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Investigation of phytotoxicity regarding copper fungicides applied to apples

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ABSTRACT

The influence of adding kaolin clay (Cutisan), pine resin (Nu-Film), and milk proteins (ProNet-alfa) to copper fungicide spray at the same levels of phytotoxicity was tested regarding copper fungicides when applied to 'Golden Delicious' apples tested during a field-trial. Copper fungicides were applied 8 times per season using a standard orchard sprayer (250 l/ha of spray), starting at the BBCH 71 stage (fruitlets with more than 15 mm diameter); and continuing throughout the season until stage BBCH 79, at the rate of 250 g Cu^{++}/ha at each application. The phytotoxicity levels of traditional copper fungicides (sulphate, oxy-chloride, oxide, hydroxide) were compared to fungicides based on copper complexes (gluconate, EDTA chelate, peptidate and aminoacid complexes). Fruit disorders were evaluated (malformations, surface-russet). The level of reduction in phytotoxicity when adding kaolin clay was 10 to 80% and reached from 10 to 40% when adding pine resin. The addition of milk proteins in some fungicides reduced phytotoxicity by 5 to 10%, whereas in others a 15 to 30% increase was observed. The content of copper was determined in the fruits at harvest-time. The use of additives generally increased the copper residues of the fruits. The highest increase was determined for pine resin (5-100%), moderate for milk proteins (5-70%), and the lowest for kaolin clay (0-30%). The results of our trial showed that the tested additives had different effects on the reductions of phytotoxicity regarding copper products, and the intake of copper ions to apple fruits. They were very specific in terms of compatibility with different formulations of copper fungicides. In regard to compatibility, kaolin clay had the most neutral effect. As the addition of pine resin and milk proteins can significantly increase the copper content of fruits, they were less suitable as additives for the reduction of phytotoxicity within certain formulations of copper fungicides.

Kew words: apple, copper, phytotoxicity, additives, kaolin clay, pine resin, milk protein

INTRODUCTION

The application of copper fungicides is still one of the important options for controlling the fire-blight disease of apples caused by *Erwinia amylovora*. The most important period regarding fire-blight control is the blooming period (Clarke et al. 1993). In Slovenia, weather conditions during the apple blooming period are often unsuitable for infections from bacteria; therefore there are fewer cases of severe blossom-blight. On the other hand, there are several cases of late twig-blight that develops during the first part of June, or later when weather conditions are much more suitable for the development of bacteria. Inoculum sources are latently infected trees from which bacteria are dispersed to the young fast-growing twigs of adjacent trees.

During that period fruit producers have less chemical options for twig-blight control. Copper fungicides are one of the few. It is well-known that the application of copper fungicides during the period of fast fruit development causes a certain level of phytotoxicity which results in fruit-russet and malformations (Lešnik et al. 2011). Russet is a very complex physiological phenomenon that is caused by cuticle cracking due to a failure to keep up with the rapid growth of a fruit's internal tissues (Ashizawa et al. 2005). Russet usually starts in the early stages of a fruit's growth - shortly after full-bloom, corresponding to the period of greatest tangential growth (Noè and Eccher 1996, Peryea 2006). Russet leads to apple market value reductions because of the non-compliance with quality standards. Russeted fruits are normally downgraded when marketing. Despite a certain level of phytotoxicity, growers still apply copper products as they do not have other alternatives. The main alternatives under Slovenian conditions are phosetyl-Al-based fungicides and *Bacullus subtilis* products. Fruit producers seek solutions on how to reduce the phytotoxicities of copper fungicides. According to the information given by pesticide manufacturers the phytotoxicities of copper sprays can be reduced to a certain level by using special additives.

The aim of our research was to test the levels of phytotoxicity regarding different copper fungicides when applied several

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times during a season to 'Golden Delicious' apples, and to check the possibility of reducing the levels of phytotoxicity by adding additives based on kaolin clay, milk proteins, and conifer resin. The tested additives are promoted by industry as suitable for organic fruit production systems because they are produced from natural substances.

MATERIALS AND METHODS

Experimental orchard and trial design

The field-trial was carried out at the experimental station of the Faculty of Agriculture and Life Sciences, University of Maribor, in nearby Hoče, Slovenia. Copper fungicides were applied to 8-year-old 'Golden Delicious' trees grafted on M9 rootstocks, planted at 0.7 m x 3.2 m tree-spacing. 'Golden Delicious' was chosen because it is a very russet-sensitive cultivar. TRV (tree row volume) was 12.000 m^3 and the maximum LAI (leaf area index) was 2.6. The trees were trained within a modified slender spindle-system. The experimental plots were arranged in a fully randomised block design with 4 replications. The plots were of approximately 300 m^2 (20m long and five rows wide). Only one row in the middle was sprayed with copper fungicides, other rows served as isolation against drift. The evaluations of copper phytotoxicity were also only performed in the middle row of trees. ANOVA analysis was performed for separation and comparison of the treatment means, followed by the use of multiple comparison tools based on Tukey's HSD test at ($P<0.05$).

Application of fungicides

The fungicides were applied using a Unigreen Turbo Teuton 5+5 standard orchard sprayer, which delivered 250 litres of spray per hectare. The droplet volume diameter (VMD) was 150-170 µm. The copper fungicides were applied 8 times per season. The rates of the fungicides per plot for all the tested formulations were adapted so that 250 grams of copper ions (Cu^{++}) per hectare were always applied. The cumulative amount of pure copper ions delivered per season per hectare amounted to 2000 g. The compositions of the tested copper fungicides are presented in Table 1. The copper fungicides were applied on May 23rd (fruit diameter 24-27 mm), June 2nd (FD 30-33 mm), June 10th (FD 35-40 mm), June 20th (FD 40-45 mm), June 27th (FD 50-55 mm), July 7th (FD 50-55 mm), July 29th (FD 55-65 mm), and August 5th (FD 65-70 mm). There were also two types of control plots. Control K1 – untreated by copper fungicides but treated 18 times with pesticides, and Control K2 – plots that were untreated with any of the preparations, neither with copper fungicides nor pesticides. At the times of applying the copper, the trees were always dry.

The K1 plots were sprayed 18 times a season with different fungicide and insecticide mixtures. They complied with the standard apple spray programme for the control of diseases and pests. We introduced these plots in order to compare the phytotoxicity levels of the copper fungicides with the level of phytotoxicity regarding the standard spraying programme. The following treatments were done at the K1 plots: 5th April (250 g thiram /ha; Tiram 80), 11th April (525 g dithianone / ha; Delan 700 WG), 17th April (200 g ciprodinyl /ha; Chorus 75 WG), 23rd April (75 g difenconazole / ha; Score 250 EC) + (400 g dodine /ha; Syllit 400), 30th April (75 g trifloxystrobin /ha; Zato 50 WG) + (1250 g captan /ha; Merpan) + (150 g tiacloprid /ha; Calypso), 10th May (same as 30th April), 18th May (same as 23rd April), 27th May (75 g flucvinconazol /ha; Clarinet) + (1250 g captan /ha; Merpan) + (90 g acetamprid / ha; Mospilan), 7th June (75 g krezoxim-methyl /ha; Stroby) + (2000 g sulphur /ha; Cosan) + (240 g tebufenozid /ha; Mimic), 20th June (525 g dithianone /ha; Delan 700 WG), 1st July (1250 g captan /ha; Merpan), 10th July (75 g trifloxystrobin ha; Zato), 20th July (1250 g captan /ha; Merpan) + (750 g chlorpyrifos /ha; Pyrinex), 28th July (2000 g sulphur /ha; Cosan), 8th August (200 g ciprodinyl /ha; Chorus 75 WG), 24th August (220 g boscalid /ha; Bellis), and 3rd September (200 g boscalid /ha; Bellis).

Table 1: Formulations of copper fungicides and additives tested during the trial

Formulation	Manufacturer	Chemical form of copper	g Cu^{++} / kg
Cuprablau Z ultra	Cinkarna d.o.o. SLO	Cu-Ca-oxychloride (nano particles)	350.0
Cinkarna EDTA	Cinkarna d.o.o. SLO	Cu-Ca- oxychloride-EDTA chelate (not fully chelated)	39.3
Coptrel 500	Yara Vita TM GB	Cu-oxide-urea complex	330.0
Copper Protein	Nova Prot GmbH DE	Cu-hydroxide-protein complex	18.0
Kupro 190 SC	Agroruše d.o.o SLO	Cu-oxo-sulphate	190.0
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
Nu-Film	Loffler AU	Conifer tree resin	
ProNet-alfa	Proagro GmbH DE	Protein based wetting agent	
Cutisan	Biofa DE	Kaolin clay	

Evaluation of phytotoxicity

For each experimental plot, samples of 150 fruits were randomly chosen from different parts of the tree-crown regions and the percentage area of fruit surfaces (%) with signs of phytotoxicity was visually determined (russet, deformation, changes of colour). The results are expressed as % of the russeted surface areas of the fruits. Each chosen fruit was observed very carefully from all sites in order to notice any change in structure of its peel. The percentage of fruit russeted surface area was calculated as an average mean value of 150 visually-estimated values for individual fruits. We used the same approach as is commonly used for estimating the percentage area of a diseased fruit's surface when studying fungicide efficacy (Laycock 2004). Russet estimation was carried out on the 29th August.

Analysis of copper content within the apples

In order to determine the copper content of the apples, the fruits were harvested at the end of September. Samples of 25 fruits were randomly chosen from different parts of trees from each experimental plot. Each fruit was manually rubbed using a plastic grater to obtain fruit pulp. The fruits were unwashed prior to rubbing. The preparation of the samples for the analysis of copper content was performed according to the procedure established by the Cinkarna Metallurgical and Chemical Industry Celje, Inc.. The pulp samples were mineralised in microwave (nitric acid + hydrogen peroxide decomposition procedure), filtrated, and the solutions prepared for the analysis carried out by an Atomic Absorption Spectrometer (Varian AA 240FS), and by reading of their absorbance at 324.8 nm.

RESULTS AND DISCUSSION

Phytotoxicity analysis

The results presented in Table 2 demonstrate that different copper fungicides have a significantly different level of phytotoxicity to apple fruits, and that addition of additives results in different levels increasing or decreasing of phytotoxicity. Systemic acting fungicides (Peptiram, Protex and Copper Protein) caused a significantly higher level of russet than contact acting ones (Cuprablau and Cupro) (see last column of Table 2). The addition of Nu-Film, on average, reduced the level of russet from 5.40% to 4.11%, significantly less than in the case of adding Cutisan (from 5.40% to 3.29%). The addition of ProNet-alfa, on average, increased the russet area of fruits (from 5.40% to 5.73%). These results demonstrate how interactions amongst copper fungicides and additives can differ and that certain additives are unsuitable for mixing with certain copper fungicides. For example, it was obvious that ProNet-alfa is unsuitable for mixing with Cuprablau (russet increased from 1.99% to 6.29%). The highest reduction of russet was achieved by adding kaolin clay. The results show that kaolin clay is the most suitable additive for reducing copper fungicide phytotoxicity from amongst the three tested.

In the literature we were unable to find extensive data about those additives that can be used for the reduction of copper fungicide phytotoxicity. During our previous research we established that Cutisan (kaolin clay) provides a considerable level of reduction in phytotoxicity (Lešnik et al. 2011). We found the reports of Privé and co-workers (2007) who established that adding of kaolin clay can reduce copper phytotoxicity to apples significantly, but not during all seasons. According to their opinion, the russet phenomenon is very much weather-related and that during certain weather conditions the addition of additives cannot prevent russet. The same was proven by Noè and Eccher (1996). They didn't, however, test additives similar to ones we tested. Researchers De Jong and Der Maas (2008) studied the russet of Elstar apples and discovered that copper treatments were less problematic in terms of phytotoxicity than treatments with Armicarb (potassium bicarbonate). Potassium bicarbonate fungicides are one of the important alternative products by which the replacement of using copper fungicides during organic production is usually carried out. They found out that copper phytotoxicity can be reduced by mixing copper fungicides with sulphur fungicides. They mentioned the possibility of adding kaolin clay but didn't present any results. Similar results on the effects of adding sulphur were determined by Mitre and Mitre (2009), but they obtained opposite results about the phytotoxicity effects of Armicarb. According to their opinion, the mixing of copper products with potassium bicarbonate can slightly reduce copper fungicide phytotoxicity. In the literature there are many manuscripts available on methods for the reduction of russet by the application of hormones (especially GA4 and GA7 gibberellins) (Taylor and Knight 1986, McArtney and Obermiller 2007). These kinds of solutions are incompatible with Slovenian-integrated and organic fruit production regulations.

In regard to Golden delicious apples, a certain level of russet is inevitable and it also appears on fruits that have been untreated with any agrochemical. During our trial we had two types of control plots. Fruits from the K1 control plots that were sprayed 18 times a season with organic fungicides and insecticides but were not sprayed with any of the copper products, also had a certain level of russet (0.3 – 2.9%). This means that organic pesticides cause a certain level of phytotoxicity. There were also control plots (K2) that were not sprayed with any of the copper fungicides, additives or standard pesticides. Russet at those plots, on average, amounted to 1.25% (from 0.2 to 2.1%), and was the result of unknown environmental and physiological factors. We cannot define which of the factors having effects on the level of russet, and known from the literature (Knoche at al. 2011; e.g. daily temperature and air humidity regime, fertilisation rate nutrients, clone-type and age of trees), played an important role during the period of our trial. According to our measurements of weather parameters, as the meteorological conditions during the summer period of 2011 didn't fluctuate much from the data from 30-year local meteorological average values.

Table 2: The rate of the fruit damage (% of the russeted and malformed surface areas of fruits) in relation to the copper fungicides and additives applied. Assessment on August 29th

Fungicide	No additives	Nu-Film	Cutisan	ProNet-alfa	Average
Cuprablau Z ultra	1.99 a A	1.41 ab A	1.17 a A	6.29 c B	2.85 a
Cinkarna EDTA	2.23 a A	1.77 b A	4.09 c A	2.00 ab A	2.52 a
Peptiram 5	9.22 c C	5.83 cd B	1.78 ab A	9.41 c C	6.72 c
Coptrel 500	5.54 b B	4.97 c AB	2.42 b A	5.35 bc B	4.57 b
Protex-CU	4.03 b AB	3.59 c A	2.84 b A	5.05 bc B	4.01 b
Kupro 190 SC	3.26 ab B	3.45 c B	1.88 ab A	3.24 b B	2.96 a
Copper Protein	9.02 c B	7.75 d A	6.88 d A	9.25 c B	8.17 d
Control K1	2.61 a B	0.80 a A	0.60 a A	1.12 a A	
Average*:	5.04 C	4.11 B	3.29 A	5.73 D	

* Control K1 excluded from calculation of average mean. Control K2 -1.25%.
** Means marked with the same letters do not differ significantly according to the Tukey HSD test (P<0.05). The capital letters serve for comparisons amongst additives and the small letters for comparisons amongst fungicides.

Table 3: Copper content of apple fruits (mg/kg fresh weight) in relation to the copper fungicides and additives applied. Assessment on September 28th

Fungicide:	No additives	Nu-Film	Cutisan	ProNet-alfa	Average
Cuprablau Z ultra	2.31 ab A	4.87 c B	3.39 b A	3.28 b A	3.46 b
Cinkarna EDTA	1.55 a A	1.90 ab A	2.00 abA	2.71 b A	2.04 a
Peptiram 5	2.52 b A	2.50 b A	2.06 ab A	2.63 ab A	2.43 a
Coptrel 500	1.93 a A	2.82 b A	2.20 ab A	2.31 ab A	2.31 a
Protex-Cu	3.03 b A	3.80 bc B	2.15 ab A	3.25 b A	3.06 b
Kupro 190 SC	1.46 a A	2.18 b AB	2.55 b B	1.70 a A	1.97 a
Copper Protein	1.91 a A	2.02 ab A	2.63 b B	2.51 ab AB	2.27 a
Control K1	1.79 a A	1.35 a A	1.25 a A	1.48 a A	
Average*:	2.21 A	2.76 B	2.42 AB	2.63 AB	

* Control K1 excluded from the calculation of average mean. Control K2-1.74 mg/kg.
** Means marked with the same letters do not differ significantly according to the Tukey HSD test (P<0.05). The capital letters serve for comparisons amongst additives and the small letters for comparisons amongst fungicides.

Copper content analysis

Additives influence copper ion adhesion to fruit surfaces and penetration into fruit tissues (Teviotdale and Viveros 1999, Orbovič et al. 2007). The aim of our study was to determine the relationship between the metallic copper concentrations of fruits and the level of phytotoxicity regarding copper fungicides. Data on the copper contents of fruits is presented in Table 3. We can see that there were some differences in the contents of copper in those fruits treated with different fungicides. When additives were omitted, statistically the differences amongst the tested fungicides were insignificant. Those fruits treated with systemic-acting fungicides (Protex and Peptiram) contained more copper than those fruits treated with contact-acting fungicides (Cuprablau, Kupro, and Coptrel). The addition of Nu-Film increased the copper content of the fruits for all copper fungicides from 2.21 mg/kg to 2.76 mg/kg. A 24.8% increase was determined, on average. This result can be explained by the Nu-Film features. Nu-Film is a resin-based additive aimed at stabilising spray deposit and extending its lifespan. Copper deposits on the fruits' surfaces remain for longer periods and the penetration of copper ions is not enhanced. The addition of Cutisan also slightly increased the copper content of the fruits (from 2.21

mg/kg to 2.42 mg/kg; 9.5% increase), but insignificantly. The increase caused by adding ProNet-alfa amounted to 19.0% (from 2.21 mg/kg to 2.63 mg/kg).

The results show that the important side-effect of adding additives is the increase of copper content in the fruits. The results do not allow us to declare any conclusive relationship between the increased copper content and the increased level of phytotoxicity, as the increased content of copper does not directly correlate with the increased level of phytotoxicity. For example, this can be seen in the results for Copper Protein when comparing data from Table 2 and 3 – no additives). The phytotoxicity of Copper Protein decreased (from 9.02% to 7.75%) by the addition of Nu-Film, despite the increase in copper content (from 1.91 to 2.02 mg/kg). Similar results were noticed in the case of Cuprablau. The phytotoxicity of Cuprablau expressed as russet was decreased from 1.99% to 1.42% by adding Nu-Film despite the increase of copper content (from 2.31 to 4.87 mg/kg). The rate of proportionality between the level of reduced russet and the level of copper content was the highest in the case of Cutisan. The addition of Cutisan decreased the level of phytotoxicity the most and increased the copper content the least of all three of the tested additives. In the literature there is very limited information available on the effects of additives on the penetration of copper ions into apple fruit tissues, therefore we cannot directly compare our results with the results of other researchers.

CONCLUSIONS

The effects of adding additives to copper fungicide sprays in order to achieve a reduction in phytotoxicity for apple fruits are quite unpredictable. Some copper fungicides have shown that they are less likely to cause phytotoxicity than others. With certain combinations of fungicides and additives, significant reduction of phytotoxicity can be achieved, but not with all, because in some cases the addition of additives can result in increased phytotoxicity.

Every combination of copper fungicide and additive needs to be tested during a small-scale trial before advised for broader use. According to our results, kaolin clay is the most suitable additive for the reduction of phytotoxicity regarding the copper fungicides tested. The determined reduction level (from 19 to 35%) was not sufficiently high enough for us to conclude that any addition of the tested additives can solve the phytotoxicity problem completely.

It is also important to take into account that the usages of certain additives can increase the intake of copper ions into apple fruit, which can result in an increased, or even too high copper content of the fruits.

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