Acta Sci. Pol. Hortorum Cultus, 18(2) 2019, 217–227

www.acta.media.pl ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2019.2.19

ORIGINAL PAPER Accepted: 13.12.2018

THE IMPACT OF TRIFENDER WP ON THE CONTENT OF CHLOROGENIC ACIDS IN POTATO PLANTS INFECTED BY *Phytophthora infestans* (Mont.) de Bary

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ABSTRACT

Five potato cultivars were grown in a micro-plot field experiment (under conditions of natural infection by pathogens). In experimental treatments, potatoes were treated with Trifender WP, whereas control plants were not treated with growth regulators. A greenhouse experiment, conducted simultaneously, involved three treatments: 1. control (no biostimulant treatment, no inoculation), 2. inoculation (potato plants inoculated with *P. infestans*), 3. Trifender WP+inoculation (soil and foliar application of Trifender WP followed by inoculation with the pathogen 2 days after the last treatment). The research material was potato petioles, in which changes in the concentration of analyzed chlorogenic acids were determined using the Waters Acquity UPLC technique. In comparison with the control treatment, higher concentrations of the 5-caffeoylquinic acid (5-CQA), 4-caffeoylquinic acid (4-CQA) and 3-caffeoylquinic acid (3-CQA) were found in potatoes treated with Trifender WP, and in cultivars with blue-purple and red-colored flesh than in those with yellow and cream-colored flesh (field experiment). In the greenhouse experiment, the content of individual chlorogenic acids increased in the petioles of potatoes inoculated with *P. infestans* and inoculated with the pathogen after the application of Trifender WP, compared with the control treatment.

Key words: *Trichoderma asperellum*, *Phytophthora infestans*, potato petioles, chlorogenic acids

INTRODUCTION

Plants growing under natural conditions are exposed to various biotic and abiotic stresses. Plants' ability to grow and develop, reproduce and generate high yields is determined by their adaptation to local environmental conditions. Plants have developed numerous mechanisms to maintain homeostasis and cope with environmental stress factors. Phenolic compounds play an important role in these processes [Wróbel et al. 2005, Weidner et al. 2009, 2011].

Phenolic compounds participate in plant defenses against pathogens such as viruses, fungi and bacteria [Hu and Lee 2001, Jiang and Joyce 2003]. Crop plants attacked by pathogens and pests synthesize phenolic compounds that exert strong cytotoxic effects [Brandt and Molgaard 2001]. Freytag et al. [1994] found that phenolic compounds, in particular chlorogenic acids, contributed to increasing the resistance of potato plants to late blight caused by *Phytophthora infestans* and wilt caused by *Verticillium albo-atrum*. Low chlorogenic acid concentrations in potato tubers stimulated the growth of *P. infestans* and *Fusarium solani* var. *coeruleum*. Barkai-Golan

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[2001] demonstrated that the inoculation of potato tubers with *Fusarium sambucinum* induced the synthesis of phenolic acids. According to the above author, phenolics, including chlorogenic acid and ferulic acid, contributed to inhibiting the growth of *Fusarium oxysporum* and *Sclerotinia sclerotiorum* under *in vitro* conditions. In another study [Cwalina-Ambroziak et al. 2015], the inoculation of potato tubers with *Colletotrichum coccodes* promoted an increase in the concentrations of phenolic acids (including the predominant chlorogenic acid) and stimulated antioxidant activities. It appears that natural phenolics could pose an alternative to pesticides used in agriculture [Kimura-Kuroda et al. 2012, Koureas et al. 2012, Morais et al. 2012, Cassault-Meyer et al. 2014).

The aim of this study was to determine the concentrations of chlorogenic acids in potato plants treated with the Trifender WP biostimulant in a micro-plot field experiment, and to analyze changes in the content of these compounds in potatoes inoculated with *P. infestans* in a greenhouse experiment, with and without prior application of Trifender WP.

MATERIALS AND METHODS

Micro-plot field experiment. The micro-plot experiment was conducted in 2013–2015 at the Agricultural Experiment Station in Tomaszkowo (53°43'02"N; 20°24'22"E) on podzolic soil with the granulometric composition of light loam, characterized by high suitability for the cultivation of rye (suitability complex 4) and quality class IIIb (WRB 2014). The following potato cultivars were grown: 'Irga' and 'Satina' with cream-colored flesh, 'Valfi' and 'Blaue St. Galler' with blue-purple-colored flesh, and 'Highland Burgundy (HB) Red' with red-colored flesh (under conditions of natural infection by pathogens). Before potato planting, Trifender WP was applied to the soil (the biostimulant contains *Trichoderma asperellum* fungal spores at a concentration of 5×10^8 g^{-1} of the product, T1 isolate, NCAIM 68/2006). During the growing season, potato plants were sprayed with Trifender WP four times at 7 to 10-day intervals beginning from stage BBCH 39 (crop cover complete). Control plants were not treated with Trifender WP.

Bulk samples of 20 petioles were collected for biochemical analyses on two dates: in the first ten days of July (7 days after the last treatment) and in the last ten days of July (21 days after the last treatment).

Greenhouse experiment. The greenhouse experiment was conducted in 2014 and 2015 at the University of Warmia and Mazury in Olsztyn, under controlled light and temperature conditions. Three potato tubers (undamaged, with no disease symptoms, 30–35 mm in size) of cvs. 'Satina' and 'Valfi' were placed in 3 dm^3 pots were filled with steamed hortisol mixed with NPK fertilizers (at the rates recommended by the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy). The experiment involved the following treatments: 1. control (no biostimulant treatment, no inoculation, plants sprayed with sterile water), 2. inoculation (plants 15 cm in height inoculated with *P. infestans*) and 3. Trifender WP + inoculation (soil application and 4 foliar applications of Trifender WP + inoculation with *P. infestans* 2 days after the last Trifender WP treatment). Each treatment consisted of 7 pots (7 replications). The inoculum [Zarzycka 1989] was prepared using the MP 1590 isolate (with high levels of virulence and aggressiveness) from the potato pathogen collection of the Institute of Plant Breeding and Acclimatization – National Research Institute, Branch in Młochów. In each treatment, the concentrations of phenolic compounds in petioles were determined 1 day, 8 days and 15 days after the inoculation with *P. infestans.*

Polyphenol analysis. Freeze-dried potato powder (1 g) was mixed with 30 ml of methanol for 30 min at 60°C. Homogenized samples were centrifuged at 10 000 rpm for 15 min at 4°C. Next, the supernatants were evaporated to dryness using a Buchi rotary evaporator. The residues were resuspended in 2 ml of a water: methanol mixture (90 : 10, v/v). The samples were filtered with 0.2 µm filters before UPLC/PDA analysis.

The concentrations of chlorogenic acids were determined using the Waters Acquity UPLC system (Milford, MA, USA) equipped with a photodiode array detector (PDA) and an HSS T3 chromatographic column (2.1 × 100 mm, 1.8 µm particle size). The eluents were 0.1% formic acid in water (line A) and

0.1% formic acid in methanol (line B) in a gradient system with a flow rate of 0.4 ml min^{-1} and 3 µl of injection volume.

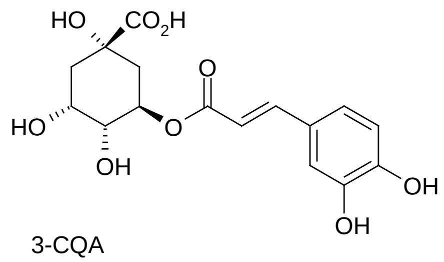
Statistical analysis. The results were processed statistically by analysis of variance (ANOVA) using STATISTICA 13.5 software. The significance of differences between means was evaluated by Duncan's test at p = 0.05. The relationships between the content of chlorogenic acids and the severity of infections caused by the *P. infestans* (infection index, %) during the growing season were determined by linear regression analysis. Coefficients of linear correlation (Pearson's r) were calculated.

RESULTS AND DISCUSSION

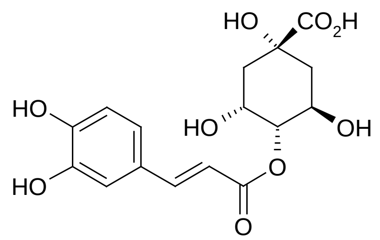
Micro-plot field experiment

Chlorogenic acids, esters of caffeic acid and quinic acid, are the predominant phenolic acids identified in potato tubers [Finotti et al. 2006, Al-Weshahy and Venket Rao 2009, Mohdaly et al. 2010, Külen et al. 2013, Amado et al. 2014]. Chlorogenic acids account for 90% of all phenolic compounds in potato skin [Schieber and Saldaña 2009] where they are present in the form of three major isomers: 5-caffeoylquinic acid (5-CQA), 3-caffeoylquinic acid (3-CQA) and 4-caffeoylquinic acid (4-CQA) [Sánchez Maldonado et al. 2014] (Fig. 1).

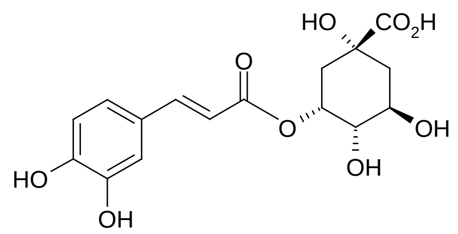
In the present study, biochemical analyses revealed that potato petioles contained 5-CQA, 4-CQA and 3-CQA, with a predominance of 5-CQA. The concentrations of chlorogenic acids in petioles were affected by treatment with the Trifender WP biostimulant and potato cultivar. The content of chlorogenic acids increased significantly in the petioles of potato plants treated with Trifender WP, analyzed 7 and 21 days after the last treatment, compared with untreated potatoes. A significant increase in the concentrations of individual chlorogenic acids (excluding 5-CQA) was noted in the petioles of potatoes treated and not treated with Trifender WP analyzed after 21 days, compared with those analyzed after 7 days. Treatment with the biostimulant led to a significant increase in the concentrations of the analyzed phenolic acids after both 7 and 21 days. The petioles of potato plants treated with Trifender WP were characterized by a significant increase in the concentrations of 5-CQA (except for cv. 'HB Red' after 7 and 21 days) and 3-CQA (except for cv. 'Satina' after 7 and 21 days and 'Valfi' after 21 days) in comparison with untreated potatoes analyzed after 7 and 21 days. The petioles of potato plants treated and not treated with Trifender WP were characterized by a significant increase in the concentrations of 4-CQA and 3 CQA after 21 days, relative to those analyzed after 7 days, (with a few exceptions); an increase in the content of 5-CQA was noted in cv. 'Blaue St. Galler' in both treatments and in cv. 'Irga' in the treatment with Trifender WP (Table 1). In a study by Zarzecka et al. [2017], biostimulants and herbicides contributed to an increase in the phenolic content of



3-CQA



4-CQA



5CQA

Fig. 1. Chemical formulas/structures of selected chlorogenic acids

potato leaves; the content of phenolic compounds was nearly 2-fold higher in potato leaves than in tubers. In our experiment, the concentrations of chlorogenic acids were higher in potato petioles analyzed 21 days after the last treatment with Trifender WP than in those analyzed on day 7. An analysis of cultivars revealed that 'Blaue St. Galler' had the highest content of 5-CQA, 'Valfi' had the highest content of 4-CQA, and 'HB Red' had the highest content of 3-CQA (Tab. 1). Nemś et al. [2015] demonstrated that the concentrations of phenolic compounds were 2-fold higher in potato cultivars with red-colored flesh ('Herbie 26' and 'Rote Emma'), and 3- to 5-fold higher in cultivars with blue-purple-colored flesh ('Blue Cango' and 'Blue Annelise') than in those with yellow- and cream-colored flesh ('Vineta' and 'Fresco'). The antioxidant activity of cultivars with colored flesh was 6- to 7-fold higher than that of cultivars with light-colored flesh. Total phenolic content is higher in potato cultivars with colored flesh due to the presence of anthocyanins which are also responsible for the intense red, purple and blue color of tubers [Ruiz et al. 2018]. In the present study, a positive interaction effect was noted for the tested biostimulant and potato cultivar. In potato petioles analyzed 7 and 21 days after the last treatment with Trifender WP, a significant increase was noted in the content of 5-CQA (excluding cv. 'HB Red' on both analytical dates), 4-CQA (cv.'Irga' on day 7 and cv. 'HB Red' on days 7 and 21) and 3-CQA (excluding cv. 'Satina' on days 7 and 21, and cvs. 'Irga' and 'Valfi' on day 21) (Tab. 2). Singh et al. [2016] observed an increase (by 44.43%) in the total phenolic content of tomato leaves in response to seed treatment with *Trichoderma asperellum* spores, relative to the control treatment. In the cited study, variation in polyphenol synthesis was noted in bioprimed tomato leaves compared with control leaves. The concentration of shikimic acid in treated leaves was approximately 3445.2 μg ml^{-1} vs. 895.1 μg ml^{-1} in control leaves, whereas the content of gallic acid in leaves treated with *T. asperellum* reached 50.16 μg ml^{-1} compared with 33.4 μg ml^{-1} in control leaves.

Table 1. Content of individual chlorogenic acids in potato petioles depending on cultivar (mg 100 g^{-1} DM) (micro-plot field experiment)

Cultivar	Treatment	5-CQA		4-CQA		3-CQA	
		after 7 days	after 21 days	after 7 days	after 21 days	after 7 days	after 21 days
'Irga'	Control	30.4 ±3.47bA	30.7 ±2.0bA	13.4 ±3.1aA	13.4 ±1.6bA	5.68 ±1.01bA	6.09 ±1.07bA
	Trifender WP	35.1 ±4.4aB	36.9 ±6.3aA	14.4 ±4.6aB	16.6 ±4.9aA	6.89 ±1.18aA	6.99 ±0.82aA
'Satina'	Control	28.2 ±3.3bA	28.0 ±4.4bA	10.7 ±3.7bA	11.9 ±3.8aA	6.90 ±0.85aB	8.92 ±0.80aA
	Trifender WP	32.7 ±6.9aA	32.9 ±7.1aA	13.4 ±4.3aA	14.3 ±3.9aA	7.36 ±1.14aA	8.41 ±1.40aA
'Valfi'	Control	49.9 ±8.1bA	51.7 ±9.7bA	29.9 ±7.9aB	33.1 ±9.7bA	9.88 ±1.44bB	13.1 ±3.1aA
	Trifender WP	56.9 ±10.1aA	54.1 ±13.8aA	33.4 ±9.3aB	37.9 ±9.9aA	11.9 ±1.8aB	13.8 ±1.8aA
'Blaue St. Galler'	Control	51.7 ±7.1bB	58.7 ±5.9aA	17.7 ±2.8bB	19.6 ±3.2bA	10.3 ±0.7bB	12.1 ±1.2bA
	Trifender WP	53.9 ±12.5aB	56.5 ±8.6bA	21.7 ±5.5aA	22.7 ±5.2aA	12.1 ±2.5aB	15.2 ±2.1aA
'HB Red'	Control	52.8 ±8.7aA	54.9 ±7.8aA	19.6 ±2.8aB	22.0 ±3.1aA	9.90 ±1.56bB	12.5 ±1.2bA
	Trifender WP	53.4 ±10.9aA	53.8 ±10.5aA	20.4 ±2.3aB	22.2 ±1.4aA	13.8 ±2.8aB	15.7 ±1.0aA

Values in columns for the same cultivar marked with different lowercase letters differ significantly ($p < 0.05$). Values in rows for the same CQA marked with different capital letters differ significantly ($p < 0.05$)

Table 2. Content of individual chlorogenic acids in potato petioles of all cultivars depending on the year of the study (mg 100 g^{-1} DM) (micro-plot field experiment)

Year	Treatment	5-CQA		4-CQA		3-CQA	
		after 7 days	after 21 days	after 7 days	after 21 days	after 7 days	after 21 days
2013	Control	42.7 ±13.0bB	46.0 ±14.9aA	18.8 ±8.1bB	21.3 ±9.9bA	8.55 ±2.54bB	11.5 ±3.5aA
	Trifender WP	49.0 ±13.4aA	47.6 ±12.9aA	21.4 ±8.7aB	23.7 ±10.5aA	9.46 ±2.67aB	12.1 ±3.5aA
2014	Control	42.9 ±16.1bB	44.9 ±15.9aA	20.7 ±10.1bA	22.1 ±11.3bA	7.97 ±2.07bB	9.61 ±2.13bA
	Trifender WP	45.1 ±17.8aA	46.3 ±16.8aA	23.7 ±11.7aB	25.7 ±12.5aA	10.7 ±4.3aB	12.2 ±4.4aA
2015	Control	42.2 ±9.1bB	43.5 ±13.0bA	15.3 ±3.7bA	16.5 ±3.6bA	9.08 ±1.95bB	10.5 ±3.4bA
	Trifender WP	45.0 ±9.5aB	46.6 ±11.0aA	17.0 ±4.5aB	18.8 ±4.5aA	11.1 ±3.0aA	11.8 ±4.1aA

Values in columns for the same year marked with different lowercase letters differ significantly ($p < 0.05$). Values in rows for the same CQA marked with different capital letters differ significantly ($p < 0.05$)

In the current study, the concentrations of individual chlorogenic acids in potato petioles were also affected by the growth regulator in successive growing seasons. Potato plants treated with Trifender WP accumulated significantly greater amounts of 5-CQA (except in the growing seasons of 2013 and 2014, analysis after 21 days), 4-CQA and 3-CQA (except in the growing seasons of 2013, analysis after 21 days) than untreated plants. The highest increase in the content of 5-CQA (by 14.75%) was noted in 2013 in petioles analyzed 7 days after the last treatment. The highest increase in the concentrations of 4-CQA and 3-CQA was observed in 2014 in petioles analyzed on days 21 (by 16.28%) and 7 (by 34.25%), respectively (Tab. 2). Zarzecka et al. [2017] demonstrated that uneven rainfall distribution during the growing season contributed to an increase in the content of polyphenols in potato tubers. The total concentration of polyphenols was lowest in wet and cold seasons. The influence of weather conditions on the levels of polyphenols in potatoes was also reported by Hamouz et al. [2013], and Zarzecka and Gugała [2011].

On both analytical dates, the total concentration of chlorogenic acids was significantly higher in potato plants treated with the biostimulant than in untreated plants (Fig. 2a). Cultivars with blue-, purple- and red-colored flesh were characterized by a significantly higher total content of chlorogenic acids than the remaining cultivars. An analysis of potato cultivars revealed that the total concentration of chlorogenic acids was significantly higher in potato plants analyzed at a later stage of the growing season (Fig. 2b). An analysis of the growing seasons, performed 7 and 21 days after the last treatment, demonstrated that the total content of chlorogenic acids in petioles was significantly higher in the first two years of the study than in the driest year of 2015 (Fig. 2c).

In the current experiment, the content of the analyzed chlorogenic acids increased in response to inoculation with *P. infestans* in both the control treatment and the treatment with Trifender WP; were higher in wet years of 2013 and 2014 (weather conditions were conducive to the infections by pathogen) than in the dry year of 2015 [Głosek--Sobieraj et al. 2018]. The strongest correlation between the analyzed factors was observed in the first year of the study (r = 0.31 in the control treatment and r = 0.35 in the treatment with Trifender WP) (Fig. 3). According to Walters et al. [2007], the presence of chlorogenic acids is associated with potato resistance to common scab caused by *Streptomyces scabies*, and the content of chlorogenic acids increases over 2-fold in carrot roots infected with *Thielaviopsis basicola*.

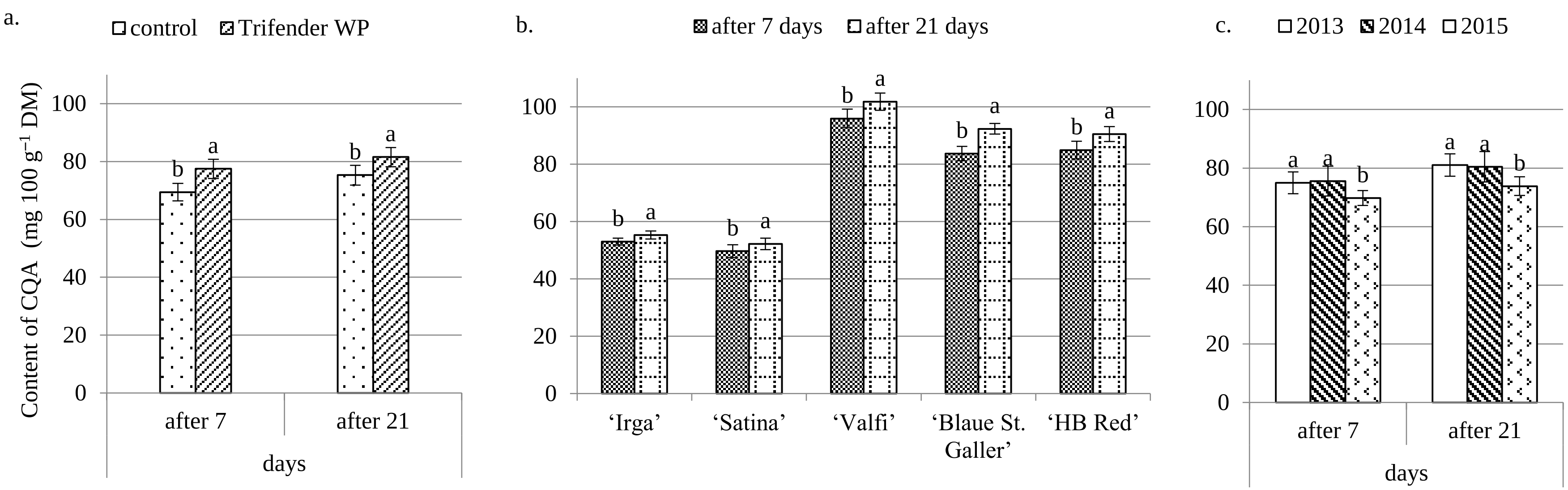


Fig. 2. Content of chlorogenic acids in potato petioles (mg 100 g^{-1} DM); values having different letters differ significantly ($p < 0.05$)

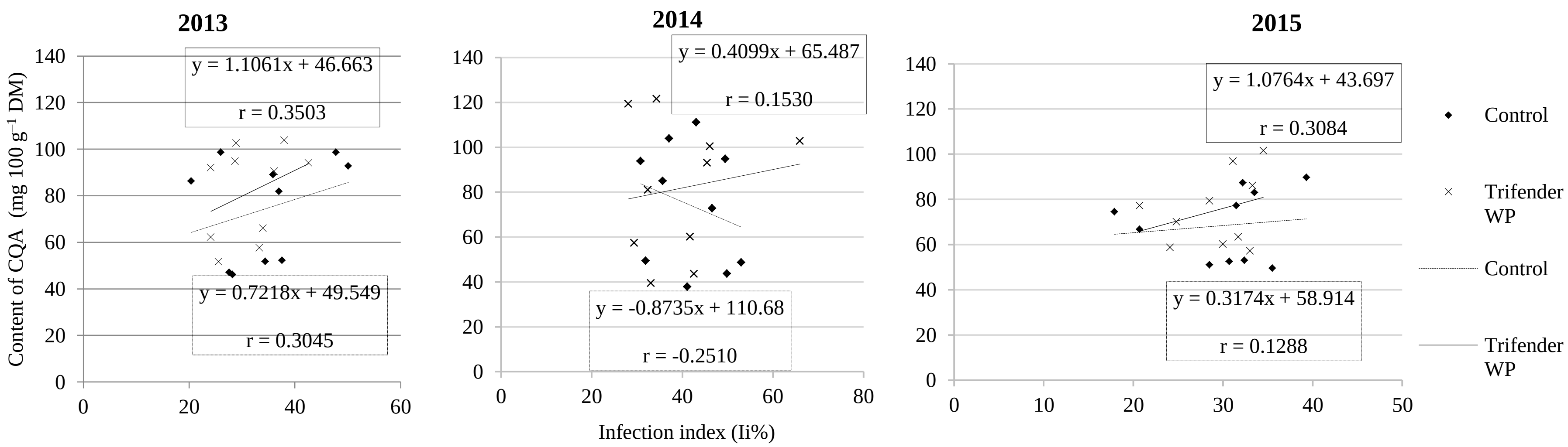


Fig. 3. Correlation between infection of potato plants by *P. infestans* and the content of analyzed chlorogenic acids in potato petioles

Greenhouse experiment

Potato plants of cv. 'Valfi' accumulated greater amounts of the analyzed chlorogenic acids compared with cv. 'Satina', and the predominant isomer was 5-CQA. A significant increase was noted in the content of individual chlorogenic acids in the petioles of cv. 'Satina' (except for 4-CQA and 3-CQA after 15 days) and cv. 'Valfi' (except for 4-CQA after 8 and 15 days) on both analytical dates in the inoculation (2) and Trifender WP + inoculation (3) treatments compared with the control treatment (1). In the petioles of cvs. 'Satina' and 'Valfi' in the inoculation (2) and Trifender WP + inoculation (3) treatments, no significant changes were found in the content of individual chlorogenic acids 8 and 15 days after inoculation except for 4-CQA after 8 days and 3-CQA after 15 days in the petioles of cv. 'Valfi'. The total concentration of chlorogenic acids in the petioles of both cultivars was significantly lower in the control treatment (1) than in the remaining treatments. The highest total content of chlorogenic acids was noted in the petioles of cv. 'Satina' 8 days after inoculation in the inoculation treatment (2) and in the petioles of cv. 'Valfi' 15 days after inoculation in the inoculation (2) and Trifender WP + inoculation (3) treatments, but the observed differences were not significant relative to the remaining analytical dates (Table 3). In the following treatments: control (1), inoculation (2) and Trifender WP + inoculation (3), the concentrations of chlorogenic acids in potato petioles increased 8 and 15 days after inoculation in comparison with the analysis performed on day 1 (except for 5-CQA content in the Trifender WP + inoculation treatment (3) and 3-CQA content in the inoculation treatment (2) in the petioles of cv. 'Satina', and 4-CQA content in the inoculation (2) treatment in the petioles of cv. 'Valfi'). However, significant differences relative to analytical date 1 were found only for the concentrations of 4-CQA in the control treatment (1) and Trifender WP + inoculation (3) treatment (only 15 days after inoculation), and 3-CQA in the control treatment (1) and the inoculation treatment (2) (only 15 days after inoculation) in the petioles of cv. 'Valfi'. On all analytical dates, the highest total concentration of chlorogenic acids in the petioles of cv. 'Satina' was noted in the Trifender WP + inoculation (3) treatment, and in the petioles of cv. 'Valfi' in the inoculation treatment (2).

Table 3. Content of phenolic compound in potato petioles (mg 100 g^{-1} DM) (greenhouse experiment)

Phenolic acid	Treatment	'Satina'			'Valfi'		
		after day	after 8 days	after 15 days	after day	after 8 days	after 15 days
5-CQA	1. Control	20.8 ±1.4bA	23.0 ±4.7bA	25.9 ±2.4bA	67.7 ±3.6bB	75.8 ±3.8bA	75.0 ±4.3bA
	2. Inoculation	32.9 ±5.1aA	35.2 ±5.7aA	34.2 ±4.8aA	86.3 ±7.4aA	92.6 ±6.8aA	92.0 ±8.3aA
	3. Trifender + inoculation	38.1 ±7.0aA	38.0 ±7.5aA	37.4 ±6.0aA	88.8 ±8.3aA	93.1 ±4.2aA	93.7 ±7.8aA
4-CQA	1. Control	6.93 ±1.47bB	10.1 ±2.3bAB	13.2 ±2.4aA	35.6 ±4.2cB	43.9 ±1.6aA	48.9 ±5.8aA
	2. Inoculation	12.1 ±2.6aA	15.7 ±3.0aA	15.1 ±2.1aA	57.7 ±3.9aA	50.5 ±2.2aB	53.5 ±2.8aAB
	3. Trifender + inoculation	11.11 ±1.0aA	14.0 ±3.1abA	13.8±2.0aA	44.2 ±6.8bA	46.3 ±6.5aA	54.5 ±17.5aA
3-CQA	1. Control	4.28 ±0.81bB	4.84 ±0.45bB	7.61 ±1.62aA	11.3 ±0.6bC	15.5 ±1.7bB	19.7 ±1.5bA
	2. Inoculation	7.50 ±0.68aA	7.04 ±1.23aA	7.41 ±0.57aA	28.7 ±4.7aB	31.0 ±1.5aAB	37.5 ±5.9aA
	3. Trifender + inoculation	8.24 ±0.41aA	8.29 ±1.52aA	8.59 ±0.34aA	27.2 ±9.8aA	27.7 ±5.0aA	32.5 ±2.4aA
Sum	1. Control	32.0 ±3.4bB	37.9 ±6.7bB	46.7 ±5.0bA	114.6 ±4.1bC	135.2 ±5.7bB	143.6 ±3.3bA
	2. Inoculation	52.5 ±2.9aB	58.0 ±2.2aA	56.7 ±3.8aAB	172.7 ±9.1aA	174.2 ±6.6aA	183.0a ±8.4aA
	3. Trifender + inoculation	57.4 ±7.7aA	60.2 ±8.5aA	59.8 ±7.3aA	160.2 ±24.6aA	167.2 ±7.9aA	180.8 ±13.2aA

Values in columns for the same CQA marked with different lowercase letters differ significantly ($p < 0.05$). Values in rows for the same cultivar marked with different capital letters differ significantly ($p < 0.05$)

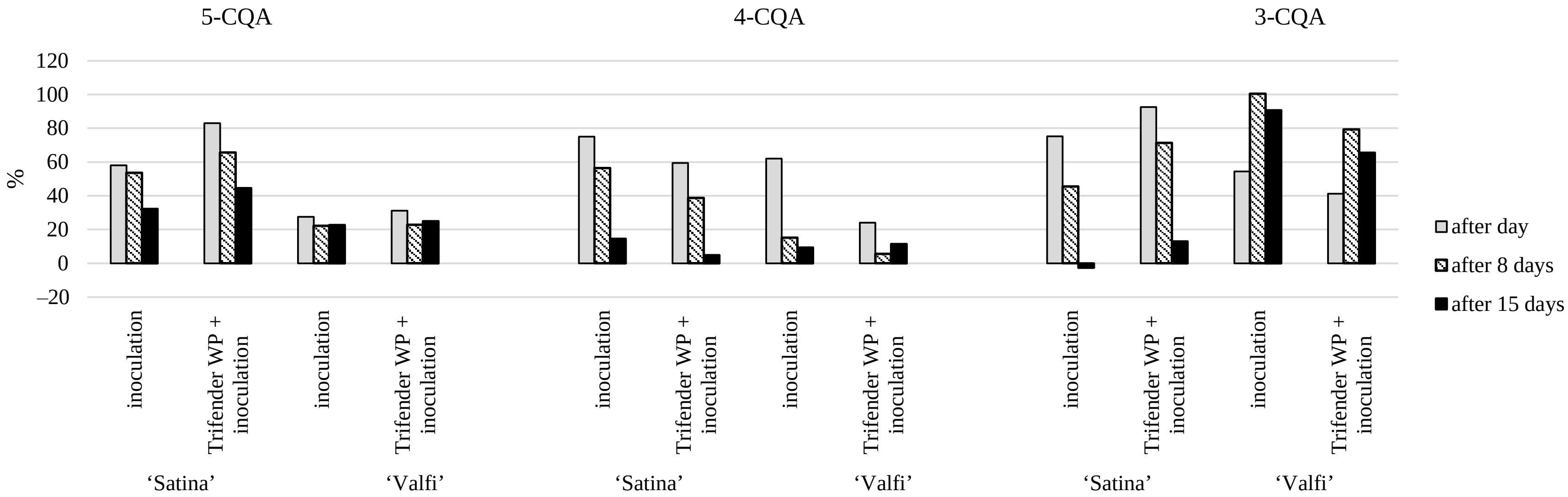


Fig. 4. Increase of chlorogenic acid content in potato petioles (%)

On all analytical dates, the petioles of the analyzed potato cultivars had a higher content of individual chlorogenic acids in the inoculation (2) and Trifender WP + inoculation (3) treatments than in the control treatment (1) (excluding 3-CQA, cv. 'Satina', inoculation treatment (2), analytical date 3). A significant increase in 5-CQA concentration in potato petioles was observed in all cultivars on all analytical dates; a significant increase in 3-CQA concentration was noted in cv. 'Valfi' on all analytical dates; a significant increase in 4-CQA concentration was found in cv. 'Valfi' one day after inoculation.

The highest increase in the content of individual chlorogenic acids in the petioles of the analyzed potato cultivars was observed in the inoculation (2) and Trifender WP + inoculation (3) treatments, relative to the control treatment (1), one day after inoculation (except for 3-CQA concentration in the petioles of cv. 'Valfi') (Fig. 4). On all analytical dates, the inoculated petioles of potato plants cv. 'Satina' treated with Trifender WP accumulated greater amounts of 5-CQA than the petioles in the inoculation treatment (2). Andreu et al. [2001] reported an increase in the total phenolic content of potato leaves after infection with *P. infestans.* Mittelstraß et al. [2006] found that *P. infestans* infection had no significant effect on the concentrations of chlorogenic acids, including flavonols and rutin, in potato plants. However, the content of chlorogenic acid and neochlorogenic acid was considerably higher in potato leaves infected with *Alternaria solani* than in control (non-infected) leaves. According to Bengtsson et al. [2014], foliar treatment with β-aminobutyric acid (BABA) contributes to a significant increase in the concentrations of three derivatives of chlorogenic acid (CQA1, CQA2 and CQA3) in the resistant potato cv. 'Ovatio' in comparison with the susceptible cv. 'Bintje'. Similar observations were made by Mittelstraß et al. [2006] who found a correlation between low levels of CQA and higher susceptibility to *P. infestans* in potato plants, thus confirming that CQA is involved in the defense response to pathogen infection. Koc and Üstün [2012] reported the highest content of phenolic compounds in the leaves of the resistant pepper cultivar PM-702 on day 6 following inoculation with *Phytophthora capsici* (10^4 zoospores ml^{-1}). The noted increase reached 42% compared with the non-inoculated control plants. According to Atanasova-Penichoen et al. [2012], the biosynthesis of chlorogenic acid and, to a lesser degree, ferulic acid in maize kernels inoculated with *Fusarium graminearum* may suggest the potential involvement of these compounds in maize ear rot resistance.

CONCLUSIONS

The results of the micro-plot field experiment show that the concentrations of chlorogenic acids were higher in potato cultivars with blue-purple- and red-colored flesh than in those with yellow- and cream-colored flesh; in potato plants treated with Trifender WP than in untreated plants; in wet years of 2013 and 2014 than in the dry year of 2015; on the later analytical date after biostimulant application.

The results of the greenhouse experiment indicate that the content of individual chlorogenic acids increased in the petioles of potatoes inoculated with *P. infestans* and inoculated with the pathogen after the application of Trifender WP, compared with the control treatment. The treatment of potato plants cv. 'Satina' with Trifender WP stimulated the accumulation of 5-CQA (predominant chlorogenic acid) in inoculated petioles.

The observed increase in the concentrations of chlorogenic acids in potato plants in response to infection with *P. infestans* and treatment with Trifender WP could provide further evidence to support the hypothesis that phenolic acids are involved in the defense mechanisms of potato plants exposed to biotic stress.

ACKNOWLEDGEMENTS

This research was financially supported by the Polish Ministry of Science and Higher Education under statutory funds (20.610.300) of the Department of Entomology, Phytopathology and Molecular Diagnosis, University of Warmia and Mazury in Olsztyn, Poland.

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