Investigation on Fungicide Residues in Greenhouse-Grown Strawberries

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Maximum residue limits (MRL's) for different agricultural food products in Norway are harmonized with EU standards. In field-grown strawberries in Norway, tolylfluanid has a 7 day quarantine from last application to harvest, while other approved fungicides have 14 days quarantine. Greenhouse production of strawberries is newly introduced to the country. Residue levels in strawberries of the cultivar Korona grown in a commercial greenhouse were investigated 4, 7, and 14 days after application of eight different fungicides at rates recommended by the manufacturers and at half rates. Iprodione, tolylfluanid, and vinclozolin were tested in two experiments, while chinomethionat, chlorothalonil, imazalil, penconazole, and triadimefon were tested once. For chinomethionat, imazalil, iprodione, penconazole, and vinclozolin, the residue levels were below MRL 2 weeks after application. Application of triadimefon in normal rate gave residues below MRL 14 days after application. However, its metabolite, triadimenol, was above MRL at the same time. Tolylfluanid gave very high residue levels, and except from half concentration in the second experiment, all other residue levels were above MRL. Seven days after application, residues in both experiments were approximately 3 times higher than MRL when normal rate of tolylfluanid was applied. For chlorothalonil at the recommended rate, the residue level was above MRL at any sampling time, while half rate gave residues below MRL 14 days after treatment. In view of the present results, tolylfluanid, chlorothalonil, and triadimefon will need longer time from last application to harvest and/or reduced application rates in greenhouse-grown compared to field-grown strawberries. In addition or as an alternative, recommended rates could be lowered.

Keywords: *Fungicides; greenhouse; residues; strawberry; HPLC; GC*

INTRODUCTION

Gray mold, caused by *Botrytis cinerea* Pers. ex Fr., and powdery mildew, caused by *Sphaerotheca alchemillae* (Grev.) Junell, are the two most important fungal diseases in field-grown strawberries (*Fragaria* × *ananassa* Duchesne) in Norway. In the field, both diseases are controlled by extensive fungicide applications. *B. cinerea* needs moist conditions for infection to occur, while infection of *S. alchemillae* is favored by warm, dry days and cool nights with high humidity, but no free water. Even if climatic conditions in a greenhouse to a large extent are controlled and can be regulated to avoid conditions favoring diseases, fungicides are often needed to control both gray mold and powdery mildew. The present authors have found neither any research reports comparing degradation of fungicides in strawberries grown under field or greenhouse conditions nor any reports on fungicide degradation in greenhouse-grown strawberries per se.

The permitted number of days from application to harvest for fungicides varies from country to country. In strawberries in Norway, tolylfluanid might be applied 7 days before harvest, while other approved fungicides have 14 days quarantine. There is a continuous focus on food safety and pesticide residues, and the current experiments were carried out to investigate the influence of the interval between application and harvest on residues for various fungicides in greenhouse-grown strawberries.

MATERIALS AND METHODS

Greenhouse Facilities and Plant Material. The experiments were carried out in a 1000 m^2 commercial greenhouse (covering material: acrylic plates) at Hobøl in Østfold county, situated approximately 50 km south of Oslo. The greenhouse had one passage in the middle and 5 m^2 shelves on each side covering the whole greenhouse area except the passage. Each shelf contained 75 plants in peat bags in a single row. The height of the shelves above the floor could be adjusted whenever necessary for picking, spraying, etc. The day temperature was kept at 18−22 °C, while the night temperature during cool nights was lowered to 10−14 °C. This temperature was kept for ca. 4 h before it was increased to day temperature. The mean daily outside temperature was approximately 11.6 and −0.8 °C during the 1994 and 1995 experiments, respectively (mean of records from two meteorological stations in Østfold county). During the first experiment (June 1994) no additional light was given. In the second experiment (February−March 1995), 18 h artificial light per day was provided by high-pressure sodium lamps at a level of 160−180 W/m^2 measured just above the canopy of the plants. The cultivar used was Korona, planted 1 September 1993 and 10 August 1994 in the 1994 and 1995 experiments, respectively. The plants grew in peat and were watered 3−5 times per day

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Table 1. Fungicides, Products, Percentage Active Ingredients (% a.i.), Recommended Spray Concentrations of the Products per Liter, and Manufacturers

fungicide	product	% a.i.	recomm concn	manufacturer
chinomethionat	Morestan	25	0.3 g	Bayer
chlorothalonil	Bravo 500	50	2.5 mL	Isk Biosciences
imazalil[a]	Fungaflor 37 SL	3.7	5.5 mL	Cillus
iprodione	Rovral Akva	50	1.5 mL	Rhône Poulenc
penconazole	Topas 100 EC	10	0.3 mL	Novartis
tolylfluanid	Euparen M	50	3.0 g	Bayer
triadimefon	Bayleton Spesial	5	1.5 g	Bayer
triadimenol[b]				
vinclozolin[c]	Ronilan FL	50	1.5 mL	BASF

[a] Not approved for strawberries in Norway. [b] Triadimefon is reduced to its corresponding alcohol triadimenol. [c] Prohibited for use in strawberries in Norway since 1996.

(during 3 min intervals) with nutrient solutions containing all macro- and micronutrients, at an electrical conductivity level around 2.0 mS/cm^2.

Fungicides, Applications, and Sampling. The following fungicides were used: chinomethionat (6-methyl-1-3-dithiolo-(4,5-*b*)quinoxalin-2-one), chlorothalonil (tetrachloroisophthalonitrile), imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)-ethyl]-1*H*-imidazole), iprodione (3-(3,5-dichlorophenyl)-*N*-(1-methylethyl)-2,4-dioxo-1-imidazoline carboximide), penconazole (1-[2-(2,4-dichlorophenyl)-pentyl]-1*H*-1,2,4-triazole), tolylfluanid (1,1-dichloro-*N*-[(dimethylamino)sulfonyl)-1-fluoro-*N*-(4-methylphenyl)methanesulfamide), triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone), and vinclozolin (3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione). In plants, triadimefon is reduced to its corresponding alcohol, triadimenol (Kidd and James, 1991). In Norway, the sum of residues for triadimefon and triadimenol (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol) is considered when residues of triadimefon in food products are determined.

Table 1 gives an overview of the various fungicides, including product names, percentage of active ingredients, and the highest fungicide concentration used in the experiments. It was sprayed with two concentrations; as recommended by the manufacturers for field-grown strawberries and half of this rate. The plants were sprayed to runoff (approximately 2.25 L/m^2) with a Ginge hand pump sprayer (Ginge-Raadvad, Brønderslev, Denmark). For each treatment, one 5 m^2 shelf with 75 plants was used. From each shelf, three parallel samples were taken at each of the three sampling days. In some of the samples in the first experiment the number of berries was relatively small. If the parallel samples were below 200 g, they were pooled into larger samples. For each sample analyzed, the size varied between 200 and 500 g. In the second experiment each sample contained approximately 1000 g. To reduce the number of samples, iprodione and vinclozolin were tank-mixed in the second experiment, and the same samples were analyzed for both fungicides. Only iprodione, tolylfluanid, and vinclozolin were tested in the second experiment. Samples were taken 4, 7, and 14 days after application. The samples were kept frozen at −18 °C from the day of sampling until they were analyzed. Small but pronounced residues of tolylfluanid and vinclozolin detected in the untreated strawberries (control) showed that the grower had sprayed with these fungicides prior to start of the second experiment. From the grower's records, it was not possible to find exact dates for these treatments.

Residue Analysis. Chinomethionat, chlorothalonil, iprodione, penconazole, tolylfluanid, triadimefon, triadimenol, and vinclozolin were analyzed by GC (Holen and Christiansen, 1994) using a Hewlett-Packard 5890 GC with electron capture and nitrogen−phosphorus detector. Strawberry subsamples of 25 g were extracted with 125 mL of acetone:petroleum ether (1:1) after addition of 100 g of anhydrous sodium sulfate. Two milliliters of the extract was evaporated to near dryness (25 μL of decane was added as a keeper), redissolved in 1 mL of acetone:isooctane (3:7), and analyzed by capillary GC. The column was HP-1, 30 m × 0.32 mm i.d., 0.25 μm film thickness, and helium was used as carrier gas. The oven was programmed from 80 °C (1.5 min) at a rate of 20 °C/min to 160 °C (0 min), then 4 °C/min to 250 °C (0 min), and finally 10 °C/min to 300 °C (8 min).

Imazalil was analyzed by HPLC (Holen and Christiansen, 1994) using a Shimadzu LC-10A System with UV detector. Strawberry subsamples of 25 g were extracted with 125 mL of acetone:petroleum ether (1:1) after addition of 1.5 mL of 6.5 M NaOH and 100 g of anhydrous sodium sulfate. Five milliliters of the extract was evaporated to dryness, redissolved in 1 mL of acetonitrile:phosphate buffer pH 2.2 (2:8), and analyzed by HPLC with UV detection at 220 nm. The column was Suplex pKb-100, 5 μ, 15 cm × 4.6 mm i.d. (Supelco), and the mobile phase was acetonitrile:0.02 M phosphate buffer with triethylamine, pH 7.1 (44:56) with flow 1 mL/min.

Certified reference standards of all pesticides were supplied by Dr. Ehrenstorfer (Augsburg, Germany). The organic solvents used for the GC analysis were of pesticide grade and acetonitrile was of HPLC grade, all from Labscan (Dublin, Ireland).

RESULTS AND DISCUSSION

Maximum residue limits (MRL's) for strawberries set by the Norwegian Food Control Authority (harmonized with current EU standards) for 1994 and 1999 are shown in Tables 2 and 3. Where no MRL's exist, an administrative guidance level has been set by Norwegian authorities. All fungicides in these experiments, except imazalil and vinclozolin, are currently approved for use in field-grown strawberries in Norway.

Limits of quantitation were calculated as the sample analyte concentration that gives a signal-to-noise ratio ≥10 (Dyson, 1990), and were 0.05 mg/kg for chinomethionat, chlorothalonil, imazalil, iprodione, tolylfluanid, and vinclozolin, 0.04 mg/kg for triadimenol, and 0.02 mg/kg for penconazole and triadimefon. The recoveries of the method, with relative standard deviations (RSD's) in parentheses, were for chinomethionat 108% (6.4%), chlorothalonil 109% (1.5%), imazalil 84% (4.2%), iprodione 95% (2.4%), penconazole 109% (8.6%), tolylfluanid 112% (8.6%), triadimefon 113% (6.8%), triadimenol 112% (6.4%), and vinclozolin 98% (4.0%).

The residue levels were somewhat higher in the second (Table 3) experiment compared to the first (Table 2). This could partly be explained by the treatments with tolylfluanid and vinclozolin by the grower prior to the second experiment. The following residue levels were found in the untreated (but sprayed by the grower) control samples in the second experiment after 4, 7, and 14 days, respectively: 0.91, 0.84, and 0.77 mg/kg vinclozolin and 0.70, 0.93, and 0.54 mg/kg tolylfluanid. However, the numbers for iprodione were also higher in the second compared to the first experiment. Time of the year could further explain the differences. The first experiment took place during summer, while the second was during late winter. Higher light intensities and temperatures during summer could increase degradation of the fungicides to a greater extent than during winter. Gennari et al. (1985) investigated degradation of vinclozolin in grapevines in four different areas of Italy. Degradation was very dependent on climatic factors, including sunlight and daily temperature fluctuations.

For iprodione, residue levels in strawberries which received both the recommended rate for field use and half rate, were under MRL at any sampling time during the two experiments (Tables 2 and 3). Full rate of vinclozolin (Tables 2 and 3) gave residue levels above

Table 2. Experiment in June 1994, Residues and MRL's for 1994 and 1999, and Fungicide Rates As Recommended for Field-Grown Strawberries and Half of This (Designated *N* and $^1/_2N$)

fungicide	residues detected (mg/kg) at different intervals after application[a] 4 days	7 days	14 days	MRL 1994	MRL 1999
chinomethionat *N*	0.45	0.15	0.06 ± 0.01	0.3	0.3
chinomethionat $^1/_2N$	0.27	0.11	<0.05		
chlorothalonil *N*	6.0 ± 0.8	2.9 ± 1.1	1.2	1[b]	*c*
chlorothalonil $^1/_2N$	2.7 ± 0.5	1.7 ± 0.5	0.53 ± 0.12		
imazalil *N*	1.9	1.1	0.66 ± 0.14	2	2
imazalil $^1/_2N$	0.94 ± 0.05	0.58 ± 0.07	0.32 ± 0.06		
iprodione *N*	3.7 ± 1.3	2.7 ± 0.7	2.2 ± 0.6	10	10
iprodione $^1/_2N$	2.7 ± 0.5	2.1	1.0 ± 0.1		
penconazole *N*	0.37	0.24	0.08 ± 0.02	*c*	0.1
penconazole $^1/_2N$	0.21	0.13	0.04 ± 0.01		
tolylfluanid *N*	12.7 ± 2.1	9.1 ± 1.5	3.9 ± 0.3	3	3
tolylfluanid $^1/_2N$	10.9 ± 2.2	7.1 ± 1.4	2.3 ± 0.5		
triadimefon *N*	0.41	0.20	0.07 ± 0.03	0.2[d]	0.1[d]
triadimefon $^1/_2N$	0.23	0.11	0.06 ± 0.02		
triadimenol *N*	0.27	0.21	0.15 ± 0.06	*c*	0.1[d]
triadimenol $^1/_2N$	0.18	0.11	0.09 ± 0.02		
vinclozolin *N*	6.5 ± 2.1	5.0	2.2 ± 0.7	10	5
vinclozolin $^1/_2N$	3.7	2.1	0.70		

[a] Results are presented as mean ± SD if three determinations existed. [b] Administrative guidance level in Norway; no MRL existed in EU or Codex. [c] No MRL existed in Norway or Codex. [d] Codex value, no MRL set for Norway. In 1994 the administrative guidance level in Norway for the sum of triadimefon and triadimenol was 0.5 mg/kg.

Table 3. Experiment in February/March 1995; Residues and MRL's in 1994 and 1999[a]

fungicide	residues detected (mg/kg) at different intervals after application 4 days	7 days	14 days	MRL 1994	MRL 1999
iprodione *N*	6.9 ± 1.0	5.9 ± 1.2	3.8 ± 0.5	10	10
iprodione $^1/_2N$	3.4 ± 0.3	3.2 ± 0.3	2.3 ± 0.1		
tolylfluanid *N*	13.3 ± 1.5	10.1 ± 0.9	7.5 ± 1.0	3	3
tolylfluanid $^1/_2N$	6.9 ± 1.0	5.6 ± 0.8	3.6 ± 0.8		
vinclozolin *N*	7.5 ± 1.0	6.3 ± 1.3	3.7 ± 0.4	10	5
vinclozolin $^1/_2N$	4.1 ± 0.2	3.8 ± 0.3	2.4 ± 0.1		

[a] Fungicide rates as recommended for field-grown strawberries and half of this (designated *N* and $^1/_2N$). Results are presented as mean ±SD of three determinations.

the current (1999) MRL after 4 days in both experiments, at or above MRL after 7 days, and below the limit after 14 days. If following the limit set in 1994, the residue levels of vinclozolin were below MRL after 4 days. Half rate of vinclozolin gave residues under MRL at any sampling time. Thus, if considering maximum residue limits, the present 14 days from application to harvest in the field for iprodione and vinclozolin could be recommended for greenhouse-grown strawberries.

Tolylfluanid (Tables 2 and 3) gave very high residue levels in both experiments. For recommended and half concentration in both experiments, the residue levels were above MRL after 4 and 7 days. After 14 days, except from half concentration in the first experiment, which was 0.2−1.1 mg/kg below MRL, the other determinations were above the limit. In both experiments when the recommended rate of tolylfluanid was applied, 7 days after harvest residues were approximately 3 times higher than the MRL, and 14 days after application in the second experiment, the residue level was more than 2 times higher than the MRL. On the basis of the high residue levels for tolylfluanid in the present experiments, 7 days from application to harvest cannot be recommended in greenhouse-grown strawberries. Tolylfluanid might need 3−4 weeks from application to harvest, very long intervals between each treatment, or even reduced rates to ensure residue levels below MRL. In another experiment in the same greenhouse (in April 1994) treated with various insecticides and miticides (unpublished data), tolylfluanid was analyzed in all samples. The grower had treated with 200 g per 100 L of the product (to runoff). Samples were taken 2, 5, and 12 days after treatment (12 samples each time). The residue level was (with SD in parentheses) 5.0 (±1.4), 4.1 (±1.0), and 2.7 (±0.6) mg/kg after 2, 5, and 12 days, respectively. In this case, with an application of two-thirds the normal rate of tolylfluanid, residues were around MRL 12 days after treatment.

The other fungicides (Table 2) were tested only once; thus the basis for recommendation is somewhat limited. However, the similarity in residue levels between experiments 1 and 2 for iprodione, tolylfluanid, and vinclozolin indicates that the residue levels for the other fungicides in the first experiment should be similar. The results indicate that 7 days from application to harvest was sufficient to keep residues of chinomethionat and imazalil below MRL. For strawberries receiving the recommended rate of penconazole, residues were just below MRL 14 days after application. Treatment with triadimefon at the full rate gave residues below MRL after 14 days. However, its metabolite, triadimenol, was above MRL at the same time. For chlorothalonil in half concentration, residues were below MRL 14 days after treatment. All other samples treated with chlorothalonil were above the limit.

The results show that fungicide degradation in strawberries grown in protected environment can differ from the field. There might be several reasons why the residue levels after application were higher than would be expected in the field. One explanation might be lack of precipitation and differences in light intensity in a greenhouse compared to the field. Zenon-Roland and Gilles (1978) in Belgium investigated degradation of vinclozolin in strawberries under field conditions in two years. One year had very little rainfall during the experiments, and degradation of the fungicide proceeded much slower than another year that had closer to normal precipitation. The half-life of vinclozolin was 12 and 22 days during the wet and dry years, respectively. It was proposed that lack of precipitation not only limited breakdown of the fungicide but also retarded the growth rate of the strawberries, which thus decreased

the disappearance rate of the chemical. In the present experiments, a further reason for a slower disappearance rate in greenhouse-grown strawberries could be a slower increase in volume of the berries and subsequently a slower decrease in fungicide-to-plant ratio masses compared to that in field-grown strawberries. Disappearance rates for pesticides are highly affected by plant growth, and as biomass increases at the beginning of vegetative periods, residues have been observed to dissipate rapidly (Spynu, 1989).

The present authors have not been able to find any other research reports on fungicide residue levels in strawberries grown under greenhouse conditions. Similar experiments as described above should be carried out for other fungicides, as, e.g., compounds in the newly developed anilinopyrimidine group, which recently have been approved for strawberries in many countries.

It can be concluded from the present experiments that chinomethionat, imazalil, iprodione, penconazole, and vinclozolin may have a 2 week quarantine from application to harvest in the greenhouse as presently in the field. On the other hand, tolylfluanid will need a much longer quarantine than the present 7 days. Furthermore, chlorothalonil and triadimefon/triadimenol may need an additional week from application to harvest than the present 2 week quarantine. One possibility to avoid residues above MRL could be to reduce the recommended fungicide rate. These experiments did not consider the effect of the chemicals on disease development; thus normal vs half rate was not investigated. Use of low fungicide rates against gray mold in field-grown strawberries are reported from Poland (Bera, 1987) and Norway (Meland, 1988; Brandsæter, 1990; Nestby, 1992; Sönsteby et al., 1996). In most cases, a reduction of 30−50% in fungicide rate could be tolerated without a significant increase in crop loss. A 50% reduction in fungicide dose in these experiments allowed chlorothalonil and triadimefon/triadimenol to reach below the residue limit 2 weeks after application; however, tolylfluanid would probably need 3 weeks quarantine, even at half rate.

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