
- Montag J., Schreiber L. & Schönherr J., 2005. An *in vitro* study on the postinfection activities of hydrated lime and lime sulphur against apple scab (*Venturia inaequalis*). *J. Phytopathol.*, **153**, 485-491.
- Palmer J.W., Davies S.B., Shaw P.W. & Wünsche J.N., 2003. Growth and fruit quality of 'Braeburn' apple (*Malus domestica*) trees as influenced by fungicide programmes suitable for organic production. *N.Z. J. Crop Hort. Sci.*, **31**, 169-177.
- Paoletti M.G. et al., 1998. Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Appl. Soil Ecol.*, **10**, 137-150.
- Schnug E., 1998. *Sulphur in agroecosystems*. Dordrecht, The Netherlands: Kluwer Academic.
- Schulze K. & Schönherr J., 2003. Calcium hydroxide, potassium carbonate and alkyl polyglycosides prevent spore germination and kill germ tubes of apple scab (*Venturia inaequalis*). *J. Plant Dis. Prot.*, **110**, 36-45.
- Simon S. et al., 2006. Does manipulation of fruit-tree architecture affect the development of pests and pathogens? A case study in an organic apple orchard. *J. Hort. Sci. Biotechnol.*, **81**, 765-773.
- Smereka K.J., MacHardy W.E. & Kausch A.P., 1987. Cellular differentiation in *Venturia inaequalis* ascospores during germination and penetration of apple leaves. *Can. J. Bot.*, **65**, 2549-2561.
- Trapman M. & Polfliet C.M., 1997. Management of primary infections of apple scab with the simulation program RIMpro: review of four years field trials. *IOBC Bull.*, **20**, 241-250.
- van Rhee J.A., 1976. Effects of soil pollution on earthworms. *Pedobiologia*, **17**, 201-208.
- Williams J.S. & Cooper R.M., 2004. The oldest fungicide and newest phytoalexin – a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol.*, **53**, 263-279.