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The widespread occurrence of overwintered conidial inoculum of *Venturia inaequalis* on shoots and buds in organic and integrated apple orchards across the Netherlands

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Abstract

A 2-year study was conducted to determine the widespread occurrence of overwintered conidial inoculum of *Venturia inaequalis* and its impact on the apple scab control in 18 apple orchards (organic and integrated) with various levels of scab in the Netherlands. Autumn assessments of scab lesions showed that the integrated orchards had a significantly lower scab incidence (< 20%) compared to that of the organic orchards (> 60%). At the bud-break phenological stage, the mean numbers of nonviable and viable conidia on 1 cm pieces of shoots ranged from 1 to about 90 and from 6 to more than 1000 in the integrated and the organic orchards, respectively, for both years. However, viable conidia on shoots were found only in 2 integrated and 6 organic orchards out of the 18 and the viability of conidia was below 2%. The mean numbers of viable and nonviable conidia per 100 buds ranged from 24 to more than 1000 and from 230 to almost 5000 in the integrated and the organic orchards, respectively, for both years. In both years, some 60–85% of the conidia was found on the outer bud scales. The percentage viability associated with the outer bud tissues was below 2% for all the orchards. However, the percentage of viable conidia within the inner bud tissues ranged from 0% to 6% in the integrated and from 2% to 11% in the organic orchards for both years. Differences between the organic and the integrated orchards were clearly demonstrated for overwintered conidia associated with both shoot and bud samples. The relationship between autumn scab incidence and numbers of overwintered conidia associated with shoots or buds was exponential. If the autumn scab incidence was above 40%, then the number of overwintered conidia markedly increased. We conclude that specific treatments for overwintering conidia of *Venturia inaequalis* may not be necessary in integrated orchards with a low scab incidence in the previous autumn. However, the risk of early scab epidemics initiated by overwintered conidia potentially is high in organic orchards. Preventative measures in early spring and also in the previous year must be established in these orchards.

Introduction

Most apple scab control strategies are based on the concept that primary infection starts with ascospores of *Venturia inaequalis* (Cooke) G. Wint from overwintered infected leaves on the ground. Therefore, ascospore maturation and release as well as the occurrence of Mills infection periods during ascospore release are key elements to be considered in scab control strategies (Szkolnik, 1969; MacHardy and Jeger, 1983; MacHardy and Gadoury, 1989).

Several older studies reported that scab conidia could overwinter on several parts of the tree such as wood, twig, shoot and bud and might cause early infections in spring (McAlpine, 1902; Cook and Schwarze, 1917; Salmon and Ware, 1931; Marsh and Walker, 1932; Dillion Weston and Petherbridge, 1933; Gloyer, 1937; McKay, 1938; Dillion Weston et al., 1952; Jeffrey, 1953; Swinburne, 1965; Cook, 1974; Hill, 1975; Jeger et al., 1982). However, these studies greatly varied in their opinion about the risk of early infection by overwintered conidia compared to the risk from ascospores. More recent studies have shown that conidia of *Venturia inaequalis* (anamorph *Spilocaea pomi* Fr.) could overwinter superficially on shoots (Kennel, 1981; Moosherr and Kennel, 1986, 1995; Moosherr, 1990) or inside buds (Becker, 1990; Becker et al., 1992). The studies of Becker (1990) and Becker et al. (1992) showed that a considerable number of conidia overwintered inside the buds if the orchard had a substantial level of scab inoculum in the previous season and the winter was mild. Under these conditions, overwintered conidia were believed to initiate early scab epidemics before ascospore infections. Investigations in the Netherlands (Holb et al., 2004) also demonstrated overwintered conidia associated with shoots or buds. Early spring infections arose mainly from conidia on the inner bud tissues. They also concluded that the risk of early scab epidemic by overwintered conidia was potentially high in an orchard with high incidence of scab in the previous years. They supposed that the effect of overwintered conidia on the spring scab epidemic would be negligible in commercial orchards, but no study has investigated the distribution of overwintered conidia in commercial apple orchards with varying incidences of scab in the previous year. Moreover, no study has evaluated the effect of widespread occurrence of overwintered conidia on apple scab control in commercial orchards. The objectives of the present study were: first, to ascertain the widespread occurrence of overwintered conidia on shoots and buds in organic and integrated apple orchards across the Netherlands; second, to classify the sampled orchards according to the presence of overwintered conidial inoculum; and third, to quantify the relationship between autumn scab incidence and conidial numbers the following spring in order to assess the impact of conidial inoculum on apple scab control in these orchards.

Materials and methods

Orchards used for sampling

Samples for the study were collected from 18 Dutch commercial apple orchards at the end of February in 2000 and 2001. The 18 sites, located in the provinces of Flevoland, Utrecht, Gelderland, Noord-Brabant and Zeeland, covered about 5000 ha of apple orchards in the Netherlands (Figure 1). Of the 18 orchards, nine followed the Dutch integrated fruit production guidelines (Anonymous, 1998a) and were designated IFP-1 to IFP-9. The other nine orchards followed the Dutch organic production guidelines (Anonymous, 1998b) and were designated ORG-1 to ORG-9. In the autumn of 1999 and 2000, fungicide applications after fruit harvest were made in two organic orchards (ORG-4 and ORG-5) and in most integrated orchards, except for IFP-5 and IFP-9 in an attempt to prevent primary scab

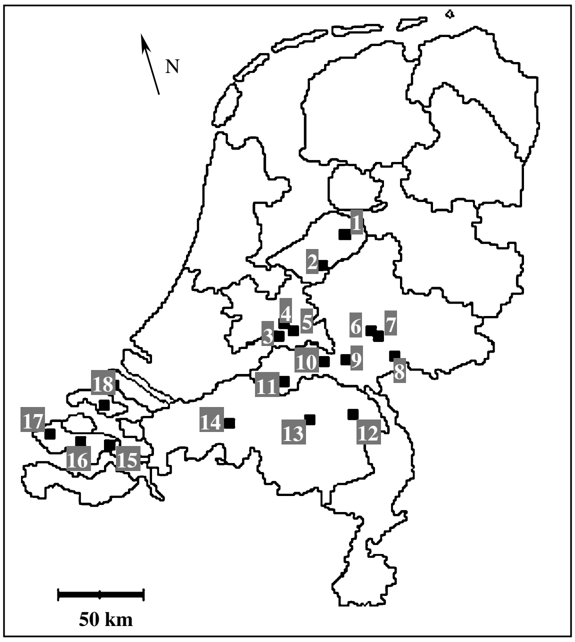

Figure 1. Map showing the location of the sampled integrated (IFP) and organic (ORG) apple orchards. Numbers on the map represent the locations of the orchards, as 1 = ORG-1 and 2 = IFP-1 (province of Flevoland); 3 = IFP-2, 4 = IFP-3 and 5 = IFP-4 (province of Utrecht); 6 = ORG-2, 7 = IFP-5, 8 = ORG-3, 9 = ORG-4, 10 = IFP-6 and 11 = IFP-7 (province of Gelderland); 12 = ORG-5, 13 = ORG-6 and 14 = ORG-7 (province of Noord-Brabant) and 15 = IFP-8, 16 = ORG-8, 17 = ORG-9, and 18 = IFP-9 (province of Zeeland).

infections the following year (Table 1). Trees in both production systems were on M.9 or M.26 rootstocks and pruned to a spindle shape. Tree numbers per hectare ranged from 1600 to 2700 across the selected orchards. Cultivars Jonagold, Elstar and Golden Delicious were grown in all orchards.

Autumn assessments

Incidence of leaf scab was determined after fruit harvest and before leaf fall in 1999 and 2000 in all orchards. Four replicate samples of 200 randomly chosen leaves were assessed in each orchard. Disease incidence was calculated as the percentage of diseased leaves.

Laboratory examinations

Sampling shoots and buds. Shoots and buds were collected in all orchards and examined for overwintering conidia of *Venturia inaequalis*. In both years, shoots and dormant flower buds of cv. Jonagold were collected at the end of February. Four replicate samples of 50 shoots, 15 cm long, and of 100 flower buds, excised approximately 2 mm below the bud scales, were examined. Before examination, samples were stored at 2 °C for no longer than 5 days. Identification was made following Sivanesan and Waller (1974) and Becker (1990); in addition, conidia found in the samples were compared in their morphological characteristics with stored conidial inoculum collected from severely infected leaves in the previous autumn.

Number and viability of overwintered conidia on shoots. Number and viability of conidia on shoots were determined in the laboratory. Each 15-cm shoot segment was cut into 1 cm pieces and labelled. Each 1 cm piece was then separately examined. They were placed in a tube with 5 ml sterilized rainfall water collected on rainy days in an iron bucket placed near the laboratory building. Rainfall water was sterilized in a Vernitron 2000 M steam sterilizer (Alfa Medical Co., Hempstead, NY, USA) at 180 °C for 1 h. Then, samples were incubated at 20 °C for 1 hour, submerged in the water bath of an ultrasonic cleaner (Bransonic Cleaning Equipment Co., Shelton, CT, USA), and sonicated for 10 min. Shoot pieces were removed, and 2 ml of the remained suspension in each tube was centrifuged on an Eppendorf 5414 microcentrifuge (Brinkmann Instrument Inc., Westbury, CT, USA) at 10,000 rpm for 7 min. The pellets were resuspended in 200 µl of rainfall water by stirring on a vortex shaker. Two 100-µl droplets of suspension per 1-cm shoot segment were placed on microslides and incubated in a moist chamber at 20 °C for 48 h. The number of conidia per drop and the number of germinated conidia were recorded by viewing the entire volume of each 100-µl droplet.

In a separate experiment, conidia from 40 sampled shoots were retrieved as described above and half of the sample was tested for viability with and the other half without sonication. A sonication correction factor (SCF) was calculated as viability without sonication divided by viability with sonication. Where viable conidia were found, data were multiplied by SCF to exclude the effect of sonication.

Number and viability of overwintered conidia associated with buds. Every single bud was labelled and separately examined in order to determine the number and viability of associated conidia. Buds were dissected by removing the outer scales, and then the inner tissues. Each dissected part was examined by stereo microscope to detect lesions on the bud scales. Outer bud scales and inner tissues were placed separately in plastic bags with 6 ml of rainfall water, immersed in the water bath and sonicated for ten min. Suspensions were then filtered through a double layer of cheesecloth into beakers, 2 ml of the suspension was placed in each of three Eppendorf microcentrifuge tubes and centrifuged at 10,000 rpm for 7 min. The supernatant was discarded and the pellets were resuspended in 100 µl of rainfall water by stirring on a vortex shaker. Each 100 µl suspension was placed on microslides and incubated in a moist chamber at 20 °C for 48 hours. The number of conidia per drop (three droplets per outer or inner bud tissue) and the number of germinated conidia were recorded. The entire volume of each 100-µl droplet was counted for the quantification of conidia.

In a separate experiment, conidia from 40 sampled buds were retrieved, the SCF was determined and all viability data were corrected as described above.

Table 1. Autumn fungicide applications after fruit harvest in the selected 18 orchards (The Netherlands, 1999 and 2000)

Orchard	1999				2000			
	Number of applications	Active ingredient	Trade name	Dosage (kg ha^{-1})	Number of applications	Active ingredient	Trade name	Dosage (kg ha^{-1})
IFP-1	3	captan, 83%	Brabant Captan	2	2	captan, 83%	Brabant Captan	3
IFP-2	2	captan, 83%	Brabant Captan	3	1	captan, 80%	Captosan 80 WG	3
IFP-3	1	captan, 80%	Captosan 80 WG	2	1	captan, 80%	Captosan 80 WG	2
IFP-4	2	captan, 83%	Luxan Captan	2	1	captan, 83%	Luxan Captan	2
IFP-5	–	–	–	–	–	–	–	–
IFP-6	2	captan, 80%	Captosan 80 WG	2	2	captan, 80%	Captosan 80 WG	2
	1	copper hydroxide, 50%	Fungaran-OH	3	–	–	–	–
IFP-7	2	captan, 80%	Captosan 80 WG	2	2	captan, 80%	Captosan 80 WG	2
	1	copper oxycloride, 50%	Brabant Koper-oxychloride	3	1	copper oxycloride, 50%	Brabant Koper oxychloride	3
IFP-8	1	captan, 83%	Luxan Captan	3	2	captan, 83%	Luxan Captan	2
	1	copper hydroxide, 50%	Fungaran-OH	3	–	–	–	–
IFP-9	–	–	–	–	–	–	–	–
ORG-1	–	–	–	–	–	–	–	–
ORG-2	–	–	–	–	–	–	–	–
ORG-3	–	–	–	–	–	–	–	–
ORG-4	2	elementary sulphur, 80%	Thiovit S		2	elementary sulphur, 80%	Thiovit S	4
ORG-5	1	elementary sulphur, 80%	Thiovit S	5	1	elementary sulphur, 80%	Thiovit S	5
ORG-6	–	–	–	–	–	–	–	–
ORG-7	–	–	–	–	–	–	–	–
ORG-8	–	–	–	–	–	–	–	–
ORG-9	–	–	–	–	–	–	–	–

Statistical analyses

Analysis of variance (ANOVA) was used to evaluate the effects of year, system (integrated or organic), orchard and their interactions on autumn scab incidence and on total number and viability of conidia associated with shoots and buds.

The selected 18 orchards formed two groups (Table 1). Group I consisted of data for the nine integrated and group II for the nine organic orchards. The two-sample Kolmogorov-Smirnov test was used (Sokal and Rohlf, 1981) to test the null hypothesis of no difference between the two groups in: percentage of autumn scab incidence, mean number of conidia on 1-cm segment shoots, viability of conidia on 1-cm segment shoots, mean number of conidia on inner budscales, mean number of conidia on outer budscales, mean number of conidia on inner plus outer budscales, viability of conidia on inner budscales, viability of conidia on outer budscales and viability of conidia on inner plus outer budscales.

Nonlinear regression was used to quantify relationships, and describe the associations between autumn scab incidence and the mean number of conidia, and between autumn scab incidence and the number of viable conidia associated with shoots or buds.

All the statistical analyses were done using the Genstat Release 4.2 statistical package (Genstat 5 Committee, 1997).

Results

Autumn scab incidence

Leaf incidences ranged from 2% to 95% in 1999 and from 0% to about 95% in 2000 across the orchards (Table 2). In both years, there were

Table 2. Autumn scab incidence in 1999 and 2000 and number of total and viable overwintered conidia of *Venturia inaequalis* on shoot before bud break in 2000 and 2001

	Autumn scab incidence				Conidia per shoot[a]							
	1999		2000		2000				2001			
Orchard	%	SE[b]	%	SE	Total[c]	SE	Viable[d]	SE	Total[c]	SE	Viable[d]	SE
IFP-1	3.0	0.4	0	0	6.7	0.8	0	–	2.4	0.3	0	–
IFP-2	44.8	5.6	43.0	5.2	22.3	1.6	0.5[d]	0.1	92.8	0.9	0.8	0.1
IFP-3	40.4	6.3	42.3	5.1	32.3	2.3	0.4	0.1	25.2	0.6	0.2	0.0
IFP-4	7.0	1.2	35.6	4.6	2.0	0.6	0	–	7.5	1.2	0	–
IFP-5	4.0	0.5	5.0	0.6	3.6	0.4	0	–	7.1	0.6	0	–
IFP-6	22.6	5.3	26.6	3.6	9.4	1.2	0	–	0.7	0.1	0	–
IFP-7	2.1	0.2	0	0	4.0	0.5	0	–	0.8	0.1	0	–
IFP-8	10.9	2.3	0.1	0.1	12.0	1.9	0	–	5.9	0.5	0	–
IFP-9	5.0	1.1	0	0	7.6	1.0	0	–	0.7	0.1	0	–
Orchard Mean	15.5	–	16.9	–	11.1	–	0.1	–	15.9	–	0.1	–
ORG-1	41.5	5.6	39.5	5.9	7.6	0.6	0	–	6.3	0.6	0	–
ORG-2	90.4	4.8	95.4	2.5	1033	79.2	10.5	1.1	581	45.3	2.1	0.1
ORG-3	72.6	7.9	51.5	9.5	51.5	1.2	2.6	0.3	43.8	2.0	0.6	0.0
ORG-4	89.3	5.5	83.3	6.3	802	32.8	7.0	1.0	302	21.7	3.5	0.4
ORG-5	59.2	8.9	45.2	6.3	63.1	5.6	1.4	0.1	21.2	2.6	0	–
ORG-6	57.5	7.6	77.5	6.8	33.4	0.9	0	–	213	26	2.1	0.1
ORG-7	39.9	8.9	46.7	5.1	12.9	1.5	0	–	14.4	2.6	0	–
ORG-8	95.0	2.5	65.5	4.9	178	11.2	3.8	0.5	59.6	4.6	0.8	0.0
ORG-9	52.5	11.2	57.5	9.3	19.8	1.2	0.3	0.0	17.5	1.3	0.2	0.0
Orchard Mean	66.4	–	62.5	–	245	–	2.8	–	140	–	1.0	–

[a] Mean number of total and viable conidia on 1-cm piece of shoot.
[b] SE = standard errors of mean.
[c] Total = mean of the total number of conidia on 1-cm piece of shoot.
[d] All viability data were multiplied with a sonication correlation factor = 1.072.

considerably lower mean incidences in the integrated orchards ($<20\%$) compared with the organic orchards ($>60\%$). Autumn scab incidence was significantly affected by the production system and orchard ($P \leq 0.001$) (Table 4). Although the year effect was not significant, there was a significant interaction with orchard ($P \leq 0.001$), for example in IFP-4 scab incidence was low in 1999 but much higher in 2000.

Number and viability of overwintered conidia on shoots

In the integrated orchards, the mean number of conidia on 1-cm pieces of shoot ranged from about 2 to 32 and from about 1 to 93 in 2000 and 2001, respectively (Table 2). In the organic orchards, it ranged from about 7 to more than 1000 in 2000 and from 6 to about 600 in 2001. Effects of production system and orchard were highly significant ($P \leq 0.001$) for the total number of conidia on shoots (Table 4).

The number of viable conidia on shoots was generally low for all orchards (Table 2). Viable conidia on shoots were found in two integrated and six organic orchards in both years. The percentage of viable conidia on shoots was extremely low, ranging from 0% to 2% for all orchards (data not shown). There were no significant effects for the viability of conidia on shoots (Table 4).

Number and viability of overwintered conidia associated with buds

No scab lesions on outer or inner bud tissues were found in either year. However, microscopic examination showed that overwintering conidia associated with buds were present in various numbers in every orchard (Table 3). In 2000, the mean of the total number of conidia per 100 buds ranged from about 24 to 210 in the integrated orchards and from 230 to about 4500 in the organic orchards. In 2001, it varied between 72 and 1040 and between 270 and almost 5000 in the integrated and organic orchards, respectively. Some 60–85% of the total numbers of conidia found was on the outer bud scales in both years. The maximum of the mean conidia number per 100 buds was 220 and 1335 conidia on the inner bud tissues in the integrated and the organic orchards, respectively. Production system was highly significant ($P \leq 0.001$) for the total number of conidia associated with buds (Table 4).

Viable conidia associated with buds were found in all organic orchards and in 6 and 4 integrated orchards in 2000 and 2001, respectively (Table 3). The mean number of viable conidia per 100 buds was low, especially in the integrated orchards. It ranged from 0 to 5.6 and from 0 to 20.4 in 2000 and 2001, respectively, in the integrated orchards. The mean values varied between 11.3 and 110.9 and between 5.9 and 65.5, respectively, in the organic orchards. There were significantly more viable conidia on the inner bud tissues than the outer bud tissues. Percent of viable conidia ranged from 0.8% to 1.8% on the outer bud tissues for all orchards in both years (data not shown). The percentage of viable conidia within the inner bud tissues ranged from 0% to 6% in the integrated and from 2% to 11% in the organic orchards for both years (data not shown). Orchard and production system had significant effects ($P \leq 0.001$) on the number of viable conidia associated with buds (Table 4).

Orchard classification and relationships

Results from the integrated and organic orchard groups were compared using the Kolmogorov-Smirnov test ($P \leq 0.05$). Autumn scab incidence and overwintered conidia of *Venturia inaequalis* in association with shoots and buds were significantly different: autumn scab incidence ($P = 0.009$); total number of conidia on a 1-cm shoot segment ($P = 0.039$); total number of conidia on the outer budscale ($P = 0.002$); viability of conidia on the outer budscale ($P = 0.008$); total number of conidia on the inner budscale ($P = 0.009$); viability of conidia on the inner budscale ($P = 0.035$); total number of conidia on the outer plus inner budscale ($P = 0.008$); viability of conidia on the outer plus inner budscale ($P = 0.003$). Viability of conidia on 1-cm shoot segments did not differ significantly between the two groups ($P = 0.345$).

The relationships between autumn scab incidence and the total number and viability of conidia associated with shoots or buds were exponential for the 2-year combined data set (Figure 2). When the percentage of autumn scab incidence was above 40% then the numbers of conidia associated with buds markedly increased. However, the increase of the number of conidia

Table 3. Number of total and viable overwintered conidia of *Venturia inaequalis* associated with buds before bud break in 2000 and 2001

		Conidia per 100 buds[a]					
		Total			Viable[b]		
Year	Orchard	Out[c] SE[d]	In[e] SE	Out + In[f] SE	Out SE	In SE	Out + In SE
2000	IFP-1	85.5 ± 1.0	23.7 ± 3.2	109 ± 4.2	0 ± 0	3.6 ± 0.6	3.6 ± 0.6
	IFP-2	175 ± 2.5	35.5 ± 5.1	210 ± 7.6	3.3 ± 0.8	2.3 ± 0.5	5.6 ± 1.3
	IFP-3	72.1 ± 0.6	9.4 ± 1.2	81.5 ± 1.8	1.5 ± 0.4	0.5 ± 0.1	2.0 ± 0.5
	IFP-4	32.3 ± 3.1	0 ± 0	32.3 ± 3.1	0 ± 0	0 ± 0	0 ± 0
	IFP-5	65.9 ± 8.9	27.2 ± 4.2	93.1 ± 13.1	0 ± 0	2.0 ± 0.2	2.0 ± 0.2
	IFP-6	22.5 ± 3.6	1.8 ± 0.1	24.3 ± 3.7	0 ± 0	0 ± 0	0 ± 0
	IFP-7	71.1 ± 6.7	5.4 ± 0.8	76.5 ± 7.5	0 ± 0	0 ± 0	0 ± 0
	IFP-8	173 ± 19.2	33.5 ± 4.2	207 ± 23.4	2.8 ± 0.8	0 ± 0	2.8 ± 0.8
	IFP-9	98.7 ± 8.6	8.7 ± 0.9	107 ± 9.5	0 ± 0	0.5 ± 0.1	0.5 ± 0.1
	Orchard Mean	88.5	16.1	105	0.8	1.0	1.8
	ORG-1	472 ± 5.2	79.3 ± 0.9	552 ± 6.1	0 ± 0	7.7 ± 2.2	7.7 ± 2.2
	ORG-2	2638 ± 11.2	583 ± 23.5	3221 ± 34.7	45.7 ± 6.6	65.2 ± 6.2	110.9 ± 12.8
	ORG-3	1962 ± 65.2	372 ± 3.2	2334 ± 68.5	14.8 ± 1.3	31.2 ± 5.4	46.0 ± 6.7
	ORG-4	3192 ± 152	582 ± 7.6	3772 ± 159	20.6 ± 1.7	28.8 ± 5.5	49.4 ± 7.2
	ORG-5	2083 ± 111	412 ± 6.1	2495 ± 117	8.1 ± 0.9	3.2 ± 0.3	11.3 ± 1.2
	ORG-6	367 ± 23.1	88.7 ± 1.6	455 ± 24.7	3.4 ± 0.2	16.0 ± 2.2	19.4 ± 2.4
	ORG-7	342 ± 15.3	59 ± 1.1	300 ± 16.4	4.7 ± 0.5	8.7 ± 1.2	13.4 ± 1.7
	ORG-8	3715 ± 156	752 ± 13.2	4467 ± 169	33.6 ± 2.2	67.8 ± 6.2	101.4 ± 8.4
	ORG-9	185 ± 12.3	46.7 ± 10.2	232 ± 22.5	4.9 ± 0.8	5.8 ± 1.3	10.7 ± 2.1
	Orchard Mean	1662	331	1993	15.1	26.0	41.1
2001	IFP-1	64 ± 2.6	21.2 ± 2.3	85.2 ± 4.9	0 ± 0	0 ± 0	0 ± 0
	IFP-2	820 ± 35.2	220 ± 15.3	1040 ± 50.5	9.5 ± 0.9	10.9 ± 0.9	20.4 ± 1.8
	IFP-3	417 ± 41.3	155 ± 19.2	572 ± 60.5	6.5 ± 0.8	10.0 ± 0.9	16.5 ± 1.7
	IFP-4	252 ± 52.1	102 ± 17.3	354 ± 69.4	4.4 ± 0.5	4.6 ± 0.3	9.0 ± 0.8
	IFP-5	67.5 ± 8.2	19.8 ± 2.8	87.3 ± 11	0 ± 0	0 ± 0	0 ± 0
	IFP-6	257 ± 31.2	114 ± 21.2	371 ± 52.4	2.9 ± 0.5	3.9 ± 0.2	6.8 ± 0.7
	IFP-7	61.2 ± 7.3	10.9 ± 2.1	72.1 ± 9.3	0 ± 0	0 ± 0	0 ± 0
	IFP-8	100 ± 11.2	39 ± 4.5	139 ± 15.7	0 ± 0	0 ± 0	0 ± 0
	IFP-9	168 ± 14.1	10.7 ± 1.6	179 ± 15.7	0 ± 0	0 ± 0	0 ± 0
	Orchard Mean	245	76.9	322	2.6	3.3	5.8
	ORG-1	378 ± 35.2	260 ± 5.2	638 ± 40.4	3.9 ± 0.6	20.5 ± 2.8	24.4 ± 3.4
	ORG-2	3622 ± 65.2	1335 ± 42.6	4957 ± 108	25.6 ± 4.5	61.8 ± 7.2	87.4 ± 11.7
	ORG-3	1482 ± 122	498 ± 23.2	1980 ± 145	13.7 ± 1.7	22.9 ± 3.7	36.6 ± 5.4
	ORG-4	3563 ± 192	1027 ± 122	4590 ± 314	25.8 ± 3.1	39.7 ± 6.6	65.5 ± 9.7
	ORG-5	1515 ± 211	433 ± 23.1	1948 ± 234	11.6 ± 1.7	13.5 ± 2.2	25.1 ± 3.9
	ORG-6	1433 ± 163	595 ± 42.5	2028 ± 205	8.6 ± 1.2	32.8 ± 4.6	41.4 ± 5.8
	ORG-7	192 ± 18.2	78.7 ± 12.3	271 ± 30.5	2.0 ± 0.4	3.9 ± 0.6	5.9 ± 1.0
	ORG-8	575 ± 15.2	350 ± 29.1	925 ± 44.3	13.6 ± 2.5	18.6 ± 1.4	32.2 ± 3.9
	ORG-9	440 ± 53.2	158 ± 19.1	598 ± 72.3	10.4 ± 1.2	17.9 ± 1.3	28.3 ± 2.5
	Orchard Mean	1467	526	1993	12.8	25.7	38.5

[a] Mean number of total and viable conidia on the outer and inner tissues of buds per 100 buds.
[b] All viability data were multiplied with a sonication correlation factor = 1.068 and 1.065 for outer and inner bud scales, respectively.
[c] Out = number of conidia on the outer bud tissues.
[d] SE = standard errors of mean.
[e] In = number of conidia on the inner bud tissues.
[f] Out + In = number of conidia on both the outer and inner bud tissues.

associated with shoots was considerably slower compared to the number of conidia associated with buds. The relationships between autumn scab incidence and the number of viable conidia associated with buds was similar to that found for total conidia.

Table 4. Analyses of variance for autumn scab incidence and for the number of total and viable overwintered conidia of *Venturia inaequalis* associated with shoots and buds

	Autumn scab			Conidia per shoot[a]						Conidia per 100 bud[b]					
	Incidence (%)			Total			Viable[c]			Total			Viable		
Source of variance	df[d]	MS[e]	*P*-value[f]	df	MS	*P*-value	df	MS	*P*-value	df	MS	*P*-value	df	MS	*P*-value
Year	1	0.47	0.927	1	15540	0.438	1	19.3	0.077	1	26606	0.713	1	0.33	0.889
System	1	44224	< 0.001	1	1267 867	< 0.001	1	0.71	0.727	1	5491 510	< 0.001	1	1957	< 0.001
Orchard	17	1304	< 0.001	17	316578	< 0.001	7	4.5	0.590	17	363318	0.053	17	139	< 0.001
Year × System	1	180	0.063	1	33828	0.254	1	0.75	0.952	1	8469	0.853	1	7.12	0.517
Year × Orchard	17	307	< 0.001	17	29721	0.315	7	2.7	0.752	17	106552	0.206	17	30.4	0.061
Orchard × System	17	0.06	0.997	17	14.3	0.995	7	0.12	0.996	17	125	0.998	17	0.05	0.996
Year × Sysytem × Orchard	17	0.05	0.998	17	11.2	0.996	7	0.09	0.998	17	106	0.998	17	0.06	0.996
Residual	55	55.8	–	76	25561	–	26	1.67	–	38	193407	–	38	16.7	–

[a] Mean number of total and viable conidia on 1-cm piece of shoot.
[b] Mean number of total and viable conidia on the outer and inner tissues of buds per 100 buds.
[c] Only orchards with viable conidia on shoots were included in the analysis.
[d] df = degrees of freedom.
[e] MS = mean square.
[f] *P*-value = the significant F probability values, considered nonsignificant at $P \geq 0.05$.

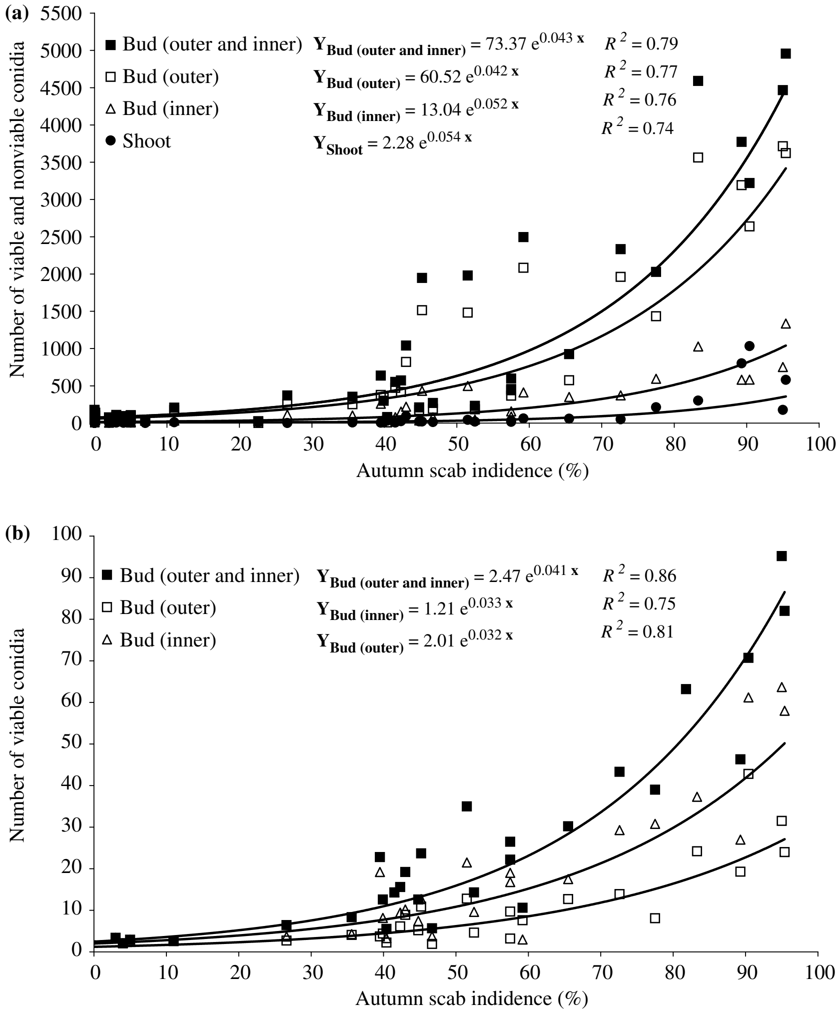

Figure 2. Relationships between autumn scab incidence in the previous year and the number (a) and viability (b) of overwintered conidia of *Venturia inaequalis* associated with buds and shoots before bud break. Data were combined for 2000 and 2001.

Discussion

The number of conidia associated with the outer surfaces of shoots or buds varied significantly among the selected orchards. Trees in orchards were possibly grown under various conditions in previous years, and differed in tree age, tree spacing, inoculum level, intensity of disease control, and cultural practices. Despite this variation, our results clearly showed that in early spring the numbers of conidia were considerably lower in the integrated orchards compared to the organic ones, for both the shoot and bud samples. These results are probably due to the different effectiveness of disease control in the two production systems. In the integrated orchards, most growers carried out an after-harvest fungicide application, whereas in the organic orchards most growers did not. Moreover, more effective fungicidal products against apple scab are allowed in integrated orchards compared with organic orchards. In the organic orchards, there is commonly one copper application (0.05–0.2%) in spring followed by one or two fungicide applications of elementary sulphur (0.2–0.7%) per week until June, then biweekly applications of elementary sulphur till harvest (annually 18–25 applications), but these are not sufficiently effective against apple scab (Ellis et al., 1994, 1998; Holb et al., 2003b). Scab infection was severe and disease development continuous in the organic orchards. Despite these frequent fungicide applications in organic orchards, disease development follows a logistic increase during the summer months (Holb, 2001; Holb et al., 2003a). Consequently, large amounts of conidial inoculum were present by autumn in the organic orchards, which could then survive the winter associated superficially with shoots and especially buds.

Although, several older studies demonstrated that conidia on scab lesions on woods or shoots overwintered in apple orchards (Cook and Schwarze, 1917; Marsh and Walker, 1932; McKay, 1938; Cook, 1974; Hill, 1975), scab lesions on branches or shoots in the sampled orchards were not found in this study. At several locations in Germany, it was demonstrated that scab conidia overwintered on the surface of shoots of scab susceptible cultivars (Kennel, 1981; Moosherr and Kennel, 1986, 1995; Moosherr, 1990). These authors assessed the germination of conidia on infected shoots as generally 12% but in some cases it reached 50% or 60%. Our results indicated that viability of conidia found on the surface of the shoots was below 2%. Viable conidia from shoots were found only in 8 orchards out of the 18, and only two of these were integrated orchards. Conidia did not survive on the surface of shoots under the climatic conditions of New York State (Becker, 1990; Becker et al., 1992). In experimental studies, Holb et al. (2004) concluded that conidia were unlikely to overwinter on the surface of apple tissues (shoots or outer bud tissues), if they were exposed to fluctuating environmental conditions. Results of the present study support those cited above and indicate that overwintered conidia on the surface of shoots are unlikely to play a role in early epidemics of apple scab in commercial apple orchards in climates typical of those found in the Netherlands.

Conidia were found on the outer or inner surface of buds in all organic and integrated orchards. Previous studies (Louw, 1951; Becker, 1990; Becker et al., 1992) demonstrated that conidia did not survive the winter on the outer tissues of buds. Conidia only overwintered in the inner tissues of buds in orchards with a high scab level at the end of the previous year. Holb et al. (2004) indicated that the risk of infection by overwintered conidia would be negligible in well-managed commercial orchards with low scab levels. Results of the present study clearly demonstrated that the number of conidia associated with buds was considerably higher in the severely diseased organic orchards compared to the integrated orchards with lower scab incidence. Moreover, our results showed that viable conidia associated with buds were rarely found in early spring in the integrated orchards with a low scab incidence in the previous year (Table 3). Therefore, we conclude that fungicide application in early spring at bud break or green tip might not be necessary against overwintered conidia of *V. inaequalis* in the integrated orchards. This conclusion has a strong connection with the control strategy suggested by MacHardy et al. (1993). If the potential ascospore dose (PAD) was below 600 ascospores per m^2 orchard floor, then the orchard could be left untreated with fungicide until the pink fruit-bud stage unless three infection periods occurred before this stage. Our results suggest that growers can safely use the strategy of MacHardy et al. (1993) in well-managed integrated orchards in the Netherlands, because overwintered conidia are unlikely to play a role in the epidemic in orchards with a low scab incidence in the previous year.

However, the numbers of viable conidia on the inner bud tissue were considerably higher in highly diseased organic orchards (Table 3), similar to those found in unsprayed orchards (Becker, 1990; Becker et al., 1992). They suggested that if the levels of scab in the previous year were high, the building up of a scab epidemic is severe next year, and large numbers of conidia would remain on the surfaces of the developing buds during the summer. These conidia could become entrapped between the bud scales through the effects of wind or rainfall water, and they could cause symptoms on the inner bud tissues. Umemoto (1991) demonstrated that pear scab conidia of *V. nashicola* produced on leaf lesions flowed down with rainfall water into the inner bud scales. Those conidia germinated and penetrated into the living part of the bud tissues under wet conditions. Each of these studies indicated that scab incidence in the previous year affects the numbers of overwintered conidia on the inner bud tissues in the following year. Although, we could not find scab lesions, only conidia, on the inner bud tissues in this study, we clearly demonstrated the strong relationships between the previous year autumn scab incidence and the total number of conidia on the inner or the outer bud tissues (Figure 2). The number of overwintered conidia increased markedly when the percentage of autumn scab incidence was above 40%. The capacity of these overwintered conidia to cause an infection was evaluated by Holb et al. (2004). They demonstrated, using plastic bag enclosure treatments in an orchard experiment, that overwintered conidia infected young green tissues, probably from the inner tissues of the buds, as also suggested by Becker (1990) and Becker et al. (1994). Therefore, regardless of the number of conidia that overwintered, organic orchards with an autumn incidence above 40% are considerably at risk the following spring. We also have to take into consideration that where the risk of overwintered conidial infection is high, the conditions also favour for ascosporic inoculum. However, conidial infection from buds can be earlier than ascospore infection (Becker, 1990; Becker et al., 1992) and ascosporic inoculum can be greatly reduced in commercial organic orchards by sanitation practices such as shredding, flaming, removal of leaves or some combinations of these strategies (Sutton and MacHardy, 1993; MacHardy et al., 2000). In such situations, conidial inoculum overwintering within buds might assume a greater importance than ascospores. It might therefore be necessary to prevent early spring infections by overwintered conidia in commercial organic orchards. Holb et al. (2004) suggested that a fungicide spray at the green-tip phenological stage would be useful against viable overwintered conidia provided the fungicide reaches the inner tissues of opening buds and kills overwintered conidia or scab lesions inside the buds. The success of this fungicide application might be partial because most fungicides used in organic orchards are 'contact' and consequently it might be difficult to reach the conidia and prevent infection. Therefore, we suggest additional winter pruning to reduce conidial inoculum inside buds. Our previous observations confirmed that most overwintered conidia (about 70–80%) would be present in the upper third part of the shoots of the tree (Holb et al., 2004; I.J. Holb and B. Heijne, unpubl.). Therefore, pruning of the upper third of the last year's extension shoot combined with a fungicide spray at the green-tip phenological stage might be more effective against overwintered conidia. Moreover, in agreement with Stensvand et al. (1996) we suggest that prevention against overwintered conidia should be initiated during the previous summer and autumn in organic orchards. Consequently, orchards should be sprayed continuously with approved products during the summer to restrict the number of lesions on leaves by the end of the summer. Autumn fungicide applications would also be important especially for those cultivars, which continue to grow late in the season and consequently with a late bud formation.

In conclusion, the present study clearly demonstrated the risk of early scab epidemics initiated by overwintered conidia in organic apple orchards with a high scab incidence in the previous year. Winter pruning and early spring fungicide application as well as preventative measures in the previous year have to be established and rigourously followed. However, more knowledge is necessary to clarify the environmental conditions favourable for the infection by overwintering conidia on cultivars with differing pomological characteristics and scab susceptibility, and their suitability for organic production systems.

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