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RESEARCH ARTICLE

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Effect of the spray volume adjustment model on the efficiency of fungicides and residues in processing tomato

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Abstract

This study compared the effects of a proportionate spray volume (PSV) adjustment model and a fixed model (300 L/ha) on the infestation of processing tomato with potato late blight (*Phytophthora infestans* (Mont.) de Bary) (PLB) and azoxystrobin and chlorothalonil residues in fruits in three consecutive seasons. The fungicides were applied in alternating system with or without two spreader adjuvants. The proportionate spray volume adjustment model was based on the number of leaves on plants and spray volume index. The modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method was optimized and validated for extraction of azoxystrobin and chlorothalonil residue. Gas chromatography with a nitrogen and phosphorus detector and an electron capture detector were used for the analysis of fungicides. The results showed that higher fungicidal residues were connected with lower infestation of tomato with PLB. PSV adjustment model resulted in lower infestation of tomato than the fixed model (300 L/ha) when fungicides were applied at half the dose without adjuvants. Higher expected spray interception into the tomato canopy with the PSV system was recognized as the reasons of better control of PLB. The spreader adjuvants did not have positive effect on the biological efficacy of spray volume application systems. The results suggest that PSV adjustment model can be used to determine the spray volume for fungicide application for processing tomato crop.

Additional key words: azoxystrobin; chlorothalonil; *Phytophthora infestans*; spray deposit; QuEChERS.

Abbreviations used: GS (growth stage); LAI (leaf area index); LN (number of leaves per plant); PLB (potato late blight); PSA (primary secondary amine); PSV (proportionate spray volume); QuEChERS (quick, easy, cheap, effective, rugged, and safe); RSD (relative standard deviation); SANCO (Health & Consumers Directorate-General of the European Commission); SV (spray volume); SVAM (spray volume adjustment model); SVI (spray volume index per leaf); SV300 (spraying 300 L/ha of fungicide suspension)

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Introduction

The biological effectiveness and the pesticide residue levels depend on precise spraying and proper adjustment of the spraying parameters (Dammer *et al*., 2009; Lehoczki-Krsjak *et al*., 2015). In order to improve the adjustment of the dose and spray volume (SV) of plant protection products the concept of "treated area" has been proposed (Weisser & Koch, 2002). The SV in agricultural crops can be finally calculated by means of the leaf area index (LAI), leaf surface area and biomass (Dammer *et al*., 2008, 2015). However, the uni-

versal model of spray volume optimization according to the crop canopy structure has not been developed due to high variability between crop plants (Derksen *et al*., 2008). As far as vegetable crops are concerned, SV is almost exclusively expressed in terms of land surface. In the last decade there have been few studies on the spray volume adjustment in vegetables, including processing tomato (Zhu *et al*., 2004; Sanjika *et al*., 2008). In the case of tomato various methods of biomass, growth measurement and canopy characteristics have been developed (Trout *et al*, 2008; Čereković *et al*., 2010; Fortes *et al*., 2015). While these measures could be useful to express "treated area" there are other, including easy tools, that may also solve this problem. One of them is BBCH scale widely used to characterize growth stages of tomato (Feller *et al*., 1995). The number of leaves is another type of easily accessible information about growth of tomato plants. However, it is not used in terms of "treated area" in tomato as well as in other vegetables. Therefore, exploring various, including easy solutions for optimisation of SV seems well-founded.

Potato late blight (PLB) caused by *Phytophthora infestans* (Mont.) de Bary belongs to the most important plant diseases in the world that can rapidly destroyed whole fields of potato and tomato (Fry, 2008). Symptoms of PLB on tomato are to be found on leaves stems and fruits. Leaf lesions or spots begin as gray-green areas that quickly enlarge and change in color to become necrotic. Stems can also exhibit necrotic lesions. Fruit symptoms include golden to brown lesions that can appear sunken. Sporulation on leaves may occur on lesions that are only four to six days old. However, the pathogen can sporulate longer on stems and fruit than on leaves. (Fry, 1998). PLB is favored by high air humidity, dew, light rain and moderate temperatures (10 to 20°C) (Harrison, 1992; Fry, 1998). Various studies showed a close relation between meteorological parameters of ambient air and PLB incidence on potato and tomato (Harrison, 1992; Sharma 2000). However, foliage abundance, agrotechnique, including crop irrigation, influence the meteorological conditions inside canopy, hence PLB potential (Olanya *et al*., 2007; Cooke *et al*., 2011). On the other hand, host resistance diminished PLB risk (Duvauchelle & Dubois, 2001). Many forecasting models for PLB were created. They are usually and primarily addressed at potato or potato and tomato (Ullrich & Schroeder, 1966; Hansen *et al*., 1995; Kleinhenz *et al*., 2007) and many of them were successfully introduced into practice (Taylor *et al*., 2003). However, Bugiani *et al*. (1993) created and validated the forecasting model especially for tomato and successfully implemented it. While many tactics of tomato protection against PLB were developed for years, the spraying with fungicides is still one of the most important.

Contact and systemic fungicides are used to protect tomato against diseases, including PLB. Chlorothalonil and azoxystrobin are widely used for tomato protection against diseases and thus they were used in our study as model fungicides. Chlorthalonil was first introduced into practice in 1969 against early blight and late blight in potato (Ballee *et al.*, 1976). Chlorothalonil is a contact fungicide used against a wide range of diseases (Tomlin, 2000). By contrast, azoxystrobin is a xylem-systemic fungicide with translaminar and weak vapor action (Wong & Wilcox, 2001; Bartlett *et al*., 2002). However Godwin *et al*. (1999) showed that 8% of the active ingredient entering the leaf was transported. The young stem of the plant could absorb it more intensively (Bartlett *et al*., 2002). The azoxystrobin molecule was selected and introduced into agriculture because of its high biological activity against a wide range of fungal pathogens (Heaney & Knight, 1994).

The spray application, biological action and residue of foliar applied fungicides, including azoxystrobin and chlorothalonil, are affected directly or indirectly by meteorological conditions: rainfall, temperature, relative humidity of air, wind speed and solar radiation. The rainfall is usually considered the overall negatively affecting factor. Due to physical and mechanical process it can cause disappearance, dispersing and redistributing of active ingredients by volume and intensity of rainfall (Bruhn & Fry, 1982b; Töfoli *et al*., 2014). Töfoli *et al*. (2014) showed that systemic, including azoxystrobin, or inherent tenacity fungicides were less affected by the simulated rainfall than contact fungicides against *P. infestans* and *Alternaria solani* on potato leaf. Chlorothalonil gave a very similar result to azoxystrobin when rain was simulated 0.5, 1 and 2 hours after application against *A. solani*. However, it was not examined against *P. infestans*. As proved in Bruhn & Fry (1982b) the logarithmic rate in function of time describes wash off of chlorothalonil from potato leaves. However, the rainfastness also depends on leaf surface properties (Reynolds *et al*., 1994) and can be increased by formulation and adjuvants (Kudsk *et al*., 1991; Ryckaert *et al*., 2007).

The temperature-mediated modifications of physicochemical properties, bioavailability, toxicokinetics of active ingredient, including chlorothalonil and azoxystrobin, can influence on their toxicity to the target organism (Bao *et al*., 2008; Rodrigues *et al*., 2015). Quin *et al.* (2016) showed a negative correlation between tolerance of *P. infestans* populations to azoxystrobin and the mean annual temperature of region of sample origin. In laboratory scale, higher temperature of fungicide mixture (50°C versus 20°C) may increase biological

effect as showed in Schirra *et al*. (2010) using four ingredients, including azoxystrobin. Simultaneously, residue accumulation significantly increased within the same temperature regime. Moreover, the degradation rate was also affected by temperature (Schirra *et al*., 2010). Bruhn & Fry (1982b) based on the study with chlorothalonil found that the linear model describes this relationship very well. On the other hand, volatilization of volatile compounds is increased with increasing temperature (Bedos *et al*., 2002).

Air humidity and wind speed can influence on biological activity and dissipation of pesticides. High relative air humidity is considered a factor favoring uptake of fungicides due to a longer time of wet form of the deposit (Taylor, 2011). On the other hand, this can be a reason of more intensive volatilization of volatile compounds from foliage (Bedos *et al*., 2010). Wind speed enhances volatilization of volatile compounds from foliage and from soil (Bedos *et al*., 2002). Chlorothalonil vapor pressure of 0.076 mPa at 25°C (Tomlin, 2000) facilitates its disappearance from plant surface. However, the results obtained in a field on potato crops showed only 5% volatilization after 7.6 days (Leistra & Van Den Berg, 2007), while on wheat crops 0.6% after 31 h (Bedos *et al*., 2010) and 2.9% after 5 days (Lichiheb *et al*., 2014). Azoxystrobin vapor pressure of 1.1×10^{-7} mPa at 20 °C (Tomlin, 2000) causes low risk of the loss of substance as a result of volatilization. The role of humidity is also connected to decomposition of chemical compounds. However, these processes are much better recognized in case of chemicals applied to a soil. It was found that the decomposition of chlorothalonil could increase with increasing of humidity (Chaves *et al*., 2007). The rate of hydrolysis increase with increasing of temperature (Braunschweiler & Koivisto, 2000).

Photodegradation is considered an important reason for decomposition of xenobiotics on the plant surface. The field-extrapolated half-life of chlorothalonil due to photolysis was estimated at 5.3 days (Monadjemi *et al*., 2011). Lichiheb *et al*. (2014) estimated photodegradation of chlorothalonil at 31.1% after 5 days. Azoxystrobin has a satisfactory ultraviolet stability in comparison to strobilurin A its parent compound in nature produced, inter alia, by *Strobilurus tenacellus* (Pers ex Fr) Singer (Bartlett *et al*., 2002). However Braunschweiler & Koivisto (2000) found that a maximum of absorption of azoxystrobin in soil is at 295 nm and photolysis can be considered an important factor in its degradation even in Nordic countries but only during summer.

Meteorological conditions also have an influence on droplets from atomization to deposition on leaf. Temperature affects droplets formation indirectly mainly by influencing viscosity and surface tension (Lefebvre, 1988; Farnham *et al.,* 2015). However, meteorological conditions have a much greater influence on droplet evaporation and drift. Generally, the losses of droplets volume due to evaporation are enhanced with increasing temperature and decreasing air humidity (Holterman, 2003; Farnham *et al.,* 2015). Wind speed enhances evaporation and is the main factor that causes droplet drift (Holterman *et al*., 1997; Czaczyk, 2012). Many models describing these relationships have been created (Holterman *et al*., 1997; Friso & Baldoin, 2015).

Adjuvants added to the tank mixture or built in the formulation can influence on the effectiveness of chemicals used for plant protection (Green & Hazen, 1998). The substances used as adjuvants may chemically activate the active ingredient (Thelen *et al.,* 1995; Green & Hazen, 1998). Usually, the improvement of the biological effect is attributed to various types of physical interactions: adhesiveness, wetting, spreading, penetrating, retention, extension of duration of action (Holloway *et al*., 2000; Taylor, 2011). Various studies showed that the efficacy of contact fungicides increased with the surface covered by sprayed droplets (Grinstein *et al*., 1997; Washington, 1997). On the contrary systemics are less influenced than contact plant protection products (Wise *et al*., 2010). Increased coverage of plant surface is considered to be the factor responsible for higher effectiveness of mixes of contact fungicides with surfactants against PLB (Prokop & Veverka, 2006). Organosilicone surfactants seem to be one of the most effective wetting agents (Nikolov *et al*., 1998). They also facilitate the infiltration of stomata and capillaries and influence on foliar penetration (Stevens *et al*., 1991; Schönherr *et al*., 1999). It would be valuable to examine fungicide mixed with adjuvants, including organosilicone surfactants, against PLB in processing tomato crop.

In view of the prominence of spraying as a technique of fungicide application to tomato protection against PLB and also considering well-characterized properties and biological effectiveness of azoxystrobin and chlorothalonil, the aim of the study was to verify the spray volume adjustment model (SVAM) on tomato infested with *P. infestans* and to determine azoxystrobin and chlorothalonil residues in fruits with a new optimized Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method.

Material and methods

Experimental model

The experimental model shown in Table 1 compares two SVAM used to apply fungicides with or without an

Table 1. The experimental model with incomplete factor structure of the treatments

Treatment	SV[a] (L/ha)	Adjuvant	Fungicide dose[b] (%)
T1	300	–	50
T2	300	polyalkyleneoxide modified heptamethyltrisiloxane	50
T3	300	multi-ingredient adjuvant	50
T4	300	–	100
T5	PSV	–	50
T6	PSV	polyalkyleneoxide modified heptamethyltrisiloxane	50
T7	PSV	multi-ingredient adjuvant	50
T8	PSV	–	100
T9 (control)	–	–	–

[a]SV, spray volume; PSV, proportionate spray volume. [b]Azoxystrobin at dose of 125 and 250 g/ha or chlorothalonil at dose of 625 and 1250 g/ha (50% and 100% of the doses recommended, respectively)

adjuvant against *P. infestans* in processing tomato. The fixed model (SV300) involves the spraying of 300 L/ha of fungicide suspension from the first to the last application. In the proportionate spray volume (PSV) model, 300 to 750 L/ha of SV was applied. The order of application and suitable SV for specific dates is shown in Table 2. The SV for the PSV model was calculated depending on to the number of fully developed leaves per plant according to the following formula:

$$PSV\ (L/ha) = LN \times SVI$$

where LN = average number of leaves per plant; SVI = spray volume index per leaf (11.16). SVI was calculated in the following way: $SVI = 300/LN_1$, where LN_1 = average number of leaves per plant on the first day of application in 2009.

It was assumed that the maximum spray volume in season would have to be maintained right until the end of the last application, despite the reduction of the number of leaves.

Azoxystrobin (methyl (E)-2-[2-[6-(2-cyanophenoxy) pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate - Amistar 250 SC; Syngenta Limited, Guildford, UK) at a dose of 125 and 250 g/ha (50% and 100% of the dose recommended, respectively) or chlorothalonil (2,4,5,6-tetrachlorobenzene-1,3-dicarbonitrile - Gwarant 500 SC; Arysta LifeScience S.A.S., Noguères, France) at a dose of 625 g/ha and 1250 g/ha (50% and 100% of the dose recommended, respectively) were applied alternatively (Table 2). Adjuvants: Slippa (Interagro (UK) Ltd., Great Notley, UK) containing polyalkyleneoxide modified heptamethyltrisiloxane (655 g/L) and Torpedo II (De Sangosse Ltd, Swaffham Bulbeck, UK) formulation of four active ingredients (alkoxylated tallow amine, 210 g/kg; alcohol alkoxylate, 380 g/kg; natural fatty acids, 75 g/kg; and polyalkylene glycol, 210 g/kg), described as multi-ingredient adjuvant, at the concentration of 1 mL/L, were added to the tank mix only when the fungicides were applied at half the dose.

Experimental set-up

Field studies were conducted for three years, from 2009 to 2011 in Poznań, Poland (16.858092 52.408529 decimal degrees). The experiment was performed using completely randomised block design, with 3 blocks in 2009 and 4 blocks in the other two years. Tomato (*Solanum lycopersicum* L.) cv. Polset F1 was cultivated in a 2-row system (0.5 m × 0.5 m × 1.5 m) on a sandy loam soil. There were 14 plants on the plot. The plants were planted from 2 to 9 June each year. The plantation was subject to standard fertilisation and chemical weed control procedures. Tomato growth stages (GS) were noted during vegetation. The BBCH identification keys according to Feller *et al*. (1995) were used to identify tomato GS.

The application of azoxystrobin and chlorothalonil commenced 5 days after the first small pale green lesion on leaf, characteristic to PLB, was observed in 2009 and after 4 days in 2011 (Table 2). In 2010 the symptoms of PLB were not observed in July, thus spraying started in August as a result of increase in accumulated risk of disease incidence due to more favourable weather condition at the beginning of this month and because of growth in leaves numbers. The spraying was conducted using a precision field knapsack sprayer equipped with horizontal spray boom with 4 nozzles, the XR 11003 (TeeJet Spraying Systems Co., Wheaton, IL, USA). A fine droplet size application was conducted at 300 kPa pressure.

Meteorological conditions

The meteorological conditions were registered with a HOBO Weather Station and dedicated sensors (ONSET Computer Corporation, Bourn, USA) and are presented in Table S1 [supplement]. The following data were registered 2 m above the ground: air temperature

Table 2. The order of application of fungicides and the number of leaves per plant and the spray volume (SV) for specific dates for proportionate spray volume (PSV) model of application

Spraying no.	2009			2010			2011		
	Fungicide and application date [a]	Leaves numbers (GS) [b]	SV [c] (L/ha)	Fungicide and application date [a]	Leaves numbers (GS) [b]	SV [c] (L/ha)	Fungicide and application date [a]	Leaves numbers (GS) [b]	SV [c] (L/ha)
1	A 20 Jul	26.88 (65-71)	300	C 06 Aug	33.35 (67-72)	372	A 13 Jul	46.65 (68-71)	521
2	A 24 Jul	30.5 (66-72)	340	A 13 Aug	47.9 (71-75)	535	C 20 Jul	55.15 (69-73)	615
3	C 31 Jul	41.67 (71-75)	465	C 24 Aug	49.9 (73-81)	565	A 25 Jul	61.5 (71-75)	686
4	A 07 Aug	55.73 (71-75)	622	A 03 Sept	53.5 (81-85)	597	C 02 Aug	50.33 (71-75)	686
5	C 14 Aug	58.2 (73-81)	668	C 18 Sept	– (85-89)	597	A 09 Aug	– (73-81)	686
6	A 25 Aug	67.6 (75-83)	754	A[d] 18 Sept; 20 Sept	– (85-89)	597	C 12 Aug	– (75-82)	686
7	C 07 Sept	63.04 (85-88)	754	–	–	–	A 16 Aug	– (75-82)	686
8	A 11 Sept	63.04 (85-88)	754	–	–	–	–	–	–
Sample collection	14 Sept			23 Sept			19 Aug		

[a]A, azoxystrobin; C, chlorothalonil. [b]GS, growth stages. [c]SV, spray volume; [d]The azoxystrobin application was repeated (20 September) due to local short heavy rainfall.

(°C) and relative humidity (%) with a S-THA-M002 sensor, total solar radiation (MJ/m^2) with a Silicon Pyranometer S-LIB-M003, wind speed (m/s) with a S-WCA-M003 sensor. The amount of rainfall (mm) was registered with a rain gauge 0.2 mm - S-RGB-M002. The meteorological dates were used to evaluate the risk of PLB development, fungicides timing and then to estimate the influence of microclimate on fungicides action.

PLB occurrence and calculation of accumulated risk of disease

Disease symptoms were evaluated visually on 10 plants per plot. The modified Horsfall-Barrat rating scale of 1 to 12 (1=0%, 12=100% disease severity) was used to evaluate PLB severity on leaves (Berger, 1980). The number of fruits with PLB symptoms was calculated as percentage, 20 fruits per plant were observed. The estimations were conducted on the last day of the application and 2 weeks later.

The accumulated value of the risk of the disease was calculated using the NegFry model according to the procedure proposed by Ullrich & Schroeder (1966). The daily risk value was calculated based on the hourly relative humidity, precipitation and the average temperature within the field of the crop. Accumulated risk value over the period of time is the result of summing daily risk values starting at the day of tomato planting.

Evaluation of azoxystrobin and chlorothalonil residues

Samples for study of residues were collected in the amount of no less than 1 kg from the plot, 3 days after the last application of azoxystrobin and 7 days after the last application of chlorothalonil in 2009 and 2011 or 2 and 5 days after the application in 2010, respectively (Table 2).

The QuEChERS pesticide multiresidue method was validated for extraction of two fungicides: azoxystrobin and chlorothalonil, according to the Health & Consumers Directorate-General of the European Commission (SANCO) requirements [SANCO 12571/2013]. The validation of this method was carried out using six pesticide-free tomato samples for each fortification level. The homogenized samples (10 g) were spiked with a small volume (<0.5 mL) of an appropriate standard mixture solution and shaken with acetonitrile (10 mL) (Payá *et al.*, 2007). As a slight modification, the obtained mixtures were frozen for two hours to save

the volatile pesticides before the addition of salt mixture consisting of anhydrous magnesium sulphate (4 g), sodium chloride (1 g), disodium hydrogen citrate sesquihydrate (0.5 g), and trisodium citrate dehydrate (1 g). After centrifugation an aliquot of the acetonitrile phase was transferred into a centrifugation tube containing a mixture of anhydrous magnesium sulphate and primary secondary amine (PSA) sorbent (0.9 g). After purification with PSA, the extracts were re-acidified by adding 50 µL of the 50 mL/L formic acid solution. The extracts were transferred to the glass tubes and carefully evaporated to dryness. The last step was to add an acetone/hexane solution (Lehotay *et al*., 2010). A mixture of azoxystrobin and chlorothalonil, amenable to gas chromatography (GC), was quantitatively recovered from spiked tomato samples and determined using gas chromatography with a nitrogen phosphorus detector and electron capture detector (GC–NPD/ECD 6890 – N Agilent Technologies, USA; column: DB-5, 30 m × 0.53 mm × 0.88 µm, Agilent J&W Scientific, USA) The spiking levels for the recovery experiments were: 0.02, 0.2 and 2.0 mg/kg for GC–NDP/ECD analyses. The method accuracy and precision were evaluated by performing recovery studies. The precision was expressed as the relative standard deviations (RSD). Accuracy was measured by analysing samples with known concentrations and comparing the measured values with the true values. A default expanded measurement uncertainty of 50% was applied according to results reported by laboratories participating in a number of EU proficiency tests [SANCO 12571/2013].

Statistical analysis

The experiment analysed 8 treatments giving incomplete three-factor structure and zero control (Table 1), therefore the study was regarded as a one-factor experiment. As a result of the Bartlett test, in case of some of the variables, the logarithmic transformation or the non-parametric Kruskal-Wallis test was necessary. A multivariate analysis of variance was conducted using the Hotelling-Lawley test to analyse the difference between the average values of the multivariate variables (R stats package).

An appropriate one-way variance analysis (ANOVA) with division into group contrasts between the treatments (so called basic contrasts) was also conducted. In order to determine the coefficients of the contrasts (normalisation and orthogonalisation) presented in Table 3, the R gmodels package was used (Warnes *et al*., 2015). Tukey's *post hoc* test was used for comparison of the studied treatments.

Diverse influence of the studied treatments on values of azoxystrobin and chlorothalonil residues implies the use of exploration techniques of analysis of those multidimensional data. To group treatments, taking into consideration average values of residues in all 3 years of studies, a cluster analysis with the use of Ward's hierarchical clustering and Euclidean distance was performed. The package agricolae (Mendiburu, 2013) for program R (3.0.2) was used for the calculations.

Results

Plants infestation analysis

Risk of PLB. The infestation of plants with PLB differed throughout the years, with its peak observed in 2011. Natural incidence of *P. infestans* was observed in 2009 and 2011 on 16 and 8 July, respectively, while in 2010 it was 1 September. However, according to the NegFry model, the accumulated risk of PLB in July was the highest in 2009, followed by 2011 and 2010 (Fig. 1). Heavy rainfalls in June and July 2009 and in July 2011 favoured the incidence of PLB in July in both years (Table S1 [supplement]). However, the lower average air temperature in July 2011 and rich foliage of tomatoes (Tables 2 and S1 [supplement]) increased

Table 3. The coefficients of basic contrast applied in the variance analysis

Contrast	T1[a]	T2	T3	T4	T5	T6	T7	T8	T9
Control	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	–0.111
Dose	–0.25	0.00	0.00	0.25	–0.25	0.00	0.00	0.25	0.00
SVAM [b]	–0.125	–0.125	–0.125	–0.125	0.125	0.125	0.125	0.125	0.00
With or without adjuvant	–0.125	0.125	0.125	–0.125	–0.125	0.125	0.125	–0.125	0.00
Adjuvant type	0.00	–0.25	0.25	0.00	0.00	–0.25	0.25	0.00	0.00
Interaction_1	0.021	0.347	0.211	–0.579	–0.021	–0.347	–0.211	0.579	0.00
Interaction_2	–0.532	–0.038	0.448	0.122	0.532	0.038	–0.448	–0.122	0.00
Interaction_3	–0.303	0.503	–0.360	0.159	0.303	–0.503	0.360	–0.159	0.00

[a]Treatments T1–T9 according to description in Table 1. [b]SVAM, spray volume adjustment model.

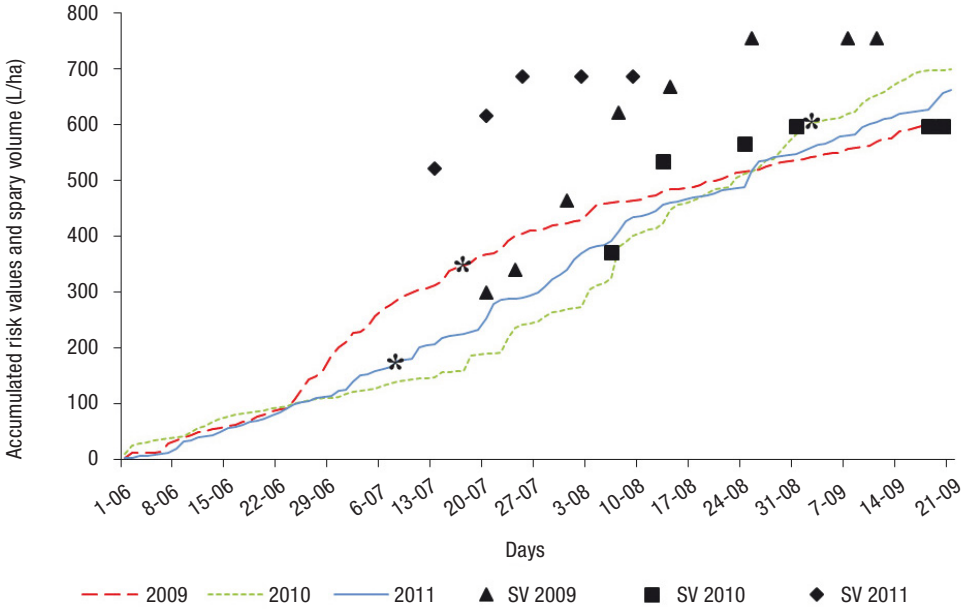

Figure 1. Accumulated risk values of potato late blight (PLB) according to the NegFry model over the year, date of the first symptoms observed (*) and applied spray volume (SV)

the risk of PLB incidence more than in other years. Within 14 days after the last application of azoxystrobin the average daily air temperature was higher and relative air humidity was lower in 2011 than in 2009 and 2010 and it reduced the risk of new infection with *P. infestans* in 2011 (Table S1 [supplement]).

Fungicide dose response. Each year applications of fungicides reduced tomato infestation with PLB (Table 4). However, there were not always significant differences between particular treatments and control (Table 5). The dose of fungicide applied without adjuvants influenced tomato infestation with PLB in 2011. Two weeks after last application in 2011 a full dose of the fungicide resulted in lower infestation of tomato than half the dose (contrast value –22.25 for leaves and – 41.5 for fruits) (Table 4). While the mean

Table 4. Values of the basic contrasts and their significance for the infestation of leaves and fruits by potato late blight (PLB) throughout the years. The data in the table columns are presented only in years, date and for leaves and fruits if could be done one-way analysis of variance

Basic contrast	2009			2010		2011			
	Leaves 11 Sept	Fruits 11 Sept	Fruits 2 weeks later	Fruits 21 Sept	Fruits 2 weeks later	Leaves 16 Aug	Leaves 2 weeks later	Fruits 16 Aug	Fruits 2 weeks later
Control	–180.67 ***	–22.92 *	–392.67 ***	–13.14 ***	–8.33 **	–558.20 ***	–232.25 ***	–429.0 ***	–390.0 ***
Dose	–2.49	–1.56	–6.67	–1.28	–0.87	–10.43	–22.25 **	–24.00 .	–41.50 ***
SVAM[a]	2.79	0.075	–8.67	–0.68	0.45	–0.50	–21.25 *	–29.50	–38.0 **
With or without adjuvant	–3.96	0.79	–8.67	0.38	3.18 **	20.15	24.25 *	54.00 **	49.00 ***
Adjuvant type	–1.62	–1.77	–6.00	0.27	–0.12	2.73	13.00 *	12.50	10.50
Interaction_1	–0.77	–0.75	–0.82	–0.062	0.28	–0.68	1.53	–2.88	0.73
Interaction_2	0.98	0.86	–0.69	–0.22	–0.01	–6.41	–7.57 *	–1.86	–8.61
Interaction_3	0.80	–0.22	–2.97	0.21	–0.50	–0.77	–2.2	–0.02	–9.60 *

[a]SVAM, spray volume adjustment model. Significance codes according to *p*-value: ***,**,*,· : 0.001, 0.01, 0.05, 0.1, respectively.

Table 5. Percentages of the infestation of tomato leaves and number of fruits with potato late blight (PLB) symptoms. The trial was performed with 3 blocks in 2009 and 4 blocks in the other two years

Treatment	2009				2010[a]		2011			
	Leaves		Fruits		Fruits		Leaves		Fruits	
	11 Sept	2 weeks later[n]	11 Sept	2 weeks later	21 Sept	2 weeks later	16 Aug[n]	2 weeks later	16 Aug	2 weeks later
T1	16.8 b	61.7 a	2.2 ab	20.7 b	3.1 b	10.6 bc	52.3 ab	74.0 b	48.0 bc	66.3 b
T2	16.5 b	49.2 a	2.4 ab	15.3 b	1.7 b	13.5 ab	52.5 ab	66.3 bcd	50.5 bc	53.8 bcd
T3	15.6 b	48.9 a	2.3 ab	14.7 b	1.7 b	23.7 ab	55.8 ab	70.8 b	56.3 b	63.0 bc
T4	17.1 b	39.2 a	2.3 ab	16.0 b	1.0 b	5.8 c	39.3 ab	56.0 e	36.5 cd	35.0 e
T5	19.1 b	52.6 a	3.0 ab	16.0 b	2.1 b	9.9 bc	40.3 ab	59.3 de	38.8 bcd	41.8 de
T6	17.0 b	45.0 a	3.3 ab	16.7 b	1.5 b	22.6 ab	45.5 ab	61.5 cde	45.0 bc	52.8 cd
T7	16.3 b	41.5 a	1.7 b	11.3 b	3.3 b	14.6 ab	52.3 ab	70.0 bc	51.8 bc	54.0 bcd
T8	16.4 b	41.7 a	1.3 b	14.0 b	0.4 b	8.3 bc	31.0 b	55.0 e	26.3 d	31.5 e
T9 (control)	47.7 a	92.4 a	5.2 a	64.7 a	18.0 a	47.7 a	99.0 a	93.1 a	97.8 a	98.5 a

[a]Infestation of tomato leaves by PLB was not showed due to serious damage to leaves caused by unpredictable appearance of *Aculops lycopersici* (Massee) in 2010; [n] Results subject to statistical analysis with non-parametric test. Means within columns with the same letter are not significantly different by $p<0.05$

severity of disease on leaves was 55.5% for full and 66.7% for half the dose, fruits were infested in 33.3% and 54.1%, respectively. Thus the corresponding reduction of plant infestation after application of fungicides was approximately 16.7% for leaves and 38.5% for fruits (Table 5). On the day of last spraying of azoxystrobin in 2011 the reduction in PLB incidence between full and half a dose was 24.1% for leaves and 27.6 for fruits. Unfortunately, contrast values for leaves reached –10.43 and –24 for fruits and were insignificant (Table 4). Similarly, contrast analysis for 2009 and 2010 showed lack of significant differences in results. However, plant infestation on suitable treatments in these years could confirm higher potential of full than half a fungicide dose for tomato protection against PLB.

SV response. The SVAM influenced the long-term infestation of tomato (Table 4). Two weeks after last spraying in 2011 the PSV resulted in lower leaves infestation of 8% (contrast value = 21.25) and fruits of 17.4% (contrast value = 38) than SV300. The corresponding values of mean infestation for PSV were 61.5% on leaves and 45% on fruits, while for SV300 66.8% on leaves and 54.5% on fruits. The post-hoc test proved that PSV system decreased tomato infestation with PLB when half of the recommended dose of the fungicide was applied without adjuvant (Table 5). The corresponding reduction of plant infestation on suitable treatments was 19.9% for leaves and 37% for fruits. However, the results in 2009 and 2010 did not differ between PSV and fixed system.

Adjuvants and SV response. The adjuvants under study did not increase tomato protection against PLB in comparison with the fungicide applied without adjuvant (Table 4). The multi-ingredient adjuvant even increased the infestation of tomato leaves by mean of 18% but only with the PSV system in 2011 (Table 5). The contrast analysis for 2011 showed that regardless of SVAM polyalkyleneoxide modified heptamethyltrisiloxane decreased leaves infestation by 10.2% to multi-ingredient adjuvant (Table 4).

Fungicide residue analysis

Validation of analytical method of fungicide residue determination. The mean recoveries were above 78.5% and 64% for azoxystrobin and chlorthalonil, respectively. The relative standard deviations were below 8.5% for azoxystrobin and 11.9% for chlorothalonil (Table 6). The detection limit was 0.02 mg/kg.

Dose response. The contrast analysis showed that the full dose of the fungicide resulted in higher residues of azoxystrobin and chlorothalonil than half the dose (Table 7). The mean residue of azoxystrobin after spray-

Table 6. Recovery of tested fungicides and appropriate relative standard deviations

Pesticide	Level of fortification (mg/kg)	Recovery (%)	RSD[a] (%)
Azoxystrobin	0.02	93.2	8.5
	0.2	78.5	8.4
	2.0	89.6	4.8
Chlorothalonil	0.02	64.0	11.9
	0.2	65.1	9.4
	2.0	91.9	5.8

[a]RSD, relative standard deviation

Table 7. Values of the basic contrasts and their significance for the azoxystrobin and chlorothalonil residues throughout the years. The data in the table columns are presented only in years and fungicides if could be done one-way analysis of variance

Basic contrast	2009	2010		2011
	C[b]	A	C	A
Control	1.49 ***	2.84 ***	1.45 *	0.92 ***
Dose	0.39 ***	0.06	0.69 ***	0.13 ***
SVAM [a]	–0.36 **	–0.20	–0.35	–0.07
With or without adjuvant	–0.90 *	–0.56 ***	–0.52 *	–0.07
Adjuvant type	–0.13	–0.13	–0.02	0.01
Interaction_1	–0.02	0.12 *	0.09	0.02
Interaction_2	–0.15 ***	–0.12 *	0.02	0.01
Interaction_3	–0.05 ***	–0.03	0.06	0.02

[a]SVAM, spray volume adjustment model. [b]A, azoxystrobin; C, chlorothalonil; Significance codes according to *p*-value: ***,**,*: 0.001, 0.01, 0.05, respectively.

ing with half the dose (0.065 mg/kg) was significantly reduced in comparison to full dose (0.1 mg/kg) only in 2011. The corresponding reduction was 35.1%. The chlorothalonil residues were significantly lower with full compared to half the dose in 2009 and 2010 by mean of 45.2% and 78.7%, respectively. The mean residues were as follows: 0.42 mg/kg with full and 0.23 mg/kg with half the dose in 2009 while in 2010 they were 0.45 mg/kg and 0.095 mg/kg, respectively. However, the post-hoc test indicated that only chlorothalonil residues can be regarded as significantly lower after application with half the dose (2009) (Table 8). The reduction was 17.8% for SV300 and even 76.9% for PSV. The cluster analysis showed that combined residues of fungicides applied at a full dose were higher than at half the dose (Fig. 2). It is noteworthy that the azoxystrobin residues in 2010 were higher than in other years because of the necessary repetition of the last application due to local short heavy rainfall (Table 2).

SV response. The SVAM significantly influenced only chlorothalonil residues in 2009 by mean of 35.9% (contrast value –0.36) (Table 7). The mean residues were as follows: 0.165 mg/kg with PSV and 0.258 mg/kg with SV300. The post-hoc test proved that PSV system significantly decreased residue only when half of the fungicide dose was applied. The cluster analysis showed that after the application of fungicides at half the dose using the PSV model the residues created a separate cluster from SV300 and were located closer to the control (Fig. 2).

Adjuvants and SV response. The statistical analysis proved that adjuvants affected residues of chlorothalonil in 2009 (Table 8) and azoxystrobin in 2010. The residues of chlorothalonil were reduced with SV300 from 0.37 mg/kg to 0.15 mg/kg in case of polyalkyleneoxide modified heptamethyltrisiloxane and even to 0.06 mg/kg for multi-ingredient adjuvant. Despite polyalkyleneoxide modified heptamethyltrisiloxane increased chlorothalonil residue of 22.2% with PSV in 2009. Moreover, chlorothalonil residue after spraying with PSV system and using last one adjuvant amounted to 0.11 mg/kg and was significantly higher than 0.07 mg/kg achieved with multi-ingredient adjuvant. The azoxystrobin residue was significantly reduced with SV300 from 0.54 mg/kg to 0.29 mg/kg only after the application of multi-ingredient adjuvant (2010).

The cluster analysis showed that combined residues of fungicides with adjuvants were roughly lower using PSV relative to SV300 system (Fig. 2). The adjuvants contributed to the reduction of fungicide residue in case of SV300 system.

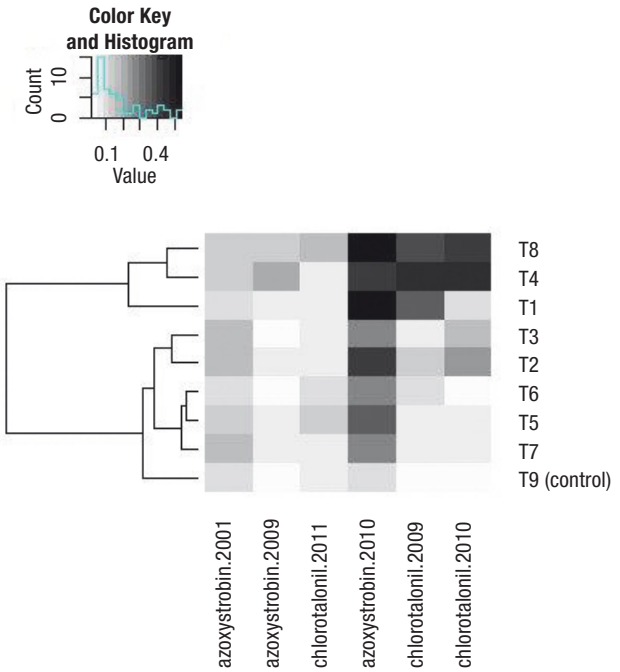

Figure 2. Results of cluster analysis of fungicides residue in tomato fruits

Discussion

Fungicide dose response

A full dose of fungicide has higher potential to increase the fungicide efficiency (Bain *et al*., 2014). This fact was confirmed by azoxystrobin and chlorothalonil residues in tomato plants under study (Table 7, Fig. 2). However, in low disease pressure circumstances a reduced dose of fungicide can even ensure similar level of plant protection. It could be seen in the results obtained in 2009 and 2010. The study showed that tomato canopy characteristics can influence the fungicide rate. In view of the fact that the tomato plants had more leaves per plant at the beginning of application in 2011 (46.7) than in 2010 (33.4) and in 2009 (26.9) (Table 2), the average fungicide rate per unit of leaf surface area should have been lower in 2011 than in 2010 and 2009. Between 2009 and 2011 the delay in the leaves number was over 2 weeks (Table 2). We assumed that lower long-term fungicide rate after spraying may result in higher ultimate tomato infestation with PLB. However, this study cannot confirm directly this thesis. Studies on other crops (Walklate *et al*., 2003; Dammer *et al*., 2008; Llorens *et al*., 2011), including potato (Cooke *et al*., 2011) indicate that crop and canopy characteristics modify the fungicide rate per leaf surface area unit and could affect fungicide efficiency.

SV response

The PSV model resulted in lower long-term infestation of fruits and leaves than 300 L/ha in 2011 when half of the fungicide dose was applied. According to Grinstein *et al*. (1997), Washington (1997) and Prokop & Veverka (2006), higher surface coverage by the contact fungicide can be a factor favouring the decrease in infestation of the leaves by disease. The interception of the fungicide and spray suspension into the canopy may confirm this dependence and can clarify the tomato infestation in 2011 both with PSV and SV300. As was proved, the spray deposit into the canopy is proportional to the SV applied (Walklate & Cross, 2013). On the other hand, this deposit into the canopy is inversely proportional to the LAI (Zhu *et al*., 2004). Thus, the estimated spray depos it should be higher with PSV than with SV of 300 L/ha. At the end of the spraying season 754 L/ha of SV was applied in 2009, 597 L/ha in 2010 and 686 L/ha in 2011 with the PSV adjustment model. The fungicide residues can also explain the spray interception into the canopy and thus, biological results. If there had been no differences in fungicide residues between both SV adjustment systems under study, the spray deposit into the canopy could have been higher in the case of higher SV. The contact fungicide residue could have verified this better than the systemic fungicide. In 2011 the residues of chlorothalonil with half the dose of fungicide ranged 0.05-0.07 mg/kg for SV300 and 0.08-0.12 mg/kg for PSV (Table 8). Therefore, after the last application of 686 L/ha in 2011, the spray deposit on fruits was higher than after the application of 300 L/ha. The spray deposit on leaves at the same level above the ground could also have been higher. Hence, the higher spray deposit into the canopy could be considered as the reason of better long-term protection of tomato with the same fungicide dose per hectare in 2011. In 2009 and 2010 the residues of chlorothalonil were poorly coherent with result of 2011. PSV system gave significantly lower chlorothalonil residues than the fixed system (Table 7). The mean residue after spraying with PSV system was 0.17 mg/kg while 0.27 mg/kg with

Table 8. Residues of azoxystrobin and chlorothalonil in tomato fruits (mg/kg). The trial was performed with 3 blocks in 2009 and 4 blocks in the other two years

Treatment	2009		2010		2011	
	A[a] [n]	C	A	C	A	C[n]
T1	0.05 ab	0.37 f	0.54 a	0.11 ab	0.12 ab	0.08 ab
T2	0.07 ab	0.15 ab	0.40 ab	0.25 ab	0.16 ab	0.07 ab
T3	0.05 ab	0.06 de	0.29 b	0.18 ab	0.15 ab	0.05 ab
T4	0.21 a	0.45 bcd	0.40 ab	0.45 a	0.19 a	0.06 ab
T5	0.08 ab	0.09 abc	0.34 ab	0.08 b	0.12 ab	0.12 ab
T6	0.05 ab	0.11 e	0.29 b	0.04 b	0.11 b	0.11 ab
T7	0.07 ab	0.07 ab	0.27 b	0.08 b	0.13 bc	0.08 ab
T8	0.15 ab	0.39 cde	0.54 a	0.44 a	0.18 ab	0.17 b
T9 (control)	0.02 b	0.03 a	0.03 c	0.02 b	0.03 c	0.03 a

[a]A, azoxystrobin; C, chlorothalonil. [n]Results subject to statistical analysis with non-parametric test. Means within columns with the same letter are not significantly different by $p<0.05$.

SV300. The corresponding residues in 2010 were: 0.16 mg/kg for PSV and 0.25 mg/kg for fixed system (Table 8). However, these results may be connected with tomato canopy shape. Tomato GS ≥ 85 appeared during the last spray application in 2009 (and also in 2010). At this time the canopy is flattened and much more transparent to spray droplets than at earlier growth stages. Thus, the plant coverage may not differ so much between both SV application systems. Moreover the SV of 300 L/ha resulted in higher concentration of the fungicide suspension on the plant surface. A high fungicide residue on the exposed fruits is in accordance with a high coverage of external part of the potato canopy by chlorothalonil (Bruhn & Fry, 1982a; Hamm & Clough, 1999). According to literature data, the results of biological action of contact fungicides against PLB were satisfactory when an SV of 160 L/ha was applied (Jensen & Nielsen, 2008). The alternate application of azoxystrobin and chlorothalonil presented in this study gave a chance to increase the probability of satisfactory biological results of PLB control using a spray volume of 300 L/ha. A systemic fungicide such as azoxystrobin may be able to maintain high performance using a mid- or low-volume spraying (Ratajkiewicz *et al.*, 2009; Wise *et al*., 2010).

In spite of the positive potential of PSV in tomato protection against PLB, this system was not able to increase biological results significantly when fungicides were applied at a full dose. On the other hand, a high pressure of *P. infestans* and leaf biomass (2011) caused high infestation of tomato with PLB despite the PSV system.

Adjuvants and SV response

Adjuvants did not have positive effect on the biological efficacy of the PSV application system or the fixed (Table 5). However, without adjuvants the PSV system was more efficient in tomato protection against PLB than the fixed system (2011). On the other hand, the cluster analysis shows that combined residues of fungicides applied at half of the recommended dose with adjuvants could be inversely associated with the SV. Hence, residues tend to be higher with the fixed rather than with the PSV system (Fig. 2). However, as was proved, the growth stage of tomato and canopy shape during last spraying had some influence on the residue in particular years. According to literature data (Schönherr *et al*., 1999; Ramsdale & Messersmith, 2001; Menéndez & Bastida, 2004), an appropriately selected adjuvant can enhance the biological action of pesticides and this effect is stronger with low rather than with high SV. The higher ratio between the adjuvant and pesticide and between the adjuvant and water can justify the stronger biological effect (Ramsdale & Messersmith, 2001; Menéndez & Bastida, 2004). In our study the same concentration of the adjuvant in water was used with both SV application systems. The ratio between the adjuvant and fungicide concentration in the spray suspension increased with increasing of SV in the PSV system, meanwhile the concentration of fungicide decreasing. Thus, this contraction could diminish the possible positive effect of adjuvant applied with the PSV system.

Other factors may also influence the fungicide action with adjuvants. The spreader adjuvants under study can increase droplet spreading and thus the coverage of the plant surface with a fungicide suspension. Therefore, the disappearance of fungicides due to volatilisation may also increase (Bedos *et al*., 2002), especially when a higher SV is applied. The photodegradation may also increase with increasing coverage. According to vapor pressure of chlorothalonil (0.076 mPa at 25 °C) and azoxystrobin (1.1×10^{-7} mPa at 20 °C) the potential risk of volatilisation was higher for the first fungicide (Tomlin, 2000). However in field trial on potato the fate of chlorothalonil due to volatilization was only 5% after 7.6 days (Leistra & Van Den Berg, 2007). Furthermore, Monadjemi *et al*. (2011) estimated half-life of chlorothalonil due to photodegradation at 5.3 days. There are studies indicating high azoxystrobin stability on tomato leaves within 1–4 days after spraying (Szpyrka & Sadło, 2008) and even after 7-10 days on fruits (Garau *et al*., 2002). Thus chlorothalonil is more susceptible to physical interactions on leaf surfaces than azoxystrobin. On the other hand, the polyalkyleneoxide-modified heptamethyltrisiloxane could have facilitated the infiltration of fungicides into cuticles (Stevens *et al*., 1991). It was shown that cuticular waxes may affect photodegradation rates and photoproducts (Ter Halle *et al*., 2006). Thus, this adjuvant has a potential to diminish the disappearance of fungicides from the plant surface. We can conclude that the key to achieve high biological effectiveness of a spray mixture is the appropriate adjustment of the adjuvant properties with the fungicide. Mitani *et al*. (2001) showed that the addition of a superspreading adjuvant to a spray liquid containing cyazofamid of stable residual activity and rainfastness properties is beneficial and necessary for the fungicide to be effective against PLB.

Verification of SV adjustment based on counting the number of leaves

The number of leaves gives valuable information about the growth of tomato. Thus, the SV proposed in

the PSV model simply reflects abundance of tomato foliage. However, we do not know how much of spray deposit is intercepted on particular stratum of canopy or on leaves. According to Bruhn & Fry (1982a) and Hamm & Clough (1999) we can assume that interception of spray into tomato plant decreased proportionally from top to bottom of canopy. However, in the end of tomato development the shape of canopy changes considerably due to stems starting to drop down and it is difficult to describe. Similarly, the interception of spray and fungicide into the canopy can change considerably. It would be valuable to optimise the PSV model according to changes in the canopy shape during fructification. Measuring the LAI, normalized difference vegetation index and fractional canopy cover would give valuable information about the tomato canopy and crop (Trout *et al*, 2008; Čereković *et al*., 2010; Fortes *et al*., 2015). A model based on a few plant and crop structure parameters would give better results of spray volume adjustment during the whole vegetation period than a PSV model based on one parameter.

However, let us hypothesize that the SV given by the proposed PSV model could be stopped or decreased from then on, as the plant canopy drops down and flattens. This thesis may be confirmed by residues of the contact fungicide (chlorothalonil). The plants at the GS 85-88 development stage (94 days after planting) (2009) had higher mean chlorothalonil residue in fruits after the application of SV300 (0.26 mg/kg) than PSV (0.17 mg/kg). In contrast, on younger plants with many numerous erect stems (GS 75-82, 70 days after planting) in 2011 the residues of chlorothalonil were opposite of that and equalled 0.07 mg/kg for SV300 and 0.12 mg/kg for PSV. On the other hand, the residues of systemic azoxystrobin were generally independent of SV applied in examined range. The mean residue of azoxystrobin in 2009 after the application with SV300 was 0.08 mg/kg and with PSV 0.09 mg/kg while in 2011 0.16 mg/kg and 0.14 mg/kg, respectively. The fungicide residue influences its biological action so it would appear to be justified to stopped or even decreased SV calculated for chlorothalonil and azoxystrobin according to PSV model as the plant canopy flattens.

On the other hand, it is worthy of attention that the residue of azoxystrobin in the end of withholding period (3 days) after spraying with SV calculated according to PSV and SV300 were substantially lower than the 3 mg/kg of maximum residue level in tomato fruits (EU, 2012a). While withholding period for chlorothalonil is also 3 days, the residues were evaluated 7 days (or 5 days in 2010) after spraying. These residues were also substantially lower than the 2 mg/kg of maximum residue level (EU, 2012b). Hence, both SVAM guaranteed food safety and consumer security.

Only one system of plant spacing and tomato variety was used in this study. The leaf number index used to calculate the SV may have varied due to different plant spacing and varieties. However, the number of leaves per plant can be easily translated into the number of leaves per hectare, thus diminishing the influence of plant spacing. Despite this, leaf morphology is influenced by tomato variety types. The proposed SVI may work properly with a tomato variety similar to Polset F1. However, according to plant morphology, this variety is classified as the most commonly used variety type in processing tomato production. In conclusion, the simplicity of PSV model can make it a useful tool in adjustment of SV in processing tomato crop.

References

Bain RA, Ritchie F, Lees A, Dyer C, 2014. Impact of fungicide input on leaf blight (Phytophthora infestans) development on different potato cultivars. Proc of the 14th EuroBlight Workshop, PPO Special Report 16, Schepers HTAM (ed.), Limassol (Cyprus), 12-15 May 2013, pp. 65-73.

Ballee DL, Duane WC, Stallard DE, Wolfe AL, 1976. Chlorothalonil. In: Analytical methods for pesticides and plant growth regulators (Vol. VIII); Zweig G, Sherma J (eds.). pp: 263-274. Academic Press, Inc. http://dx.doi.org/10.1016/b978-0-12-784308-7.50020-x.

Bao VW, Koutsaftis A, Leung KM, 2008. Temperature-dependent toxicities of chlorothalonil and copper pyrithione to the marine copepod Tigriopus japonicus and dinoflagellate Pyrocystis lunula. Australas J Ecotoxicol 14(2/3): 45-54.

Bartlet DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B, 2002. The strobilurin fungicides: a review. Pest Manag Sci 58(7): 649-662. http://dx.doi.org/10.1002/ps.520.

Bedos C, Cellier P, Calvet R, Barriuso E, Gabrielle B, 2002. Mass transfer of pesticides into the atmosphere by volatilization from soils and plants: overview. Agronomie 22(1): 21-33. http://dx.doi.org/10.1051/agro:2001003.

Bedos C, Rousseau-Djabri MF, Loubet B, Durand B, Flura D, Briand O, Barriuso E, 2010. Fungicide volatilization measurements: Inverse modeling, role of vapor pressure, and state of foliar residue. Environ Sci Technol 44(7): 2522-2528. http://dx.doi.org/10.1021/es9030547.

Berger RD, 1980. Measuring disease intensity. Proc of EC Stakman Commemorative Symposium on Crop Loss Assessment; Teng PS, Krupa SV (eds.), Minneapolis, MN (US), Aug 20-23. pp: 28 31.

Braunschweiler H, Koivisto S, 2000. Fate and effects of chemicals in the Nordic environments related to the use of biocides. Nordic Council of Ministers, Copenhagen. 135 pp.

Bruhn JA, Fry WE, 1982a. A statistical model of fungicide deposition on potato foliage. Phytopathology 72: 1301-1305. http://dx.doi.org/10.1094/Phyto-72-1301.

Bruhn JA, Fry WE, 1982b. A mathematical model of the spatial and temporal dynamics of chorothalonil residues on potato foliage. Phytopathology 72: 1306-1312. http://dx.doi.org/10.1094/Phyto-72-1306.

Bugiani R, Cavanni P, Ponti I, 1993. An advisory service for the occurrence of Phytophthora infestans on tomato in Emilia-Romagna region. Bull OEPP 23: 607-613. http://dx.doi.org/10.1111/j.1365-2338.1993.tb00557.x.

Čereković N, Todorović M, Snyder RL, 2010. The relationship between leaf area index and crop coefficient for tomato crop grown in southern Italy. Euroinvent 1 (1): 3-10.

Chaves A, Shea D, Cope WG, 2007. Environmental fate of chlorothalonil in a Costa Rican banana plantation. Chemosphere 69 (7): 1166-1174. http://dx.doi.org/10.1016/j.chemosphere.2007.03.048.

Cooke LR, Schepers HTAM, Hermansen A, Bain RA, Bradshaw NJ, Ritchie F, Shaw DS, Evenhuis A, Kessel GJT, Wander JGN, et al. 2011. Epidemiology and integrated control of potato late blight in Europe. Potato Res 54 (2): 183-222. http://dx.doi.org/10.1007/s11540-011-9187-0.

Czaczyk Z, 2012. Influence of air flow dynamics on droplet size in conditions of air assisted sprayers. Atomization Spray 22 (4): 275-282. http://dx.doi.org/10.1615/AtomizSpr.2012003788.

Dammer K, Wolny J, Giebel A, 2008. Estimation of the leaf area index in cereal crops for variable rate fungicide spraying. Eur J Agron 28: 351-360. http://dx.doi.org/10.1016/j.eja.2007.11.001.

Dammer KH, Thöle H, Volk T, Hau B, 2009. Variable-rate fungicide spraying in real time by combining a plant cover sensor and a decision support system. Precis Agric 10 (5): 431-442. http://dx.doi.org/10.1007/s11119-008-9088-7.

Dammer KH, Hamdorf A, Ustyuzhanin A, Schirrmann M, Leithold P, Leithold H, Tackenberg M, 2015. Target-orientated and precise, real-time fungicide application in cereals. Landtechnik 70 (2): 31-43.

Derksen RC, Frantz J, Ranger CM, Locke JC, Zhu H, Krause CR, 2008. Comparing greenhouse handgun delivery to poinsettias by spray volume and quality. T ASABE 51: 27-33. http://dx.doi.org/10.13031/2013.24214.

Duvauchelle S, Dubois L, 2001. The evolution of the foliar late blight resistance of the cultivar- Two years of trials in northern France (1999-2000) – The main results 2001. PAV-Special Report 7: 239-244.

EU, 2012a. Commission Regulation (EU) No. 270/2012 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for amidosulfuron, azoxystrobin, bentazone, bixafen, cyproconazole, fluopyram, imazapic, malathion, propiconazole and spinosad in or on certain products. 26 March 2012. Official Journal EU 89: 5-63.

EU 2012b. Commission Regulation (EU) No. 441/2012 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for bifenazate, bifenthrin, boscalid, cadusafos, chlorantraniliprole, chlorothalonil, clothianidin, cyproconazole, deltamethrin, dicamba, difenoconazole, dinocap, etoxazole, fenpyroximate, flubendiamide, fludioxonil, glyphosate, metalaxyl-M, meptyldinocap, novaluron, thiamethoxam, and triazophos in or on certain products. 24 May 2012. Official Journal EU 135: 4-56.

Farnham C, Nakao M, Nabeshima M, Mizuno T, 2015. Effect of water temperature on evaporation of mist sprayed from a nozzle. J Heat Island Inst Int 10: 35-44.

Feller C, Bleiholder L, Buhr H, Hack M, Hess M, Klose R, Meier U, Stauss R, Van den Boom T, Weber E, 1995. Phänologische Entwicklungsstadien von Gemüsepflanzen. I. Zwiebel-, Wurzel-, Knollen- und Blattgemüse. Nachrichtenbl Deut Pflanzenschutz 47 (9): 217-232.

Fortes R, Prieto MH, García-Martín A, Córdoba A, Martínez L, Campillo C, 2015. Using NDVI and guided sampling to develop yield prediction maps of processing tomato crop. Span J Agric Res 13 (1): e02-004. http://dx.doi.org/10.5424/sjar/2015131-6532.

Friso D, Baldoin C, 2015. Mathematical modelling and experimental assessment of agrochemical drift using a wind tunnel. Appl Math Sci 9 (110): 5451-5463.

Fry WE, 1998. Vegetable crops. Late blight of potatoes and tomatoes. Cornell Univ Coop Ext, NY State, USA. http://vegetablemdonline.ppath.cornell.edu/factsheets/Potato_LateBlt.htm. [7-1998].

Fry W, 2008. Phytophthora infestans: The plant (and R gene) destroyer. Mol Plant Pathol 9 (3): 385-402. http://dx.doi.org/10.1111/j.1364-3703.2007.00465.x.

Garau VL, Angioni A, Del Real AA, Russo M, Cabras P, 2002. Disappearance of azoxystrobin, pyrimethanil, cyprodinil, and fludioxonil on tomatoes in a greenhouse. J Agr Food Chem 50 (7): 1929-1932. http://dx.doi.org/10.1021/jf011219f.

Godwin JR, Bartlett DW, Heaney SP, 1999. Azoxystrobin: implications of biochemical mode of action, pharmacokinetics and resistance management for spray programmes against Septoria diseases of wheat. In: Septoria on cereals: A study of pathosystems; Lucas JA, Bowyer P, Anderson HM (eds.). pp: 299-315. CABI Publ.

Green JM, Hazen JL, 1998. Understanding and using adjuvant properties to enchance pesticide activity. Proc Fifth Int Symp on Adjuvants for Agrochemicals, Vol 1; McMullan PM (ed), Memphis (TN), August 17–21. pp: 25-36.

Grinstein A, Riven Y, Elad Y 1997. Improved chemical control of botrytis blight in roses. Phytoparasitica 25: 87-92. http://dx.doi.org/10.1007/BF02980335.

Hamm PB, Clough GH, 1999. Comparison of application methods on deposition and redistribution of chlorothalonil in a potato canopy and potential for control of late blight. Plant Dis 83 (5): 441-444. http://dx.doi.org/10.1094/PDIS.1999.83.5.441.

Hansen JG, Andersson B, Hermansen A, 1995. NEGFRY – A system for scheduling chemical control of late blight in potatoes. Proc Phytophthora 150 Sesquicentennial Sci Conf; Dowley LJ, Bannon E, Cooke LR, Keane T, O'Sullivan E, (eds), Dublin, Ireland, Sept 10-16. pp: 201-208.

Harrison JG, 1992. Effects of the aerial environment on late blight of potato foliage - A review. Plant Pathol 41 (4): 384-416. http://dx.doi.org/10.1111/j.1365-3059.1992.tb02435.x.

Heaney SP, Knight SC, 1994. ICIA 5504: A novel broad-spectrum systemic fungicide for use on fruit, nut and horticultural crops. Proc Brighton Crop Protection Conference – Pests and Diseases, Brighton (UK), Nov 21-24. pp: 509-516.

Holloway PJ, Ellis B, Webb DA, Western NM, Tuck CR, Hayes AL, Miller PCH, 2000. Effects of some agricultural tank-mix adjuvants on the deposition efficiency of aqueous sprays on foliage. Crop Prot 19 (1): 27-37. http://dx.doi.org/10.1016/S0261-2194(99)00079-4.

Holterman HJ, 2003. Kinetics and evaporation of water drops in air. IMAG report 2003-12/ Wageningen UR. IMAG, Wageningen, NL. 67 pp. http://www.holsoft.nl/idefics/pdf/kinevap.pdf.

Holterman HJ, Van De Zande JC, Porskamp HAJ, Huijsmans JFM, 1997. Modelling spray drift from boom sprayers. Comput Electron Agr 19 (1): 1-22. http://dx.doi.org/10.1016/S0168-1699(97)00018-5.

Jensen PK, Nielsen BJ, 2008. Influence of volume rate and nozzle angling on control of potato late blight with flat fan, pre-orifice and air-induction nozzles. In: Effects of climate change on plants: Implications for agriculture; Halford N, Jones HD, Lawlor D (eds). pp: 447-452. Assoc Appl Biol, UK.

Kleinhenze B, Falke K; Kakau J, Rossberg D, 2007. SIMBLIGHT1 – A new model to predict first occurrence of potato late blight. Bull OEPP 37: 339-343. http://dx.doi.org/10.1111/j.1365-2338.2007.01135.x.

Kudsk P, Mathiassen SK, Kirknel E, 1991. Influence of formulations and adjuvants on the rainfastness of maneb and mancozeb on pea and potato. Pestic Sci 33 (1): 57-71. http://dx.doi.org/10.1002/ps.2780330107.

Lefebvre A, 1989. Atomization and sprays. Tayor & Francis, US. 423 pp.

Lehoczki-Krsjak S, Varga M, Mesterházy Á, 2015. Distribution of prothioconazole and tebuconazole between wheat ears and flag leaves following fungicide spraying with different nozzle types at flowering. Pest Manag Sci 71 (1): 105-113. http://dx.doi.org/10.1002/ps.3774.

Lehotay SJ, Son KA, Kwon H, Koesukwiwat U, Fu W, Mastovska K, Hoh E, Leepipatpiboon N, 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. J Chromatogr A 16: 2548-2560. http://dx.doi.org/10.1016/j.chroma.2010.01.044.

Leistra M, Van Den Berg F, 2007. Volatilization of parathion and chlorothalonil from a potato crop simulated by the PEARL model. Environ Sci Technol 41 (7): 2243-2248. http://dx.doi.org/10.1021/es0627242.

Lichiheb N, Personne E, Bedos C, Barriuso E, 2014. Adaptation of a resistive model to pesticide volatilization from plants at the field scale: Comparison with a dataset. Atmos Environ 83: 260-268. http://dx.doi.org/10.1016/j.atmosenv.2013.11.004.

Llorens J, Gil E, Llop J, 2011. Ultrasonic and LIDAR sensors for electronic canopy characterization in vineyards: Advances to improve pesticide application methods. Sensors 11 (2): 2177-2194. http://dx.doi.org/10.3390/s110202177.

Mendiburu F, 2013. Agricolae: Statistical procedures for agricultural research. R Package Version 1.1-4. http://CRAN.R-project.org/package=agricolae.

Menéndez J, Bastida F, 2003. The correlation of the spraying volume with herbicide adherence and herbicide penetration in glyphosate treatments. Comm Agr Appl Biol Sci 69 (4): 815-820.

Mitani S, 2001. RANMAN® (cyazofamid) - A novel fungicide for the control of oomycete plant diseases. Agrochemicals Japan 78: 17-20.

Monadjemi S, El Roz M, Richard C, Ter Halle A, 2011. Photoreduction of chlorothalonil fungicide on plant leaf models. Environ Sci Technol 45 (22): 9582-9589. http://dx.doi.org/10.1021/es202400s.

Nikolov AD, Wasan DT, Koczo K, Policello GA, 1998. Mechanisms for superspreading: role of surface tension gradient and surfactant adsorption. Proc Fifth Int Symp on Adjuvants for Agrochemicals, Vol 1; McMullan PM (ed), Memphis (TN), August 17–21. pp: 125-130.

Olanya OM, Starr GC, Honeycutt CW, Griffin TS, Lambert DH, 2007. Microclimate and potential for late blight development in irrigated potato. Crop Prot 26 (9): 1412-1421. http://dx.doi.org/10.1016/j.cropro.2006.12.002.

Payá P, Anastassiades M, Mack D, Sigalova I, Tasdelen B, Oliva J, Barba A, 2007. Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. Anal Bioanal Chem 389: 1697-1714. http://dx.doi.org/10.1007/s00216-007-1610-7.

Prokop M, Veverka K, 2006. Influence of droplet spectra on the efficiency of contact fungicides and mixtures of contact and systemic fungicides. Plant Prot Sci 42: 26-33.

Qin CF, He MH, Chen FP, Zhu W, Yang LN, Wu EJ, Guo ZL, Shang LP, Zhan J, 2016. Comparative analyses of fungicide sensitivity and SSR marker variations indicate a low risk of developing azoxystrobin resistance in Phytophthora infestans. Scientific reports 6: 20483. http://dx.doi.org/10.1038/srep20483.

Ramsdale BK, Messersmith CG, 2001. Nozzle, spray volume, and adjuvant effects on carfentrazone and imazamox efficacy 1. Weed Technol 15 (3): 485-491. http://dx.doi.org/10.1614/0890-037X(2001)015[0485:NSVAAE]2.0.CO;2.

Ratajkiewicz H, Kierzek R, Karolewski Z, Wachowiak M, 2009. The effect of adjuvants, spray volume and nozzle type on azoxystrobin efficacy against Leptosphaeria maculans and L. biglobosa on winter oilseed rape. J Plant Prot Res 49(4): 440-445. http://dx.doi.org/10.2478/v10045-009-0070-9.

Reynolds KL, Reilly CC, Hotchkiss MW, 1994. Removal of fentin hydroxide from pecan seedlings by simulated rain. Plant Dis 78 (9): 857-860. http://dx.doi.org/10.1094/PD-78-0857.

Rodrigues ET, Moreno A, Mendes T, Palmeira C, Pardal MÂ, 2015. Biochemical and physiological responses of Carcinus maenas to temperature and the fungicide azoxystrobin. Chemosphere 132: 127-134. http://dx.doi.org/10.1016/j.chemosphere.2015.03.011.

Ryckaert B, Spanoghe P, Haesaert G, Heremans B, Isebaert S, Steurbaut W, 2007. Quantitative determination of the influence of adjuvants on foliar fungicide residues. Crop Prot 26(10): 1589-1594. http://dx.doi.org/10.1016/j.cropro.2007.02.011.

Sanjika TM, Jayasuriya HPW, Salokhe VM, 2008. An investigation into the effect of nozzle-canopy interaction on spray deposition: a study to optimize nozzle selection and minimize chemical use in crop production. Environ Sci Technol 3 (2): 28-34.

Schirra M, Palma A, Barberis A, Angioni A, Garau VL, Cabras P, D'Aquino S, 2010. Postinfection activity, residue levels, and persistence of azoxystrobin, fludioxonil, and pyrimethanil applied alone or in combination with heat and imazalil for green mold control on inoculated oranges. J Agr Food Chem 58 (6): 3661-3666. http://dx.doi.org/10.1021/jf904521f.

Schönherr J, Baur P, Buchholz A, 1999. Modelling foliar penetration: its role in optimising pesticide delivery. In: Pesticide chemistry and bioscience, The food-environment challenge; Brooks GT, Roberts TR (eds.), pp: 134-154. The Royal Society of Chemistry, Cambridge. http://dx.doi.org/10.1533/9781845698416.3.134.

Sharma KK, 2000. Influence of meteorological factors on potato late blight development in North-Western plains of India. J Indian Potato Assoc 27: 1-3.

Stevens PJG, Gaskin RE, Hong S, Zabkiewicz JA, 1991. Contributions of stomatal infiltration and cuticular penetration to enhancements of foliar uptake by surfactants. Pestic Sci 33: 371-382. http://dx.doi.org/10.1002/ps.2780330310.

Szpyrka E, Sadło S, 2009. Disappearance of azoxystrobin, cyprodinil, and fludioxonil residues on tomato leaves in a greenhouse. J Plant Prot Res 49 (2): 204-208. http://dx.doi.org/10.2478/v10045-009-0030-4.

Taylor MC, Hardwick NV, Bradshaw NJ, Hall AM, 2003. Relative performance of five forecasting schemes for potato late blight (Phytophthora infestans) I. Accuracy of infection warnings and reduction of unnecessary, theoretical, fungicide applications. Crop Prot 22 (2): 275-283. http://dx.doi.org/10.1016/S0261-2194(02)00148-5.

Taylor P, 2011. The wetting of leaf surfaces. Curr Opin Colloid In 16 (4): 326-334. http://dx.doi.org/10.1016/j.cocis.2010.12.003.

Ter Halle A, Drncova D, Richard C, 2006. Phototransformation of the herbicide sulcotrione on maize cuticular wax. Environ Sci Technol 40 (9): 2989-2995. http://dx.doi.org/10.1021/es052266h.

Thelen KD, Jackson EP, Penner D, 1995. The basis for the hard-water antagonism of glyphosate activity. Weed Sci 43: 541-548.

Töfoli JG, Domingues RJ, de Melo PCT, Ferrari JT, 2014. Effect of simulated rain on the efficiency of fungicides in potato late blight and early blight control. Semina: Ciências Agrárias 35 (6): 2977-2990. http://dx.doi.org/10.5433/1679-0359.2014v35n6p2977.

Trout TJ, Johnson LF, Gartung J, 2008. Remote sensing of canopy cover in horticultural crops. HortScience 43 (2): 333-337.

Ullrich J, Schrödter H, 1966. Das Problem der Vorhersage des Auftretens der Kartoffelkrautfaule (Phytophthora infestans) und die Möglichkeit seiner Lösung durch eine Negativprognose. Nachrichtenblatt Deut Pflanzenschutz 3: 33-40.

Walklate PJ, Cross JV, 2013. Regulated dose adjustment of commercial orchard spraying products. Crop Prot 54: 65-73. http://dx.doi.org/10.1016/j.cropro.2013.07.019.

Walklate PJ, Cross JV, Richardson B, Baker DE, Murray RA, 2003. A generic method of pesticide dose expression: Application to broadcast spraying of apple trees. Ann Appl Biol 143: 11-23. http://dx.doi.org/10.1111/j.1744-7348.2003.tb00264.x.

Warnes GR, Bolker B, Lumley T, Johnson RC, 2015. Contributions from Randall C. Johnson are Copyright SAIC-Frederick, Inc. Funded by the Intramural Research Program, of the NIH, National Cancer Institute and Center for Cancer Research under NCI Contract NO1-CO-12400. Gmodels: Various R Programming Tools for Model Fitting. R package version 2.16.2. https://CRAN.R-project.org/package=gmodels.

Washington JR, 1997. Relationship between the spray droplet density of two protectant fungicides and the germination of Mycosphaerella fijiensis ascospores on banana leaf surfaces. Pestic Sci 50: 233-239.

Weisser PY, Koch H, 2002. Expression of dose rate with respect to orchard sprayer function. Aspect Appl Biol 66: 353-358.

Wise JC, Jenkins PE, Schilder MC, Vandervoort C, Isaacs R, 2010. Sprayer type and water volume influence pesticide deposition and control of insect pests and diseases in juice grapes. Crop Prot 29: 378-385. http://dx.doi.org/10.1016/j.cropro.2009.11.014.

Wong FP, Wilcox WF, 2001. Comparative physical modes of action of azoxystrobin, mancozeb, and metalaxyl against Plasmopara viticola (grapevine downy mildew). Plant Dis 85 (6): 649-656. http://dx.doi.org/10.1094/PDIS.2001.85.6.649.

Zhu H, Dorner JW, Rowland DL, Derksen RC, Ozkan HE, 2004. Spray penetration into peanut canopies with hydraulic nozzle tips. Biosyst Eng 87 (3): 275-283. http://dx.doi.org/10.1016/j.biosystemseng.2003.11.012.