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Efficacy of azoxystrobin for the control of cucumber downy mildew (*Pseudoperonospora cubensis*) and fungicide residue analysis

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ABSTRACT

During a five-year trial (2007–2011), the efficacy of azoxystrobin (Quadris, 250 g a.i. L^{-1}, Syngenta) in two doses (187.5 g a.i. ha^{-1} and 250 g a.i. ha^{-1}) and chlorothalonil (Bravo 720-SC, 720 g a.i. L^{-1}, Syngenta) at a rate of 1.44 kg a.i. ha^{-1} was tested for the control of cucumber downy mildew (CDM). Cultivars that were susceptible or resistant to CDM (Regal and Haros, respectively) were tested for their response to fungicide applications. Differences in both disease severity and yield of the cultivars among resistance levels and fungicide treatments were observed. A highly significant and negative correlation was obtained between AUDPC and yield. Higher yields can be achieved by planting more resistant cultivars in combination with lower doses of fungicides. This is an indication that CDM contributes significantly to yield losses in cucumber production in Serbia. While monitoring the degradation of azoxystrobin residues, a decrease in residue levels to 1.0 mg kg^{-1} below the maximum residue level (MRL) was observed at the end of the pre-harvest interval (PHI).

1. Introduction

Cucumber downy mildew (CDM), caused by *Pseudoperonospora cubensis* (Berkeley & Curtis) Rostovtsev, is the most common and economically important cucumber disease in the Republic of Serbia (Bagi et al., 2009). In 2011, approximately 9000 ha of processing and fresh market cucumbers were produced in Serbia with an average yield of approximately 8 t ha^{-1} (Faostat, FAO Statistics Division, 2013). Since the first disease epidemic in Serbia in 1978, CDM has occurred every year and has caused significant losses and, in some cases, complete crop failures. The first symptoms often appear in the later part of June or the beginning of July. Symptoms occur almost exclusively as chlorotic lesions on the adaxial surface of the leaves (Savory et al., 2011). Lesions are restricted by leaf veins, which give them an angular appearance. As the infection progresses, the chlorotic lesions expand and may become necrotic.

Fungicide applications are currently necessary for adequate disease control (Gisi, 2002). The use of genetically resistant cultivars has to be combined with other integrated pest management (IPM) practices to minimize the risk that the pathogen will overcome resistance (Urban and Lebeda, 2006).

The discovery of systemic fungicides was a major advance over the use of protectant fungicides in the control of CDM. Systemic fungicides (metalaxyl, azoxystrobin, propamocarb, fosetyl Al and oxadixyl), alone or in combination with protective fungicides (chlorothalonil, zineb, mankozeb and copper), proved to be very successful in the control of CDM (Keinath et al., 2007; Call et al., 2013).

Azoxystrobin (QoI – Quinone outside Inhibitor) is a systemic fungicide which inhibits cell respiration by binding to the protein subunits in the mitochondrial cytochrome bc1 complex (Jordan et al., 1999; Fisher and Meunier, 2008). Fungicides from this group (FRAC, 2013) do not persist in the environment, are safe to non-target species, and are highly suitable for inclusion in IPM programs (Ishii et al., 2001). In cucumbers, the pre-harvest interval (PHI) is 4 days for azoxystrobin (Quadris, 250 g a.i. L^{-1}, Syngenta) applied at the recommended rates. In terms of toxicity, the acceptable daily intake (ADI) for an adult man is 0.1 mg kg^{-1} b.wt. day^{-1}, and the maximum tolerable content of this fungicide in cucumbers is 1.0 mg kg^{-1} in the European Union and the Republic of Serbia (Official Gazette RS 25/2010; Regulation EC 396/2005).

Chlorothalonil is a broad range, protective fungicide with multisite contact activity. It is a non-specific, general enzyme inhibitor, which leads to the disruption of glycolysis and energy production. Chlorothalonil is part of a group of fungicides considered to be low

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risk, and there are no signs that target organisms are developing resistance (FRAC, 2013).

In recent years, public concern has increasingly focused on food safety issues, especially the effects of pesticide residues (Vuković et al., 2012). Taking into account that cucumbers are harvested every second or third day, the presence of fungicide residues and their degradation rates are a limiting factor in the production of high quality and residue free fruits. To evaluate the potential risk associated with the use of pesticides, it is important to know the fate of a pesticide on a plant (Pucarević et al., 2013).

The objective of this study was to evaluate the effects of azoxystrobin and chlorothalonil in combination with cucumber cultivars with different levels of resistance on CDM disease severity and yield. Additionally, residue content of azoxystrobin in fruits was determined at several time points after fungicide application.

2. Materials and methods

2.1. Evaluation of azoxystrobin efficacy

2.1.1. Location of field trials

Experimental trials were conducted in Bačko Petrovo Selo, northern Serbia, over a 5-year period (2007–2011). This location has a long history of growing cucumbers, which are a significant source of income for the local population. The climate in northern Serbia is typically continental and favorable to cucumber production as well as the occurrence of CDM. Bačko Petrovo Selo is situated by the Tisa River, which promotes the spread of the disease. Over the course of the trials (June, July), the average daily temperatures and precipitation were recorded (Table 1).

2.1.2. Cucumber genotypes

The trials were carried out using two cultivars, Regal (Harris Moran Seed Company, Hungary) and Haros (ZKI, Hungary). Regal is an early-maturing, high-yield cultivar with dark green fruits that are 3–5 cm in length and suitable for both fresh consumption and processing. Due to its high adaptability to growing conditions, Regal is widely used in Serbia although it is susceptible to CDM. Haros is a newer generation cultivar that falls into an intermediate category in terms of its maturation time, vigorous growth and moderate branching. The fruits are dark green and 3–4 cm in length. According to the breeder's data, Haros has a certain level of resistance to CDM.

2.1.3. Field trial design

The production practices employed in the trial were similar to those used in commercial cucumber production. In Serbia, cucumbers are still grown in the field by sowing at a distance of 140 cm between rows and 30 cm between plants. Producers rely on natural precipitation, and plants are grown without irrigation.

Azoxystrobin (Quadris, 250 g a.i. L^{-1}, Syngenta) was applied at 187.5 g a.i. ha^{-1} and 250 g a.i. ha^{-1} to control CDM. Clorothalonil (Bravo 720-SC, 720 g a.i. L^{-1}, Syngenta), at a rate of 1.44 kg a.i. ha^{-1}, was used as a standard for comparison because protective chlorothalonil-based fungicides are commonly used against CDM in Serbia. Untreated plots were used as negative controls.

Table 1
Average daily temperatures (°C) and sum of precipitation (mm) for June and July 2007–2011.

Year	Temperature (°C)		Precipitation (mm)		
	June	July	June	July	Sum
2007	22.1	23.3	71.1	38.8	109.9
2008	21.9	27.7	115.9	41.6	157.5
2009	19.6	22.8	127.2	58.1	185.3
2010	20.2	19.9	171.8	99.0	270.8
2011	22.0	23.0	30.0	84.0	114.0

The experimental plots consisted of six 5 m rows, spaced 140 cm apart, at an average density of four plants per meter after emergence. The experimental design was completely randomized factorial design with four replications. Two meter buffer zone between the treatments prevented drift during pesticide applications. Fungicides were applied using a backpack mist blower (Solo 423) at 400 L ha^{-1} of fungicide mixture in the first, and 600 L ha^{-1} in the second and third applications.

Each year, the first fungicide treatment was applied at the onset of disease (2 July 2007, 28 June 2008, 9 July 2009, 5 July 2010 and 24 June 2011).

By the time of the first fungicide application, the crop had developed the fourth true leaf on the main stem in 2007, 2008 and 2010 (Biologische Bundesanstalt, Bundessortenamt and CHemical industry, BBCH 104 – Meier, 2001) and the fifth in 2009 and 2011 (BBCH 105). Every year a total of three fungicide applications for each treatment were conducted, with the second and third applications carried out eight days after the previous.

Over the course of trial, disease severity was assessed three times during vegetation, eight days after each application based on a scale ranging from 0 to 10, where 0 is a completely healthy leaf and 10 a completely necrotic leaf (EPPO, 2004). Each evaluation comprised 100 young leaves (leaf diameter at its widest part up to 5 cm), 100 medium leaves (leaf diameter 5–10 cm) and 100 older leaves (leaf diameter over 10 cm). The area under the disease progress curve (AUDPC, Karaoglanidis et al., 2003) was calculated from the individual leaf ratings using the disease index (McKinney, 1923). The yield for the individual plots was determined by harvesting fruits longer than 3 cm every third day from the moment of fruit formation and weighing them to within 1 g of precision. The fruits necessary for residue analysis were left on the plants, and those plants were subtracted from the yield evaluation. Altogether, there were eight harvests during the trial.

2.2. Evaluation of azoxystrobin residues

2.2.1. Field trials and sampling

Sampling aimed at detecting residues and the degradation dynamics of azoxystrobin was performed each year following the last fungicide application. The samples were collected at 2 (after the pesticide deposit had dried), 12, 24 and 48 h as well as 3, 4, 5, 7 and 8 d after fungicide application. Fruits (2 kg) were collected from each replicate, placed in polyethylene bags, and immediately transported to the laboratory. Each sample was divided into five sub-samples and stored at −20 °C until it was analyzed.

2.2.2. Materials

All solvents used were chromatography grade and obtained from Merck (Darmstadt, Germany). The certified analytical azoxystrobin standard, with purity of 99.7%, was purchased from Dr. Ehrenstorfer, GmbH (Augsburg, Germany). The standard stock solution of azoxystrobin (100 μg mL^{-1}) was prepared in toluene and stored at −18 °C. Helium, which was used as a carrier for all analyses, had purity greater than 99.9995%.

2.2.3. Sample preparation

Extracts were obtained using the method described by Lentza-Rizos et al. (2006). Azoxystrobin was extracted from a 25 g homogenized sample with a mixture of 50 mL of toluene and 25 mL of 2-propanol using an Ultra-Turrax (Heidolph DIAX 900, Germany). The propanol layer was removed by rinsing twice with a 2.0% Na_2SO_4 solution. The pesticide-containing toluene layer was rinsed