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Influence of copper fungicide/fertilizer formulations applied during the blooming period on a fruit set of apples

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Two trials were carried out on apple plantation in order to determine the phytotoxicity level of several copper formulations (fungicides or foliar fertilizers) which were applied during the apple blooming and shortly after it. Tests were performed with formulations based on copper calcium oxychloride (trial standard), copper sulphate, and complexes or chelates of them with amino acids, peptides, EDTA, urea and gluconic acid. Different formulations were applied 8 or 10 times in a rate of 200 g pure copper ions (Cu++) per hectare to trees of seven cultivars (Golden Delicious, Gala, Fuji, Idared, Breaburn, Jonagold and Elstar). Evaluations of fruit set (i.e. number of fruits developed per number of flowers) and fruit disorders (malformations, surface russeting …) were done. The highest decrease in fruit set was observed when applying systemic Cu-gluconate and soluble Cu-sulphate to Fuji and Idared trees. The most severe fruit disorders occurred as a consequence of the application of systemic Cu-gluconate, Cu-protein complexes and soluble Cu-sulphate to Jonagold and Golden Delicious trees. The highest portion of damaged fruit (18-22%) was observed after the application of systemic acting Cu-gluconate and Cu-protein complexes. New systemic copper formulations are thought to have higher biological efficacy to control the fungal and bacterial diseases of apples than the traditional fungicide preparations. In the case of high efficacy of newly tested copper formulations, especially against bacteria, the observed level of phytotoxicity could be tolerated in terms of acceptable economic losses due to decreased quality of fruits. The phytotoxicity levels vary significantly in different apple cultivars, which shows the need for development of specific cultivar related instructions for use of new copper formulations.

Key word: copper formulations, apple, phytotoxicity, yield quality

INTRODUCTION

Copper preparations have been widely used in agriculture for many decades. As a result of a long term, intensive use a big depot of copper was formed in soils of fruit and grapevine plantations, which led to serious and negative ecological impacts with detrimental effects on soils fertility (Georgopoulos et al. 2001, Van Zwieten et al. 2004). The existing system of how to use the copper preparations must, therefore, be changed significantly. Some EU countries have already decided to ban all copper products, while others decided to significantly reduce their use. Significant reduction in use of copper compounds can be achieved by partial replacement of copper products with other active substances, by reducing the number of applications per year or by reducing the hectare rates of copper products on individual plant treatment (Golba 2001, Goebel et al. 2004, Jamar and Lateur 2007).

Reduction of hectare rates can be achieved by changing the formulation of preparations and by increasing their biological efficacy. The ultimate goal of development of new copper formulations is to keep or even to increase the biological efficacy in control of pathogens with significantly lower hectare rates of pure copper ions. There are many new formulations available on EU market nowadays, which are still to be tested for efficacy against different diseases, traditionally controlled by copper fungicides (such as apple scab, apple nectria twig blight, several bacterial diseases caused by bacteria from genus *Pseudomonas* and *Erwinia*). One of many diseases that were originally controlled by copper preparations is also the fire blight of pipe fruits, caused by the bacterium *Erwina amylovora* Burr. (Clarke et al. 1993).

Apple trees are most exposed to infection by bacteria during their flowering. Products containing antibiotics are not allowed to be used in Slovenia. Our control strategies are mainly based on the use of products containing fosethyl-Al or biological products based on *Bacillus subtilis* Cohn or *Aureobasidium pullulans* (de Bary) G. Arnaud (Lešnik et al. 2005). All currently available products on the market are not in particularly efficient and are quite expensive. Because copper products are, on the other hand, quite cheap and efficient, farmers are now showing the increased interest in their use, even during the apple blooming period.

It is well known that the copper products are, to a certain extent, phytotoxic in all growth stages of pome and stone fruit (Holb 2008). When testing certain new copper products (foliar fertilizers) lately, we noticed that the phytotoxicity level was not as high as we would have expected. In theory, the phytotoxicity of copper products is mentioned a lot, but very limited amount of detailed data is actually available for specific cultivars and rates of pure copper per hectare. There-

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fore, we have decided to test the phytotoxicity level of new products, which could then potentially be available on Slovenian market in years to come, and to compare them with the standard copper formulations that are already available on our market. Our hypothesis was that the level of phytotoxicity of new copper formulations applied during blooming of apples for flowers and fruitlets is not higher than the phytotoxicity level of traditional standard formulations (Cu-hydroxide or Cu-oxychloride).

MATERIALS AND METHODS

Field trial design

Our trial was carried out in 2008 and 2009 on trees of 7 apple cultivars, grown in a special collection of apple cultivars, at the experimental station of Faculty of Agriculture and Life Sciences in Hoce near Maribor. Trees of different cultivars were planted in a randomised block design so that the statistical experiment design was a two factorial trial in four replications of randomised blocks. The first trial factor was copper formulation, the second apple cultivar. Each experimental plot consisted of 14 trees in a row. Trees were grafted on MM9 rootstock and were planted at 0.7 m distance in a row and at 2 m distance between rows. They were trained in a modified super spindle system resembling an espalier system. The green wall was 2.2 m high and 40 cm wide. In 2008 the plantation was 6 years old. For separation and comparison of treatment means, the ANOVA analysis was performed, followed by the use of multiple comparison tools based on Tukey's HSD test at (α=0.05).

Copper application and phytotoxicity assessments

All copper formulations were applied prior to the start of blooming, 3 times during the blooming of apples and four to five times after the petal fall. No other plant protection products were applied to trees during the whole period of this trial in order to avoid any effect of other chemicals on the trial results. We always applied 200 grams of free copper ions (Cu^{++}) per hectare. The rates of copper preparations were adapted so that we always applied the same amount of copper ions in all preparations. Formulations were applied in a 650 l/ha spray volume by a special sprayer with vertical boom (Technoma EuroPulve, France). Trees were sprayed both sides. The spray system was driven by electric pump. We used Teejet 800067 flat fan nozzles. Droplet volume median diameter (VMD) was 120 microns.

At the early stage of blooming, individual clusters of blooms were chosen randomly in the different regions of tree crowns. Hundred clusters per individual experimental plot were selected. Clusters of blooms were marked by placing colour ribbons (sticky tape for electrical isolation) on the basis of each selected cluster. Different colours marked different numbers of flowers in the cluster (4, 5, 6 etc.). This helped us to record, later in the season, how many fruits developed from the specific cluster of flowers. Fruit set was calculated and expressed in percentage using the following formula: **FS(%) = ((number of fruits after natural June drop per cluster / number of flowers in cluster at bloom time) * 100).** As an example: when 4 fruits develop out of 6 flowers in a cluster, fruit set was 66.6 percent.

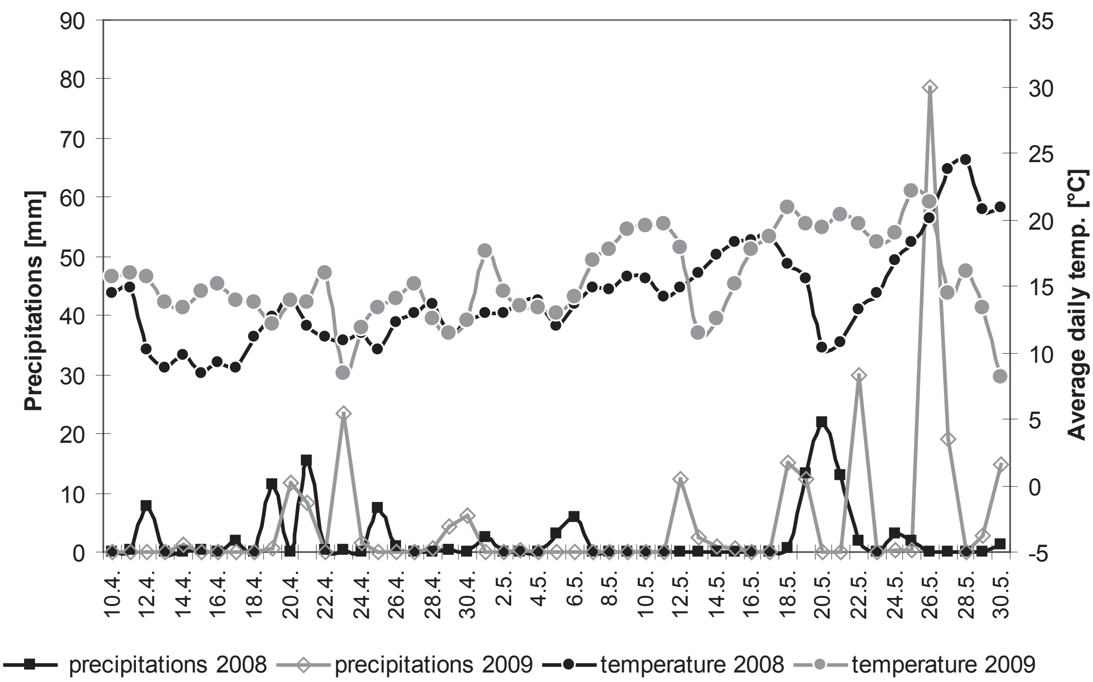

Figure 1: Average daily temperatures and precipitations during the main period of flowering in years 2008 and 2009. Data from ADCON meteorological station at close vicinity of experimental orchard plots.

Evaluations of the fruit set were performed approximately four weeks after the end of blooming, when natural June fruitlet drop was completed. We also carried out very accurate observations of fruit shape and any possible surface disorders (malformations, russeting etc.).

Weather conditions during the experimental period are presented in Figure 1. Conditions during the flowering in 2009 were better than in 2008, where temperatures were lower and rainy periods were longer. In 2008 flowering lasted 3.5 weeks. During the flowering period average day temperatures varied between 10 and 16 °C with night temperatures between 5 and 8 °C. In 2008 trees developed quite a lot of flowers. Cultivars Idared, Elstar and Bareburn developed between 180 and 400 flowers per tree while others developed from 100 to 350 flowers per tree. In 2009 the experimental orchard fell in an alternative yielding. The number of flowers developed per tree was lower. In cultivars Gala, Fuji and Jonagold from 120 to 300 flowers per tree were developed and 80 to 250 per tree in others. Flowering was faster due to higher temperatures. During the flowering period average day temperatures varied between 10 and 18 °C with night temperatures between 6 and 10 °C. The precipitation rate was also higher in 2009 and therefore we needed to apply copper products more frequently than in 2008. The leaching of products from trees was consequently more intensive than in year 2008.

Tested formulations and their composition

The composition of products tested is presented in Table 1. Hectare rates of all researched products we calculated so that we always applied 200 grams of free copper ions (Cu++) per hectare. We selected formulations of different chemical backgrounds in order to determine the difference in phytotoxic response of apple cultivars to different chemical forms of copper. In 2008 copper formulations were applied on April 16, April 22, April 26, April 29, May 8, May 14, May 19 and June 2. In 2009 they were applied on April 10, April 15, April 19, April 24, April 28, May 8, May 14, May 21, May 26 and June 4. When applying copper formulations, control plots were always spayed with pure water.

In 2009 we decided to add two more experimental variants - spray programme of organic production and spray programme of integrated apple production. We did that so that we would be able to perform additional comparison of effects of copper formulations and effects of plant protection products, used in organic or integrated production. The organic spray program consisted of the following applications: April 6 (400 g Cu++ /ha; Cuprablau Z ultra), April 10 (200 g Cu++ /ha; Cuprablau Z ultra), April 15 (1500 g potassium hydrogen carbonate /ha; SaluKarb) + (1600 g sulphur /ha; Cosan), April 19 (*Bacillus subtilis*, 4 kg /ha; Serenade), April 21 (1600 g sulphur /ha; Cosan) + (200 g Cu++ /ha; Cuprablau Z ultra), April 24 (1500 g potassium hydrogen carbonate /ha; SaluKarb) + (2000 g caolin clay /ha; Cutisan), April 28 (1600 g sulphur /ha; Cosan) + (2000 g caolin clay /ha; Cutisan), May 8 (8 kg acidified clay minerals /ha; Ulmasud) +(1600 g sulphur /ha; Cosan), May 18 (same like 24. April), May 26 (same like 28. April), June 4 (same like April 24).

The integrated spray program consisted of the following applications: April 6 (400 g Cu++ /ha; Cuprablau Z ultra), April 10 (240 g thiram /ha; Tiram 80), April 19 (200 g ciprodinyl /ha; Chorus 75 WG), April 24 (75 g difenconazole / ha; Score 250 EC) + (400 g dodine /ha; Syllit 400), May 30 (75 g trifloxystrobin /ha; Zato 50 WG) + (240 g thiram /ha; Tiram 80), May 8 (same like April 24), May 18 (75 g flucvinconazol /ha; Clarinet) + (75 g krezoxim-methyl /ha; Stroby), May 26 (525 g dithianone /ha; Delan 700 WG), June 4 (the same like April 24).

RESULTS AND DISCUSSION

Fruit set (FS)

The effect of applications of different copper formulations to trees of different apple cultivars during the pre-blooming, blooming and after blooming period on fruit set is shown in Table 2. In 2008 all studied apple cultivars developed quite a significant number of flowers. The number of developed fruits per flower cluster was the highest in Elstar apples. Differences among other cultivars were not significant. When comparing average FS among different formulations we can see that all of them reduced the number of developed fruits in relation to untreated control (FS = 17.9 %). The highest and most noticeable decrease (FS = 12.8 %) was obtained by application of ProtexCU (soluble Cu-sulphate). This decrease can also be considered as causing a potentially important yield reduction. The reduction in number of developed fruits at all other

Table 1. Formulations of copper used in trials

Formulation:	Manufacturer:		Chemical form of copper:	g Cu++ / kg
Cuprablau Z ultra	Cinkarna d.o.o.	SI	Cu-calcium oxychloride (with the addition of nano particles)	350.0
Cinkarna PF1	Cinkarna d.o.o.	SI	Cu-Ca oxychloride-EDTA chelate (not a fully chelated)	32.0
Cinkarna PF2	Cinkarna d.o.o.	SI	Cu- Ca oxychloride-titanium dioxide amino complex	27.0
Cinkarna PF3	Cinkarna d.o.o.	SI	Cu-Ca oxychloride-EDTA chelate (not a fully chelated)	39.3
Cinkarna PF4	Cinkarna d.o.o.	SI	Cu-Ca oxychloride amino complex	27.5
Coptrel 500	Yara Vita TM	GB	Cu-oxide in urea complex	330.0
Copper Protein	Nova Prot GmbH	DE	Cu-hydroxide-protein complex	18.0
Labicuper	Macasa S.L.	ES	Cu-gluconate complex	65.0
Peptiram 5	Sicit 2000 S.p.a.	I	Cu-sulphate-peptide complex	50.0
Protex-CU	Ares Europe BV	NL	water soluble Cu-sulphate	60.0

Table 2. Fruit set (% of flowers that were developed to fruit) in relation to type of applied copper preparation and apple cultivar. Ida – Idared, Jon – Jonagold, G.del – Golden Delicious, Gal – Gala, Bra- Braeburn, Fuj – Fuji.

Preparation:	Ida	Jon	G.del	Els	Gal	Bra	Fuj	Average:
FRUIT SET 2008								
Cuprablau ZU	13.3^{aa}	14.5^{aab}	16.7^{aa}	12.7^{ac}	16.6^{aa}	11.5^{ab}	13.7^{aa}	14.2^{AB}
Copper Protein	12.1^{aa}	12.6^{aab}	15.0^{aa}	16.2^{ab}	13.2^{ab}	22.5^{aa}	12.3^{aa}	14.8^{AB}
Cinkarna PF1	12.8^{aa}	14.0^{aab}	14.6^{aab}	22.9^{aa}	14.0^{aab}	11.7^{ab}	10.1^{aa}	14.3^{AB}
Peptiram	14.2^{aa}	12.5^{aab}	17.4^{aa}	13.5^{abc}	18.9^{aa}	14.0^{ab}	12.3^{aa}	14.7^{AB}
Coptrel	12.5^{aa}	12.7^{aab}	18.2^{aa}	16.6^{ab}	14.1^{aab}	10.5^{ab}	10.9^{aa}	13.6^{AB}
Protex CU	12.1^{aa}	8.8^{ab}	9.2^{ab}	14.4^{ac}	20.7^{aa}	11.7^{ab}	13.1^{aa}	12.8^{B}
Control	14.5^{aa}	17.4^{aa}	18.4^{aa}	24.4^{aa}	17.9^{aa}	17.7^{aa}	15.6^{aa}	17.9^{A}
Average	$13.1^{\mathbf{A}}$	$13.2^{\mathbf{A}}$	$15.6^{\mathbf{A}}$	$17.2^{\mathbf{A}}$	$16.5^{\mathbf{A}}$	$14.3^{\mathbf{A}}$	$12.6^{\mathbf{A}}$	14.6
FRUIT SET 2009								
Cuprablau ZU	15.6^{bb}	17.1^{bb}	20.1^{aa}	14.7^{bab}	20.1^{aa}	13.0^{cab}	16.1^{bab}	16.7^{AB}
Cinkarna PF2	14.8^{aab}	16.5^{aab}	17.3^{aab}	10.7^{ab}	16.4^{aab}	13.3^{aab}	14.0^{aab}	14.7^{AB}
Cinkarna PF3	14.5^{bab}	14.7^{bb}	22.2^{aa}	19.9^{aa}	16.6^{bab}	11.7^{cb}	12.2^{bcb}	16.5^{AB}
Cinkarna PF4	16.8^{aa}	18.2^{aa}	21.3^{aa}	14.9^{bab}	15.5^{bab}	20.4^{aa}	16.7^{aa}	17.7^{AB}
Copper Protein	11.3^{ab}	11.3^{ab}	11.3^{ab}	11.3^{ab}	11.3^{ab}	13.1^{aab}	11.3^{ab}	15.5^{AB}
Labicuper	13.7^{aa}	9.2^{ab}	$9.8.3^{ab}$	16.7^{aa}	15.2^{aab}	13.3^{aab}	15.2^{aab}	13.4^{B}
Coptrel	16.7^{ba}	14.4^{bab}	15.1^{bab}	15.7^{bb}	23.2^{aa}	16.5^{ba}	14.1^{bb}	16.5^{AB}
Control	17.2^{aa}	21.1^{aa}	22.5^{aa}	18.7^{aa}	21.8^{aa}	21.6^{aa}	18.7^{aa}	20.2^{A}
Organic prod.	19.0^{aa}	19.5^{aa}	18.2^{aa}	17.0^{aa}	20.8^{aa}	19.6^{aa}	19.0^{aa}	19.1^{A}
Integrated prod.	18.1^{aa}	17.3^{aa}	19.7^{aa}	18.1^{aa}	18.6^{aa}	20.4^{aa}	19.9^{aa}	18.9^{AB}
Average	$16.1^{\mathbf{A}}$	$16.3^{\mathbf{A}}$	$18.4^{\mathbf{A}}$	$16.7^{\mathbf{A}}$	$18.3^{\mathbf{A}}$	$16.3^{\mathbf{A}}$	$16.0^{\mathbf{A}}$	16.9

*Averages marked with small letters serve as comparison between apple cultivars sprayed with the same preparation and averages marked with the small bold letters as comparison within the preparations applied to the same apple cultivar. Average values marked with the same letter do not, according to the Tukey's HSD test (α=0.05), differ significantly.

Table 3. The extent of surface damage on fruit (% of fruit surface area russeted/other types of disorders). Ida – Idared, Jon – Jonagold, G.del – Golden Delicious, Gal – Gala, Bra- Braeburn, Fuj – Fuji.

Preparation:	Ida	Jon	G.del	Els	Gal	Bra	Fuj	Average:
FRUIT SURFACE DAMAGE 2008								
Cuprablau ZU	4.1^{cb}	11.3^{bbc}	23.2^{aa}	9.8^{bab}	2.4^{cb}	3.2^{cab}	3.6^{cb}	8.2^{B}
Copper Protein	23.5^{aa}	30.4^{aa}	32.7^{aa}	14.8^{ba}	11.2^{ba}	6.7^{ca}	9.6^{ba}	18.4^{C}
Cinkarna PF1	2.6^{ab}	2.2^{ac}	1.3^{ab}	5.3^{ab}	4.6^{ab}	2.3^{ab}	2.5^{ab}	2.9^{A}
Peptiram	23.2^{aa}	28.3^{aa}	30.4^{aa}	19.6^{ba}	13.8^{ba}	5.4^{cab}	10.8^{bca}	18.8^{C}
Coptrel	1.7^{ab}	4.4^{aab}	1.9^{ab}	2.4^{ab}	0.9^{bab}	1.5^{abb}	1.1^{abc}	2.0^{A}
Protex CU	16.5^{aab}	11.5^{abb}	4.7^{bb}	10.1^{aba}	8.8^{aba}	8.4^{aba}	9.8^{aba}	10.1^{B}
Control	1.1^{ab}	1.2^{ac}	1.6^{ab}	0.9^{ac}	1.4^{ab}	1.4^{ab}	1.2^{ac}	1.2^{A}
Average	$10.4^{\mathbf{BC}}$	$12.8^{\mathbf{C}}$	$13.7^{\mathbf{C}}$	$9.1^{\mathbf{BC}}$	$6.2^{\mathbf{AB}}$	$4.1^{\mathbf{A}}$	$5.5^{\mathbf{AB}}$	8.8
FRUIT SURFACE DAMAGE 2009								
Cuprablau ZU	4.9^{ba}	10.4^{aa}	10.7^{ab}	7.4^{aa}	2.9^{bab}	4.2^{ba}	2.5^{ba}	6.1^{C}
Cinkarna PF2	3.3^{bab}	3.8^{bb}	12.7^{ab}	8.9^{aa}	1.6^{bab}	3.3^{ba}	1.7^{bab}	5.1^{BC}
Cinkarna PF3	4.7^{aba}	9.0^{aa}	14.4^{ab}	10.2^{aa}	4.4^{aba}	1.5^{bb}	2.6^{ba}	6.7^{C}
Cinkarna PF4	3.3^{bab}	3.2^{bb}	7.3^{ac}	7.1^{aa}	0.8^{bb}	1.5^{bb}	1.3^{bb}	3.5^{ABC}
Copper Protein	2.0^{bb}	3.5^{ab}	7.3^{ac}	6.9^{aa}	3.6^{aba}	4.6^{aba}	3.4^{aba}	4.5^{ABC}
Labicuper	6.3^{ba}	18.5^{aa}	27.8^{aa}	7.5^{ba}	3.4^{ca}	2.4^{ca}	1.6^{cab}	9.7^{D}
Coptrel	6.3^{aa}	4.5^{ab}	5.1^{acd}	4.4^{aab}	3.6^{aa}	1.8^{bab}	2.8^{aa}	4.1^{ABC}
Control	0.7^{bb}	2.6^{abc}	1.5^{ac}	1.4^{ac}	1.8^{aab}	1.5^{ab}	2.0^{aa}	1.6^{A}
Organic prod.	1.8^{ab}	1.5^{ac}	1.8^{ad}	2.1^{abc}	1.7^{aab}	1.2^{ab}	2.3^{aa}	1.8^{AB}
Integrated prod.	2.9^{ab}	1.8^{bb}	3.7^{acd}	3.3^{ab}	3.1^{aa}	2.7^{aa}	2.3^{aa}	2.8^{ABC}
Average	$3.6^{\mathbf{A}}$	$5.9^{\mathbf{B}}$	$9.4^{\mathbf{C}}$	$5.9^{\mathbf{B}}$	$2.7^{\mathbf{AB}}$	$2.5^{\mathbf{A}}$	$2.2^{\mathbf{A}}$	4.6

*Averages marked with small letters serve as comparison between apple cultivars sprayed with the same preparation and averages marked with the small bold letters as comparison within the preparations applied to the same apple cultivar. Average values marked with the same letter do not, according to the Tukey's HSD test (α=0.05), differ significantly.

formulations was not significant and can be considered as not important in terms of final total yield.

In 2009 we included two additional control plots (organic and integrated spray programme) in order to compare the effects of other plant protection chemicals. It is well known that other plant protection products can interfere with flower fecundation and early fruitlet development (Church 1983, Teviotdale and Viveros 1999, Holb 2008). Also, in 2009 there was no massive fluctuation observed in FS between the studied apple cultivars. The highest FS rate was obtained at Golden Delicious and Gala apples. Overall number of flowers per tree was lower than in 2008. Some trees fell in alternative yielding cycle. Although the organic and integrated spray programs decreased the FS in most studied cultivars, the effect in comparison to untreated control plots (see Table 2) was not statistically significant.

The highest decrease of FS was observed in trees treated with Labicuper (Cu-gluconate). This formulation is highly systemic. We believe that the FS reduction caused by systemic active formulations would normally be higher than the one with formulations with no systemic activity. In the case of Coptrel (Cu-oxide), which is almost completely insoluble in water and can not enter plant tissues, we observed a relatively high decrease in FS, which does not support our assumptions.

The second highest decrease (FS = 14.7 %) was observed in trees sprayed with Cinkarna experimental formulation PF4 (Cu-Ca oxychloride amino complex) which was expected to be partially systemic. The Copper-Protein formulation containing copper in a copper-protein complex caused a moderate decrease in FS which was, aside of Labicuper formulation, not significantly different from others. We predicted higher reduction in FS upon the application of Cinkarnas' experimental formulation PF3 (Cu-Ca oxychloride EDTA chelate), but that turned out not to be the case. EDTA-Cu chelates were expected to be systemic. Also the pH value of sprays probably had an important influence on the level of phytotoxicity. The pH of spray containing Cu-EDTA chelate was 6.0 in contrast to sprays containing Labicuper (pH = 9.0) or Protex-Cu (pH = 4.7). We think that formulations with highly basic or acid character are more phytotoxic than formulations whose pH value is close to neutral. The pH value of the spray deposit on the surface of the apple organs influences significantly the solubility of the copper and through that also the level of penetration of copper ions into the tissue of apple organs. The pH value of Cuprablau Z Ultra (7.7) was much closer to the neutral and the phytotoxicity level was also lower.

Reactions of individual cultivars to different copper formulations were not the same. For an example, the Cinkarna's PF3 did not influence FS of Golden Delicious flowers at all, but it caused a significant reduction in FS in Breaburn and Fuji apples. There are several similar cases in which the reactions of different cultivars to the same formulations are different. That shows the interaction among cultivars and formulations. We are at this stage unable to provide the explanation for this phenomenon. However, the same facts were discovered by other researchers, who also proved that responses of different apple cultivars to copper fungicides could be very different (Straub and Kienzle 1992). The reasons for this may be connected to the morphological structure of flowers, to the chemical composition of the surface of the flowers and to the way the pollen

Table 4. Percent of damaged fruit. Ida – Iadred, Jon – Jonagold, G.del – Golden Delicious, Gal – Gala, Bra - Braeburn, Fuj – Fuji.

Preparation:	Ida	Jon	G.del	Els	Gal	Bra	Fuj	Average:
% DAMAGED FRUIT 2008								
Cuprablau ZU	19.9^{aa}	22.1^{aa}	23.4^{aa}	19.9^{aa}	17.1^{aa}	18.6^{aa}	16.1^{ab}	19.6^{C}
Copper Protein	23.1^{aa}	24.5^{aa}	26.6^{aa}	21.3^{aa}	23.8^{aa}	20.3^{aa}	20.6^{aa}	22.9^{D}
Cinkarna PF1	17.8^{aa}	16.8^{aab}	17.8^{aab}	18.2^{aa}	19.9^{aa}	16.1^{ab}	19.9^{aa}	18.1^{C}
Peptiram	21.7^{aa}	22.4^{aa}	22.4^{aa}	22.4^{aa}	21.3^{aa}	17.5^{bab}	16.4^{bb}	20.6^{CD}
Coptrel	15.1^{ab}	12.9^{ab}	11.2^{abb}	9.8^{bb}	10.8^{ab}	8.7^{bc}	11.2^{ac}	11.4^{B}
Protex CU	22.4^{aa}	17.5^{bab}	19.2^{aab}	19.5^{aa}	20.6^{aa}	18.2^{aa}	21.3^{aa}	19.8^{CD}
Control	5.7^{ac}	5.9^{ac}	8.1^{bc}	5.1^{ac}	5.6^{ac}	5.6^{ad}	5.6^{ad}	5.9^{A}
Average	17.9^{AB}	17.4^{AB}	18.4^{B}	16.6^{AB}	17.0^{AB}	15.0^{A}	15.9^{AB}	16.9
% DAMAGED FRUIT 2009								
Cuprablau ZU	9.0^{abb}	18.5^{aa}	16.2^{aa}	11.5^{ab}	7.2^{bb}	8.0^{bb}	8.5^{aba}	11.2^{B}
Cinkarna PF2	11.7^{abb}	13.5^{ab}	16.5^{aa}	12.2^{ab}	9.5^{bb}	10.7^{aba}	9.0^{ba}	11.8^{B}
Cinkarna PF3	12.2^{abb}	11.0^{bb}	15.7^{aa}	15.7^{aa}	4.4^{cc}	14.5^{aa}	9.2^{ba}	12.7^{B}
Cinkarna PF4	11.2^{ab}	14.5^{ab}	14.0^{aa}	14.5^{aa}	8.0^{cb}	8.0^{cb}	9.5^{aba}	11.3^{B}
Copper Protein	7.5^{bb}	8.7^{bbc}	14.2^{aa}	17.2^{aa}	15.0^{aa}	10.5^{ab}	11.0^{aba}	12.3^{B}
Labicuper	16.0^{aa}	19.5^{aa}	19.7^{aa}	18.7^{aa}	12.2^{aab}	15.2^{aa}	9.7^{ba}	15.8^{C}
Coptrel	10.0^{abb}	12.0^{ab}	13.5^{ab}	14.5^{aa}	9.5^{bab}	10.5^{aba}	11.0^{aba}	11.6^{B}
Control	4.0^{ac}	4.0^{ac}	3.5^{ac}	5.0^{ac}	3.5^{ac}	3.0^{ac}	3.5^{ac}	3.8^{A}
Organic prod.	3.5^{abc}	3.5^{abc}	4.5^{ac}	4.0^{ac}	2.0^{bc}	3.5^{abc}	5.0^{ac}	3.7^{A}
Integrated prod.	4.5^{abc}	6.5^{ac}	6.0^{ac}	4.0^{bc}	6.5^{ac}	8.0^{ac}	7.0^{ac}	6.1^{A}
Average	8.9^{AB}	11.2^{BC}	12.4^{C}	11.7^{C}	8.8^{A}	8.7^{A}	8.3^{A}	10.1

*Averages marked with small letters serve as comparison between apple cultivars sprayed with the same preparation and averages marked with the small bold letters as comparison within the preparations applied to the same apple cultivar. Average values marked with the same letter do not, according to the Tukey's HSD test (α=0.05), differ significantly.

germinates at the surface of the stigma (Church et al. 1983). It is expected that high concentrations of copper could hinder normal germination of pollen and therefore also the fertilisation of flowers (Church et al. 1983, Watters and Sturgeon 1990, Holb 2008).

We can not directly extrapolate the influence in reduction of FS on the final yield of apples. It is thought that there is a certain level of compensation between a number of fruit and weight of individual fruit at formation of final apple yield (Lauri and Terouanne 1999). The compensation characteristics of certain cultivars are unknown. The effect of copper treatment on the yield formation can not be completely excluded from many other factors that influence the final yield and are acting on the fruits throughout the entire growing season. In our trial, we did not apply any plant protection products, so the yield losses were much more dependent on the damage done by fungal diseases and insects than by copper formulations. Somehow with the application of copper products, we performed a chemical thinning of apples. We need to thin the apples on yearly basis anyway, therefore, the limited thinning by application of copper could and should be tolerated.

Fruit surface disorders

Results from observations of fruit surface damages (russeting and other disorders) are presented in Table 3. In 2008 there were statistically significant differences observed between the tested formulations and apple cultivars. Applications of copper formulations caused obvious surface disorders on most of the fruit in question. The Golden Delicious and Jonagold apples were shown to be the most susceptible. More than 15 % of fruit surface area was damaged, mostly in a form of shallow surface russeting. The least susceptible cultivar was Braeburn. The highest level of phytotoxicity was observed in formulations of Peptiram and Copper Protein, which are based on copper-protein complexes and have systemic properties. The rate of fruit russeting in case of Coptrel (very low soluble copper oxide form) was lower. The biggest differences among preparations were observed at most susceptible cultivars Golden Delicious and Jonagold.

The rate of fruit damage in 2009 was slightly higher than in 2008. The main reason is probably due to the two additional applications of copper products (10 times in 2009 vs. 8 times in 2008). It is interesting that we have also noticed a high surface damage in fruits developed in trees, which were managed according to organic or integrated plant protection systems. It means that those products (insecticides and fungicides) are also capable of causing a large amount of different disorders, especially if applied frequently. With regard to this fact, the russet caused by copper products should not be judged too strictly.

Russeting of apple fruit surface is a very complex physiological phenomenon. It is known that sometimes a significant surface russeting of fruits may appear simply due to bad weather. Intensive rains in combination with high fluctuation of temperatures can cause severe russeting. It can also be caused by certain plant protection products that contain aggressive organic solvents as well as hormones, or by pests (mites, aphids …) and saprophytic bacteria and yeasts, which inhabit fruit surface naturally or are applied as a biological plant protection agents (Heidenreich et al. 1997). Several fungi including *Aureobasidium pullulans* (de Bary) G. Arnaud and *Rhodotorula glutinis* (Fresenius) F. C. Harrison can russet fruits severely. The mentioned two fungi (yeasts) are common on the surface of apple fruit and leaves. Apples are susceptible to this kind of russet during bloom and at least 4 weeks after petal fall.

The microclimate of the orchard influences the russeting significantly (Noe and Eccher, 2000). We are aware of this fact due to experiences gathered in orchards planted in the slopes. Fruits on trees grown at the foothills have much more russet than fruits developed on the trees at the top of the hill. It is often not easy to distinguish between russeting caused by an unknown physiological background and the actual russeting caused by applied chemicals.

Russeting and other fruit disorders can significantly affect the apple fruit market value. Russeted fruits are normally downgraded at marketing. Copper formulations can significantly accelerate the russeting, which is mainly caused by cuticle cracking due to its failure to keep up with the rapid growth of fruits internal tissues (Ashizawa et al. 2005). Russet usually starts in the early stages of fruits growth - shortly after full bloom, corresponding to the period of greatest tangentional growth (Peryea 2006). Many spray materials (including surfactants), especially emulsifiable concentrates and materials containing copper, zinc, or calcium applied at that time can produce the russet if not evenly distributed by the spray equipment.

Percentage fruits with disorders

Data on percent of fruits with disorders is shown in Table 4. In season 2008 applications of copper formulations caused visible disorders in high portion of fruit (11 to 22 %). We estimated that approximately 50% of all fruit, where disorders were noticed, could not be marketed as a first class fruit. The accurate extrapolation of data on percent of damaged fruit in comparison to the final percent of fruit, which is first class fruit at harvest, is not possible. Therefore, the economic consequences of the phytotoxicity can not be fully estimated either. The most severe damage was caused by the application of Peptiram and Copper Protein. Both formulations are able to penetrate into the tissue of the fruit. The lowest level of damage was observed in the case of Coptrel. Results from 2009 are similar to those from 2008. The highest portion of fruit with disorders was observed in the case of Labicuper (Cu-gluconate), Copper Protein and Cinkarna PF3 (CuCa oxychloride EDTA chelate).

Results show that there is some sort of correlation existing between the level of copper penetration into the fruit and the frequency of disorders that appear as a result. It is interesting that the percentage of damage in fruit increased in plots that were sprayed according to the integrated spray schedule. This demonstrates that standard fungicides and insecticides have limited detrimental effect on development of fruit as well.

We can not directly compare our results with the results of other researchers, because we have not found any manuscripts describing experiments with the same copper formulations. On average, the percentage of fruit with disorders was not higher, in specific cultivars that we tested, it was even lower, than the one established by other researchers in their trials using similar copper formulations in the past (Straub and Kienzle 1992, Kienzle et al. 1995, Kelderer et al. 1997, 2000, Holb and Heijne 2001, Lešnik et al. 2005).

CONCLUSIONS

The application of different formulations of copper preparations (fungicides / foliar fertilizers) influences the fruit set of apples in different ways, depending on the combination between the formulation and the apple cultivar. We estimate that most new studied formulations do not decrease the quantity of fruit set to such an extent, that the reduction in number of developed fruit would significantly reduce the final yield of apples. The expected level of phytotoxicity to flowers and early stages of fruitlets is higher in cases of formulations which have partial or good systemic activity. When applying these formulations during the flowering period, the hectare rate of pure copper should not exceed 200 grams of pure copper per hectare per individual application, or they should not be applied at all, if there is a case of more susceptible cultivars and a small number of developed flowers per tree.

Most standard copper fungicides form relatively insoluble deposit of copper compounds on the surface of treated plants. Solubility of copper compounds in modern formulations (fungicides and fertilizer) is much higher. Because of that the release of copper ions is much faster and the amount of ions that penetrate into the plant tissue is significantly higher. This of course has good as well as bad consequences. On the one hand it increases biological efficacy against pathogens, on the other hand, ions that enter the plant tissue without being complexed with other molecules, can cause severe phytotoxic effect to plant. During the development of new formulations a specific equilibrium on copper solubility, which is suitable for specific plants, must be found. Regarding to the susceptibility of cultivars and types of fruit, the best way to do that would be to develop formulations based on mixtures of copper in different types of complexes with specific ratios between them. Many additional studies need to be carried out in order to gather information on optimal ratios between different copper forms. Optimised new formulations could therefore, significantly lower the copper input to the agricultural environment and could provide the same level of apple diseases control as existing standard formulations.

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