

## Influence of different disease control pesticide strategies on multiple pesticide residue levels in apple

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(Accepted 26 August 2009)

### SUMMARY

Seven pesticide application strategies were investigated to control apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) and, at the same time, fulfil the new quality standards implemented by some German retailers. These demand that pesticide residues should be below 80% of European Maximum Residue Levels (EU MRL) and that the number of residues present at levels above 0.01 mg kg<sup>-1</sup> should be limited to a maximum of four. The strategies fulfilled the requirement to use combinations of different active substances in order to prevent the emergence of resistance to pesticides. The trials were conducted at two sites in Switzerland, in 2007, and all strategies and applications were in accordance with actual practice. Four replicates of apple samples from each strategy were then analysed for pesticide residues. The incidence of infection with apple scab and powdery mildew were monitored during the season in order to evaluate the efficacy of the different strategies. The efficacies of the different strategies against apple scab and powdery mildew were between 84% and 100% successful. In general, the level of pesticide residues found correlated with application rate and time, and no measured residue level exceeded the EU-MRL. The numbers of residues present at > 0.01 mg kg<sup>-1</sup> were between two and five.

Pesticides are widely used during the production of fruits, vegetables, and cereals. In apple production, fungicides and insecticides are used both pre- and post-harvest to protect the fruit from a range of pests and fungi, and to preserve quality. Food authorities, [e.g., the Commission of the European Communities (CEC)] have established Maximum Residue Levels (MRL) for all the pesticides used in the production of food and feed. (European Community, 2005). Monitoring programmes for pesticides have shown that fungicide and insecticide residues are often found in apples. An EC-coordinated monitoring programme in 2004 showed residues of pesticides in 61% the apple samples analysed (European Commission, 2006). Although, residues above the MRLs were found in only 2% of samples, many consumers and non-governmental organisations (NGOs) are concerned about any exposure to pesticide residues. One of the major criticisms is that pesticide residues are evaluated individually and MRLs are set for each pesticide, individually, although more than 25% of all fruit and vegetables analysed had residues of more than one pesticide (European Commission, 2007). The NGO, Greenpeace, has published a survey of pesticide residues in fruit and vegetables collected in German supermarkets (Greenpeace, 2007). The results from this investigation contributed to the implementation of new quality management systems by major retailers with the aim of reducing both the number and concentration of pesticide residues in fruits and vegetables (i.e., that

residue levels must not exceed 80% of the MRL, and there must not be more than four residues present at > 0.01 mg kg<sup>-1</sup> per sample).

This new quality standard has created a conflict of interest between retailers and producers. In modern integrated pest management (IPM) systems, farmers are encouraged to rotate the use of plant protection products (PPP) or to use mixtures of several active substances to control a specific pest or disease. This is done to prevent resistance emerging in the pest or pathogen against the PPP, which may lead to economic losses or to a need for higher rates of application of the PPP. Many new PPPs are specific against one, or one group, of weeds, pests or pathogens and, because simultaneous attacks from different organisms are often seen, application of a mixture of different PPPs, at lower application rates of individual active substances, can control these organisms while simultaneously prevent resistance to each active substance (Brent and Hollomon, 2007).

Apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) are important pathogens in apple production. Both are controlled by timely applications of fungicides, supported by an on-line forecasting system. To prevent resistance against anilino-pyrimidines, strobilurins, and triazoles arising in *V. inaequalis*, the number of applications per season is limited and a mixture with folpet or captan is prescribed. In years with a high disease pressure, the control of powdery mildew requires the addition of bupirimate or sulphur to the anti-scab products.

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TABLE I  
Pest and disease control strategies carried out at Wädenswil.

Application date (days before harvest)	Strategy 1	Strategy 2	Strategy 3
156	Captan (1.33), Sulphur (2.56)	Captan (1.33), Sulphur (2.56)	Captan (1.33), Sulphur (2.56)
143	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)
133	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Dithianon (0.6), Sulphur (2.56)
127	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Pyrifenoxy (0.08), Captan (0.96)
120	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)
106	Difenoconazole (0.06), Captan (1.33), Pirimicarb (0.32)	Difenoconazole (0.06), Captan (1.33), Pirimicarb (0.32)	Trifloxystrobin (0.08), Captan (1.33), Pirimicarb (0.32)
90	Captan (1.99), Bupirimate (0.22)	Captan (1.99), Bupirimate (0.22)	Difenoconazole (0.06), Captan (1.33)
77	Captan (1.99), Bupirimate (0.22)	Captan (1.99), Bupirimate (0.22)	Difenoconazole (0.06), Captan (1.33)
64	Folpet (1.6)	Folpet (1.6)	Folpet (1.6)
45	Trifloxystrobin (0.08)	Folpet (1.6)	Folpet (1.6)

Numbers in brackets represent the amount of active substance sprayed in kg ha<sup>-1</sup>.

In the present study, seven different application strategies were investigated to control apple scab (*V. inaequalis*) and powdery mildew (*P. leucotricha*) and to fulfil the new quality standards being implemented by retailers. The strategies also fulfilled the requirement to use different active substances in order to prevent the emergence of resistance to each pesticide.

## MATERIALS AND METHODS

### Field trials

The trials were conducted at two sites in Switzerland (Wädenswil and Güttingen) in 2007, by applying a total of seven different fungicide strategies against apple scab (*V. inaequalis*) and powdery mildew (*P. leucotricha*). Strategies 1 – 3 were applied at Wädenswil and Strategies 4 – 7 at Güttingen. Pesticide application rates

and times are shown in Table I and Table II. In addition, spraying with dithianon was performed two-three times for all strategies 169 – 189 d before harvest (DBH). Applications were made using a Holder reverse-axial blower, equipped with flat-jet nozzles, in water, equivalent to 400 l ha<sup>-1</sup>. All strategies and applications were in accordance with actual practice. Harvesting was on 21 September at Güttingen and on 27 September at Wädenswil. Four replicate samples of apples (n = 10 per replicate) from each Strategy were collected and transported immediately to the laboratory for pesticide residue analysis. After harvest, the apples were stored for 6 months to investigate the degradation of pesticide residues during the chain from producer to consumer (manuscript in preparation).

During the 2007 growing season, the incidence of infection with apple scab and/or powdery mildew were

TABLE II  
Pest and disease control strategies carried out at Güttingen

Application date, (days before harvest)	Strategy 4	Strategy 5	Strategy 6	Strategy 7
158	Trifloxystrobin (0.08), Captan (1.22)	Cyprodinil (0.24), Captan (1.22), Bupirimate (0.22)	Trifloxystrobin (0.1), Tolyfluanide (1)	Dithianon (0.6)
148	Bupirimate (0.22)	Bupirimate (0.22)	Bupirimate (0.22)	Bupirimate (0.22)
137/134 <sup>1</sup>	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Pyrifenoxy (0.08), Captan (1.22), Sulphur (2.56)
128/126 <sup>1</sup>	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Dithianon (0.6), Sulphur (2.56), Pirimicarb (0.32)
123	Pirimicarb (0.32)	Pirimicarb (0.32)	Pirimicarb (0.32)	Pirimicarb (0.32)
114/119 <sup>1</sup>	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.08), Captan (1.22), Sulphur (2.56)	Trifloxystrobin (0.1), Tolyfluanide (1), Sulphur (2.56)	Dithianon (0.6), Bupirimate (0.22)
97/99 <sup>1</sup>	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)	Folpet (1.6), Bupirimate (0.22)
81/85 <sup>1</sup>	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)	Difenoconazole (0.06), Captan (1.33)	Folpet (1.6)
63	Captan (1.33)	Captan (1.33)	Captan (1.33)	Trifloxystrobin (0.08), Folpet (1.6)
52	Captan (1.33)	Captan (1.33)	Captan (1.33)	Captan (1.33)
37	Captan (1.33)	Trifloxystrobin (0.08)	Captan (1.33)	Captan (1.33)

<sup>1</sup>The application date before the slash refers to Strategies 4–6, and the date after the slash refers only to Strategy 7. Numbers in brackets represent the amount of active substance sprayed in kg ha<sup>-1</sup>.

monitored 500 leaves to evaluate the efficacy of the different pesticide strategies. Furthermore, a rating of storage diseases was done for all three pesticide strategies performed at Wädenswil. This rating was done after 6 months storage by sorting 100 kg of the apples into the categories: “undamaged”, “apple scab lesions”, “*Gloeosporium* rot”, or “other damage”.

#### Pesticide residues analysis

Analyses were carried out according to Anastassiades *et al.* (2003) as modified by Payá *et al.* (2007). Briefly, the analytical procedure used 15.0 g homogenised apple sample extracted with 15.0 ml of acetonitril (Rathburn, Walkersburn, UK) by shaking for 30 s. After extraction, 6.0 g MgSO<sub>4</sub> (J.T Baker, Phillipsburg, NJ, USA), 1.5 g NaCl (Merck, Whitehouse Station, NJ, USA) were added and the tube was shaken for 1 min. Then 1.5 g sodium citrate dihydrate and 0.75 g sodium citrate sesquihydrate (both from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) were added and, after additional 1 min of shaking and centrifugation at 4,400 × g for 10 min, 1.0 ml of clarified extract was transferred to a small centrifuge tube containing 0.05 g primary secondary amine (PSA) adsorbent and 0.15 g MgSO<sub>4</sub> (UCT, Bristol, PA, USA). After shaking for 30 s and centrifugation at 4,400 × g for 5 min, the extract was analysed by GC/MS/MS using a Quattro Micro Tandem GC-MS/MS system (Waters, Milford, MA USA). The instrument was operated with electronic ionisation, and detection was performed for bupirimate, difenoconazole, folpet, pirimicarb, trifloxystrobin, captan and the degradation product of captan, and tetrahydrophthalimid, in the multiple reaction monitoring (MRM) mode with two transitions.

## RESULTS AND DISCUSSION

The efficacy of the different pesticide strategies against apple scab and powdery mildew was between 84% and 100%, compared to an unusually low incidence of both diseases (< 10%) on the untreated controls. Under these conditions, single infections may have a strong impact on efficacy and any comparison of strategies is not reliable. However, no significant

differences were found for the main storage diseases scab or *Gloeosporium* within each orchard, but the level of protection against storage diseases was generally better on apples from Wädenswil than from Güttingen. This may reflect differences in the quality of the applications at the two sites. A final application of trifloxystrobin, without a mixing agent, is permitted, in order to protect against storage diseases and is common in practice as it causes no spots on the fruit. This single application is favourable in the context of residues, but is highly questionable in the context of the prevention of the emergence of resistance.

The pesticide residues found on the various apple samples are shown in Table III. The active substance, captan, was applied in all strategies and residues of captan and its degradation product, tetrahydrophthalimid, were found in all strategies. The levels of these residues found in apples correlated with the amount of captan applied ha<sup>-1</sup>. Captan residues were below the EU-MRL (at 3 mg kg<sup>-1</sup>) for all strategies.

Folpet was applied in Strategies 1, 2, 3, and 7 and its residue levels indicated a relationship between application rate and time, with the highest concentrations in apples from Strategies 2 and 3, where folpet was applied 45 d before harvest. However, folpet could also be found in apples from Strategies 4 and 6, and even in control apples from Güttingen. The trial was performed as an efficacy evaluation trial and the tank was emptied, but not cleaned, between applications of the different substances. The results showed that even minor residues in the sprayer tank can lead to detectable pesticide residues in apples, which could cause growers problems in complying with retailer regulations.

The active substance, bupirimate was applied to control powdery mildew in Strategies 1, 2, 4, 6, and 7. It is notable that significant residues of bupirimate were found only when it was applied in the second part of the growing season (i.e., in Strategies 1, 2, and 7). However, the levels were very low.

Trifloxystrobin residue levels seemed to be highly correlated with application rate and time. The highest amount was detected in fruit from Strategies 1 and 5, where it was applied as a final treatment 45 d and 37 d prior to harvest, respectively. When trifloxystrobin was

TABLE III  
Residue levels (in mg kg<sup>-1</sup>) of captan, tetrahydrophthalimid, pirimicarb, folpet, bupirimate, or trifloxystrobin detected in apples produced at Wädenswil (Strategy 1–3) and at Güttingen (Strategy 4–7)

Pesticide (EU MRL)	Strategies at Wädenswil				Strategies at Güttingen				
	1	2	3	Control	4	5	6	7	Control
Captan (3.0 mg kg <sup>-1</sup> )	0.045 (27%)	0.016 (42%)	0.019 (17%)	n.d.	0.016 (45%)	0.012 (53%)	0.010 (28%)	0.012 (28%)	n.d.
Tetrahydro-phthalimid (not included)	0.084 (6%)	0.051 (44%)	0.042 (18%)	n.d.	0.076 (33%)	0.075 (43%)	0.060 (17%)	0.031 (27%)	n.d.
Pirimicarb (2.0 mg kg <sup>-1</sup> )	0.033 (11%)	0.032 (30%)	0.022 (15%)	n.d.	0.006 (8%)	0.008 (28%)	0.007 (18%)	0.010 (27%)	0.008 (10%)
Folpet (3.0 mg kg <sup>-1</sup> )	0.019 (51%)	0.036 (52%)	0.086 (35%)	n.d.	0.010 (23%)	n.d.	0.007 (19%)	0.015 (50%)	0.005 (10%)
Bupirimate (0.2 mg kg <sup>-1</sup> )	0.004 (14%)	0.003 (18%)	n.d.	n.d.	n.d.	n.d.	n.d.	0.002 (20%)	n.d.
Trifloxystrobin (0.5 mg kg <sup>-1</sup> )	0.012 (21%)	n.d.	n.d.	n.d.	n.d.	0.010 (42%)	n.d.	0.007 (17%)	n.d.

n.d. = not detected.

Number in brackets after each result refers to the relative standard deviation of four repetitions per sample. The number in brackets after the pesticide name refers to the EU MRL.

applied earlier than 107 DBH (i.e., in Strategies 2, 3, and 6), no residues could be found.

Pirimicarb was applied in all Strategies, and residues of this insecticide were detected in all cases. Concentrations were correlated with the time of application.

Difenoconazole was not detected above its detection limit of 0.005 mg kg<sup>-1</sup>.

The present results confirm that the levels of PPP residues detected on apples are highly dependent on the chemistry of the active ingredient, as well as the timing and dose applied.

None of the residue levels detected exceeded EU-MRLs. However, newly implemented regulations by some German and Swiss retailers limit the number of residues that can exceed a threshold of 0.01 mg kg<sup>-1</sup>, to five. The plant protection strategies tested here corresponded to those advised by consultants and agrochemical companies. Strategy 1 did meet retailer regulations. The number of residues detected at > 0.01 mg kg<sup>-1</sup> in the other strategies was between two and four.

#### FUTURE WORK

An artificial supply chain from producer to consumer, will be investigated in order to elucidate the degradation of pesticide residues and the formation of patulin in apples. This chain will consist of cold storage at 4°C for 6 months, at 97% relative humidity. After storage, the apples will be passed through a warm water bath, and will finally be kept at room temperature for 14 d. In addition, the influence of white light (400 – 800 nm) and UV light (300 nm) will be investigated, as well as tolerance to *Penicillium expansum* and the resulting concentrations of patulin.

The ISAFRUIT Project is funded by the European Commission under Thematic Priority 5 – Food Quality and Safety of the 6th Framework Programme of RTD (Contract No. FP6-FOOD-CT-2006-016279).

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