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Impact of Stolbur Phytoplasmas on Potato Tuber Texture and Sugar Content of Selected Potato Cultivars

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Abstract Potato stolbur is a phytoplasmal disease that seriously affects yield and tuber quality in South Eastern Europe, Russia and the Mediterranean areas. In 2007 and 2008, field experiments were carried out to determine stolbur resistance of processing potato cultivars at Sannicolau Mare (Romania) by determining consistency and concentration of reducing sugars (fructose and glucose), sucrose and phytoplasmas in potato tubers. In both years, non-symptomatic potato tubers showed sucrose levels in the range of 3,000 mg kg^{-1} fresh weight (FW). In contrast, sucrose concentrations were up to 11,820 mg kg^{-1} FW in 2007 and 19,560 mg kg^{-1} FW in 2008 in tubers showing severe symptoms. These high values severely affect suitability of tubers for processing as sucrose serves as substrate for the formation of reducing sugars that are the limiting factor in fried potato production for Maillard-related discolouration. The cultivars examined differed considerably in susceptibility to stolbur disease. Whereas cvs. 'Courage' and 'Lady Rosetta' showed high numbers of diseased tubers and high sucrose concentrations, 'Lady Claire' had a lower incidence of symptomatic tubers and lower sucrose concentrations. However, fully resistant cultivars were not observed. Across all cultivars examined, phytoplasmal concentration was significantly higher in symptomatic tubers than in non-symptomatic ones.

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Introduction

Potato stolbur is a serious disease in South Eastern Europe, Russia and the Mediterranean areas (Eroglu et al. 2010). Since 2006, it has also been observed in Germany (EPPO 2006). Depending on time of infection and environmental conditions, stolbur phytoplasmas cause considerable yield losses and reduce tuber quality (Fargašová and Bojňanský 1992; Ember et al. 2011). Phytoplasmal infection severely impairs assimilate translocation, might be responsible for subtle changes in the bioenergetics of the phloem and influences sugar metabolism (Lepka et al. 1999). Studies of Albertazzi et al. (2009) and Hren et al. (2009a) demonstrated that phytoplasmas affect carbohydrate production in infected grapevines. Similarly, carbohydrate metabolism in potatoes may also be affected which is the likely cause of the quality losses of processed potato products like crisps and French fries. In recent years, phytoplasma-induced discolouration of fried potatoes has been discussed more intensively. The most common phytoplasmas responsible for this phenomenon belong to the three phytoplasma groups: stolbur (16SrXII), aster yellows (16SrI-A/B) and clover proliferation (16SrVI) (Secor 2007). Phytoplasmas of groups 16SrII, 16SrIII and 16SrXVIII and closely related strains thereof have also been associated with potato phytoplasmoses (Lee et al. 2009). Other phytoplasmas have been identified, namely new species associated with discolouration of potato tubers (Lee et al. 2006; Munyaneza et al. 2009). Furthermore, a newly described disease of potatoes, Zebra chip (Secor et al. 2009), also induces crisp discolouration (Gao et al. 2009).

The dark brown discolouration that occurs during frying is a non-enzymatic reaction (Maillard reaction; Haase 2007) caused by an interaction between free aldehyde groups of reducing hexoses like glucose and fructose and free α-amino groups of amino acids and tuber proteins during high-temperature processing. Therefore, the level of reducing sugars is a critical parameter for crisps production (Hoover and Xander 1962; Kyriacou et al. 2008) as crisps may become dark at high concentrations of reducing sugars (Marquez and Anon 1986) during the frying process. A maximum tolerable value of reducing sugars depends on the processing technique, but it should always be lower than 1,500 mg kg^{-1} FW (fresh weight) for crisp production (Putz 1989; Blenkinsop et al. 2002), while an upper limit of 1,000 mg kg^{-1} FW was described for best product quality (Dale and Mackay 1994; Biedermann-Brem et al. 2003). In contrast to the reducing sugars, sucrose, a disaccharide of glucose and fructose, was described as a poor indicator of crisp quality (Roe and Faulks 1991; Rodriguez-Saona and Wrolstad 1997). However, sucrose was found to be (partially) involved in crisp discolouration (Clegg and Chapman 1962) and should thus be involved in the study.

The objective of this study was to determine the impact of stolbur phytoplasmas on sugar content in potato tubers of selected cultivars and to evaluate this criterion for ranking resistance to phytoplasma infection. Our work focused on the two reducing sugars (glucose and fructose) and sucrose. Disease incidence was determined by the severity of plant disease symptoms and the texture of potato tubers. Phytoplasmal infection frequency and intensity were analysed by PCR.

Material and Methods

Field Cultivar Screening Trials

The aim of the trials was to determine the response of selected potato cultivars to stolbur infection by visual inspection (shortly before flowering, at the beginning of flowering and at the end of flowering) and by tuber texture. In 2007 and 2008, field experiments were conducted at Sannicolau Mare near Timisoara (Banat) in Romania. In 2007, the potato cvs. 'Agria', 'Bintje', 'Hermes', 'Lady Claire', 'Pirol' and 'Saturna' were examined. In 2008, cvs. 'Albata', 'Atlantic', 'Courage' and 'Salome' were included in the experiment. 'Lady Claire' served as the standard cultivar to which the other cultivars were compared to provide consistency over a number of years. On April 24, 2007, for each cultivar, 100 tubers were planted in 26-m^2 plots in a randomised complete block design with four replications. Twelve weeks after planting, all plants were visually inspected for symptoms of potato stolbur such as stunting, rolling or yellowing of upper leaflets, proliferation of axillary buds, formation of aerial tubers or swollen stems, cortical necrosis on lower stems, and sloughing of tissue and premature death (Harding and Teakle 1985; EPPO/CABI 1996). Three leaves of all symptomatic potato plants per replication from each cultivar were collected, stored in a cool box and subjected to PCR diagnosis using the primers fStol/rStol (Maixner et al. 1995) to confirm stolbur phytoplasmal infections. Ten days later, 1 week before harvest, a second visual observation took place. At the beginning of August, all tubers of ten randomly selected plants from each replication were harvested. These tubers were visually examined for rubbery consistency, a symptom of the stolbur disease, and divided into three groups: non-symptomatic tubers, tubers with mild symptoms (no visible symptoms but rubbery texture), and tubers with severe symptoms (clearly visible rubbery, shrivelled texture). In most cases, tubers with clearly visible rubbery texture showed browning around the vascular ring area when cut into slices. Three tubers with severe symptoms from each cultivar were subjected to PCR diagnosis.

The 2008 large-scale experiment was conducted in plots of 5–10 ha in size. On April 25, potatoes were planted in adjoining plots. In the middle of August, four rows per cultivar of each plot were randomly chosen for evaluation per cultivar and all tubers of ten plants from each of these four rows were harvested and divided into three groups according to symptom severity as described above.

DNA Extraction and Conventional PCR

DNA extraction from leaves and tubers was performed using the DNeasy Plant Mini kit (Qiagen). To detect stolbur phytoplasma, the main midribs were cut from three randomly selected leaves of all symptomatic potato plants per replication from each cultivar as well as slices from the vascular ring from three also randomly selected tubers with severe symptoms from each cultivar. About 100 mg of leaf and potato tuber tissue were ground in liquid nitrogen with a sterilized mortar and pestle. DNA extraction was carried out following the instructions of the kit's manufacturer. For PCR, the DNA was diluted 1:50.

PCR diagnosis was carried out utilizing the thermal cyclers MJ Research PTC-100 and peqSTAR 96 Universal Gradient (PeqLab). All PCR assays were run as per Maixner et al. (1995) using primers fStol/rStol. Reaction mixture (20 μl) was modified as

follows: 2.5 μl of extracted DNA was added to the PCR mixture, which contained 1.875 mM $MgCl_2$; 0.125 mM each dNTP (Fermentas); 0.5 μM each primer and 0.7 U Taq DNA Polymerase (Qiagen). The PCR product was analysed by electrophoresis of 10 μl of each reaction mixture in a 1.5% horizontal agarose gel in TBE buffer.

DNA Extraction and Quantitative Real-Time PCR

DNA extraction and quantitative real-time PCR (Q-PCR) were carried out on the cultivars included in the 2007 field experiments. All harvested tubers were divided into tuber samples without and with severe symptoms per cultivar and replication and cut into cubes of 10×10×20 mm with a vegetable cutter (model TR 21, Pefra, Germany). An aliquot of approximately 500 g was lyophilised in a freeze dryer (model alpha 1–4, Christ, Germany), and after drying, ground for further analysis with a hammer-type cyclone mill (Laboratory mill 3100, Falling Number AB, Sweden). Phytoplasma-DNA was extracted from 20 mg of freeze-dried potato tuber material using the DNeasy Plant Mini kit (Qiagen).

Q-PCR was carried out using Bio-Rad iCycler IQ. The template was amplified employing the phytoplasma-specific, rDNA-based primer/Taqman probe system designed by Christensen et al. (2004). PCR mixture contained the following in a final volume of 50 μl: 2.5 U of hot-start Taq polymerase, 1× polymerase buffer with 3 mM $MgCl_2$ (both Amplicon), 0.2 mM dNTPs, 0.4 μM each primer, 0.2 μM Taqman probe and 1 μl of DNA extract. The parameters used for amplification were 15 min at 95 °C followed by a two-step protocol consisting of 40 cycles at 95 °C for 30 s and 60 °C for 1 min. The samples were run in duplicates.

To generate a standard curve for phytoplasma quantification, a PCR-amplified 16S rDNA fragment of *Candidatus* phytoplasma mali containing the target sequence was cloned into the pGEM-T EASY vector system (Promega) and multiplied in *Escherichia coli* XL1 Blue cells (Stratagene). Recombinant plasmids were extracted using the Qiagen miniprep kit. Based on the size of the recombinant plasmid, a ten-fold dilution series ranging from 10^7 to 10^1 copies per PCR was prepared (Bisognin et al. 2008). From the Ct values of the standards and the templates, the phytoplasma concentration in the plant tissue was estimated, considering the dilution of the templates and the fact that phytoplasmas have two rRNA operons.

Analysis of Carbohydrates, Potato Crisp Colour and Sugar Distribution

Sugar analysis was conducted on the cultivars from both the 2007 and 2008 experiments. For analysis, samples were freeze-dried and ground as described above. In addition to the tuber samples with and without severe symptoms, potato tubers with minor symptoms were also analysed. Tuber concentration of reducing sugars (D-glucose, D-fructose) and sucrose was determined using an enzymatic test (Bergmeyer et al. 1974; Boehringer 1995). D-Glucose concentration in the lyophilised samples was determined before and after enzymatic sucrose hydrolysis (β-fructosidase) by phosphorylation of D-glucose with hexokinase in the presence of adenosine-5′-triphosphate (ATP) to G6P and oxidation of G6P by G6P-dehydrogenase in presence of nicotinamide-adenine dinucleotide phosphate ($NADP^+$) with the stoichiometric formation of reduced NADPH and measurement at 365 nm against a blank with a U

1100 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan). Sucrose content was calculated from the difference between the D-glucose concentrations before and after enzymatic conversion. D-fructose was phosphorylated to F6P, converted by phosphoglucose isomerase to G6P and further treated as described above.

Dry matter concentration of the samples was calculated by weight loss during freeze-drying. The remaining moisture content of the lyophilised and ground samples was measured as weight loss at 105 °C in an oven dryer (AACC 1993).

For sugar distribution in tubers and crisp colour determination, potatoes of the cv. 'Lady Rosetta' (2007 season) were analysed. The tubers came from a ware potato production field. All the tubers of ten plants from each of four randomly chosen rows were harvested in July and divided into three symptom classes as described above and split into two subsamples: one for analysing the sugar distribution in potato tubers, and one for crisp colour determination.

Sugar Distribution in Tubers

In addition to whole tuber analysis, mildly and severely diseased tubers were divided into three distinct parts: (1) bud ends, (2) core and (3) stem ends, using a cork borer. These parts were freeze-dried, ground and treated for sugar analyses as described above.

Crisp Colour Determination

Ten tubers in each disease symptom class (non-symptomatic, mild and severe symptoms) were randomly selected and one slice was taken from the centre of each tuber. After frying in peanut oil at 175 °C for 3 min (table top fryer; model TFeco 4, frifri, Germany), slices were rated using a four-group colour scale for quality evaluation of potato crisps ranging from above 7 (best – white slice) to below 4 (worst – brown slice). Results were reported like 3–0–5–2, that means, 3 crisps with grade ≥7; 0 crisps with grade 6, 5 crisps with grade 5 and 2 crisps with grade ≤4 according to the Swiss Frying Test 86 (Mini et al. 2004).

Statistical Analysis

Presence of Stolbur Phytoplasma in Potato Tubers

Number of phytoplasma cells per milligramme FW of potato tuber were calculated for each potato cultivar tested in 2007. Due to the high variance of data points obtained by real-time PCR, statistical analysis was done by the PROC MIXED method (SAS® 9.2) using least squares means. Means of phytoplasmal concentrations were compared between non-symptomatic tubers and tubers with severe symptoms for all tested cultivars and also for each cultivar. The level of significance was set at $P=0.05$.

Sugar Concentration of Potato Tubers

Concentrations of sugars and dry matter were calculated for each potato cultivar tested in 2007 and 2008. Statistical analysis was carried out employing SAS® 9.1 using Student–Newman–Keuls test. The level of significance was set at $P=0.05$.

Results

Susceptibility of Potato Cultivars to Stolbur Disease

By visual inspection 12 weeks after planting, disease incidence ranged from 27% to 55% for the different cultivars tested in 2007. 'Agria' had the lowest disease rate whereas 'Lady Claire', 'Saturna' and 'Bintje' showed the highest level of incidence (Fig. 1). In the PCR examination, only 52% of symptomatic leaves tested positive. The most probable reason for that seems to be that phytoplasmas are often unevenly distributed within infected plants (Berges et al. 2000) and can therefore be absent in the sampled tissue or present in a very low number, while the plant as a whole will still show symptoms and will behave as if infected. Apart from that, we do not rule out that senescence at an early plant stage because of the abnormal climatic conditions and some other pathogens were also able to cause stolbur like symptoms.

Almost all plants showed stolbur symptoms when they were inspected 10 days later (1 week before harvest). To determine the response of potato cultivars to stolbur infections, the texture of the tubers was evaluated by assigning tubers to three tuber texture classes (no, mild and severe symptoms). Three tubers with severe symptoms from each cultivar were subjected to PCR analysis. All these potato tuber samples tested positive. In contrast to the visual observation 12 weeks after planting, disease values determined by tuber inspection were high for cvs. 'Agria', 'Hermes' and 'Pirol' and low for 'Lady Claire' and 'Bintje' (Fig. 1).

In 2008, disease incidence (determined on the basis of tuber texture) was lower than in 2007 (Fig. 2). 'Lady Claire' had only 2% tubers with severe and 10% tubers with mild symptoms. In contrast, the same cultivar showed 16% tubers with severe and 21% tubers with mild symptoms in 2007. 'Albata' had moderate disease incidence, whereas for 'Courage', a high disease incidence was observed.

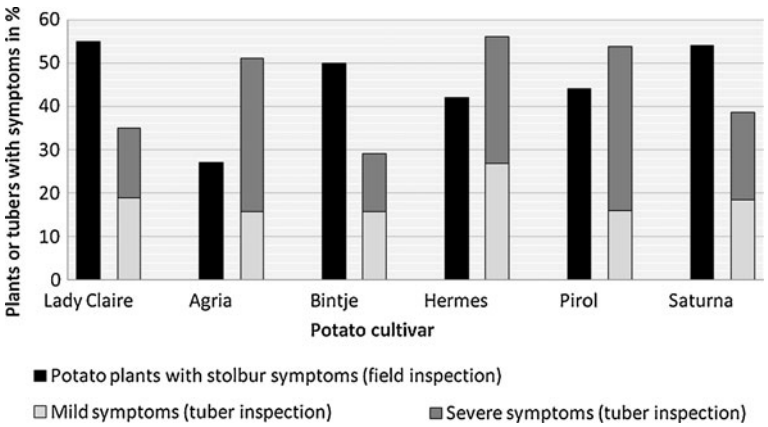

Fig. 1 Incidence of foliar stolbur symptoms and percentage of tubers with mild and severe symptoms regarding tuber texture for the different potato cultivars tested in field experiment 2007

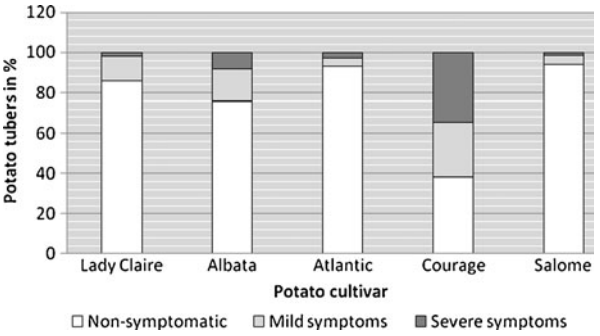

Fig. 2 Percent of tubers with stolbur symptoms of different potato cultivars tested in field experiment 2008

Quantification of Phytoplasmal Infection

Q-PCR assay of phytoplasmal 16S DNA was used to determine the presence and concentration of stolbur phytoplasmas in non-symptomatic potato tubers and tubers with severe symptoms by examining tubers of the 2007 trial and comparing them between all analysed potato cultivars (Fig. 3). Using Q-PCR, phytoplasmas were not detected in non-symptomatic potato tubers of cv. 'Lady Claire' (Table 1). All non-symptomatic tubers of the further analysed cultivars were positive for phytoplasmas by Q-PCR. Concentration of stolbur phytoplasmas varied from 14 cells per milligramme FW (cv. 'Hermes') to 873 cells per milligramme FW (cv. 'Agria'). Concentrations of phytoplasmas in tubers with severe symptoms ranging between 890 cells per milligramme FW (cv. 'Hermes') and 8,470 cells per milligramme FW (cv. 'Saturna') were considerably higher compared to non-symptomatic ones. The only exception was cv. 'Pirol' with a higher phytoplasma count in non-symptomatic tubers than in tubers with severe symptoms. Although phytoplasma numbers were mostly higher in severely affected tubers than in non-symptomatic tubers, the differences were, with the exception of 'Lady Claire', not statistically significant in individual cultivars. The reason for that seems to be the small number of data points and their high variance per individual cultivar. However, for all cultivars, significant differences in phytoplasma concentration could be established between non-symptomatic potato tubers and tubers with severe symptoms using the simulate method (SAS® 9.2) at P=0.05.

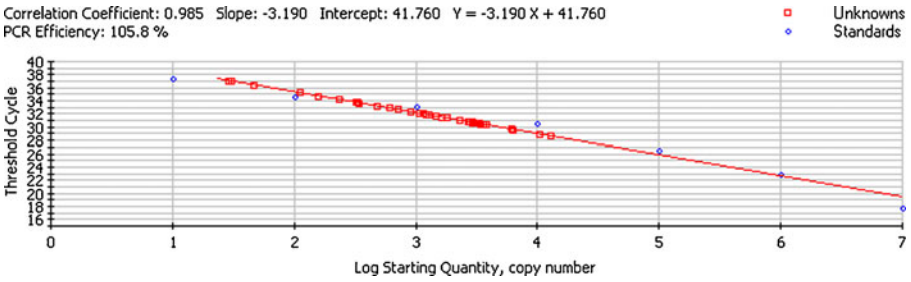

Fig. 3 Standard curve generated by the cycler software. The threshold numbers of PCR cycles (C_T value; means of duplicates) of sample DNA (*unknowns*) are plotted against a ten-fold dilution series (10^1–10^7 copies) of a recombinant plasmid containing the target (*standards*)

Table 1 Determination of phytoplasma concentration in non-symptomatic tubers and tubers with severe symptoms of the cultivars tested in the field experiment of 2007 using quantitative real-time PCR (cells/mg fresh weight of potato tuber; n=4)

Cultivar	C_T means of positive values (range)	Tubers with severe symptoms normalized means quantity (cells/mg fresh weight)	Ls Means[a]	C_T means of positive values (range)	Non-symptomatic tubers normalized means quantity (cells/mg fresh weight)	Ls Means[a]	p[b]
Agria	31.1 (30.6–31.9)	2,425	7.7262	33.6 (30.7–35.2)	873	5.8791	0.9524
Bintje	30.8 (30.2–31.6)	2,525	7.7784	32.7 (31.4–34.0)	620	6.2092	0.9851
Hermes	33.5 (33.4–33.5)	890	6.8818	37.1 (n.d.–37.1)	14	0.6305	0.0818
Lady Claire	31.7 (n.d.–32.1)	1,075	4.8630	n.d.	n.d.[c]	−2.3026	0.0006*
Pirol	32.8 (31.4–33.7)	870	6.5219	31.9 (30.8–32.7)	1,440	7.1120	1.0000
Saturna	29.3 (28.7–29.7)	8,470	9.0015	34.3 (31.8–37.0)	520	5.3731	0.4483
All cultivars		2,709	7.0737		578	3.8764	0.0031*

[a] Least squares means

[b] Probability for exceeding

[c] no detectable effect

*P=0.05 significantly different when tested with the Simulate Method

Sugar Concentration

Concentrations of fructose were on a low level and glucose remained mostly stable in all tuber classes (field experiment 2007; Table 2). The sucrose level increased threefold from healthy to severely infected tubers. 'Bintje' had the highest concentration, followed by 'Hermes' and 'Saturna' (Fig. 4). In contrast, 'Lady Claire', 'Agria' and 'Pirol' had levels below 10,000 mg kg^{-1} FW. 'Lady Claire' had the lowest sucrose concentration in stolbur-infected tubers (6,420 mg kg^{-1} FW).

Reducing sugar concentration of the 2008 samples without stolbur symptoms had a higher variability among single data points compared with 2007 samples (data not shown), probably caused by weather and crop management. However, there was no significant influence of phytoplasmal infection on reducing sugars. Sucrose levels were much higher in tubers with severe symptoms than in non-symptomatic tubers in 2008. Sucrose concentration in non-symptomatic tubers of all analysed cultivars was almost the same, but severe tuber symptoms resulted in different increases. 'Atlantic' and 'Courage' had the highest sucrose concentration (19,500 and 15,580 mg kg^{-1} FW, respectively) and 'Lady Claire' the lowest one (9,800 mg kg^{-1} FW; Fig. 5).

Crisp Colour

A frying experiment with tubers of cv. 'Lady Rosetta' revealed quality shifts between different symptom classes. Tubers from plants without visual stolbur symptoms had moderate processing quality with some dark-coloured slices (3–3–2–2). Samples with mild tuber symptoms tended to give more darkly coloured slices, while severely diseased tubers showed the poorest results (Table 3).

Sugar Distribution within Tubers

The sucrose concentration in whole tubers of cv. 'Lady Rosetta' with mild and severe symptoms was 11,011 and 25,500 mg kg^{-1} FW, respectively. Within both disease classes, sucrose distribution was nearly uniform between the different tuber parts.

Table 2 Average dry matter content and sugar concentration in potato tubers of the cultivars, infected with stolbur phytoplasma tested in field experiment 2007. The tubers were grouped into the symptom categories non-symptomatic, mildly and severely affected. (mean±std. dev.; mg kg^{-1} Fresh weight (FW); n=4)[a]

Tuber class[b]	Dry matter (%)	Glucose	Fructose	Sucrose	Reducing sugars	Total sugars
Non-symptomatic	23.9±2.29 b	341±354 a	70±107 a	3,080±558 b	408±453 a	3,490±693 b
Mild symptoms	23.8±2.28 b	453±495 a	82±108 a	4,350±1,830 b	532±596 a	4,880±2,030 b
Severe symptoms	26.2±2.46 a	473±459 a	81±90 a	9,750±3,180 a	554±536 a	10,300±3,400 a

[a] Values within a single column followed by the same letter were not significantly different when tested with the Student–Newman–Keuls test at P=0.05

[b] Tubers of ten plants were harvested and rated for disease severity in three classes

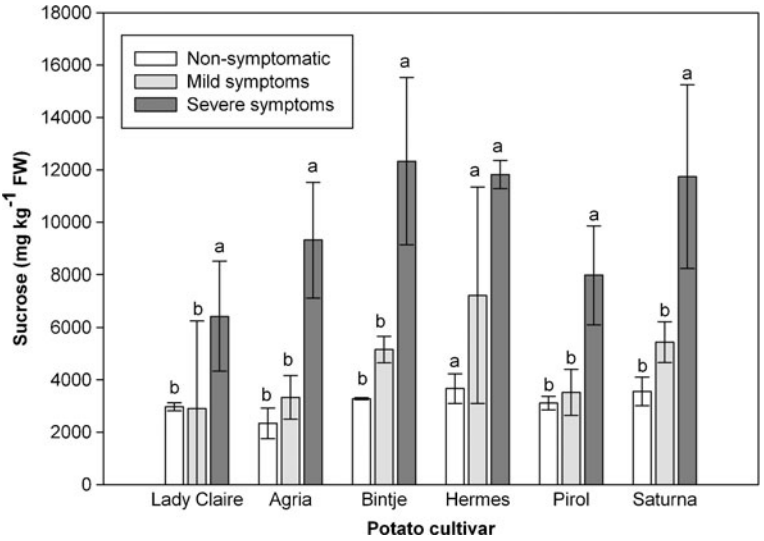

Fig. 4 Sucrose concentration (milligramme per kilogramme FW; mean±std. dev.) in tubers of cultivars tested in field experiment 2007. The tubers were grouped into the symptom categories non-symptomatic, mildly and severely affected. For each cultivar, *bars* indexed with the same letter were not significantly different at $P=0.05$ when analysed with the Student–Newman–Keuls test

Tubers with severe stolbur symptoms had a higher dry matter concentration than tubers with mild symptoms. In both disease classes, the core region showed a lower dry matter concentration than the bud or stem end. Glucose and fructose

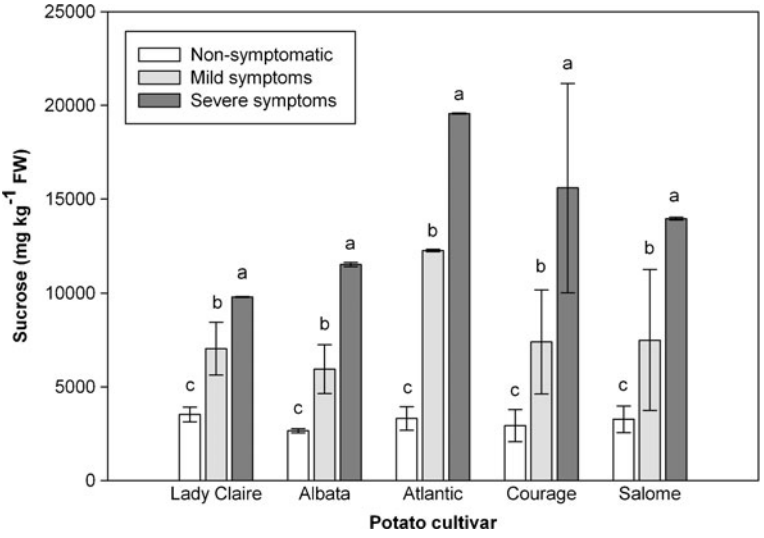

Fig. 5 Sucrose concentration (milligramme per kilogramme FW; mean±std. dev.) in tubers of cultivars tested in field experiment 2008. The tubers were grouped into the symptom categories non-symptomatic, mildly and severely affected. For each cultivar, *bars* indexed with the same letter were not significantly different at $P=0.05$ when analysed with the Student–Newman–Keuls test

Table 3 Quality of crisp slices in relation to stolbur symptoms on potato tubers of cv. 'Lady Rosetta' in 2007 (Swiss Frying Test; Mini et al. 2004)

Sample	Description	Score			
		≥7[a]	6	5	≤4[b]
		Number of slices			
1	Non-symptomatic tubers	3	3	2	2
2	Tubers with mild symptoms	0	1	7	2
3	Tubers with severe symptoms	0	0	0	10

[a] Best score—white to yellow colour

[b] Worst score—brown colour

concentrations were comparable in both symptom classes; concentrations were highest in the bud end portion of the tuber (Table 4).

Discussion

This paper focuses on the response of potato cultivars to stolbur phytoplasmal infection with respect to tuber consistency and sucrose concentration in tubers. The effect of phytoplasmal infection on sucrose concentration was considered to be an important evaluation criterion in the resistance screening because it can be assumed that an increased content of reducing sugars is not the reason for crisp discolouration of products from stolbur phytoplasmal-infected potato tubers. This is evidenced by the fact that the concentration of these components is not or only slightly increased by infection. Instead, it is very likely that the increased sucrose concentrations associated with infection is the reason for the discolouration of crisps during frying.

Table 4 Dry matter (DM) content and sugar concentration in potato tubers of 'Lady Rosetta' with stolbur symptoms, separated into sections in 2007

Stolbur	Section	DM [%]	Glucose[a]	Fructose[a]	Sucrose[a]
Mild symptoms	Whole tuber	25.9	520	190	11,011
	Bud end	26.1	2,060	690	11,165
	Core	22.5	210	120	9,720
	Stem end	26.2	1,100	200	8,710
Severe symptoms	Whole tuber	30.9	510	170	25,500
	Bud end	33.6	2,520	1,490	23,250
	Core	28.2	380	170	24,510
	Stem end	34.6	890	130	24,850

[a] Concentrations were expressed as milligramme per kilogramme FW

These results are based on the allocation of all analysed potato tubers to three tuber texture classes that could be associated with a stolbur infection in the following way: For tubers with severe symptoms (clearly visible rubbery, shrivelled texture), a high infection frequency was confirmed using conventional PCR. The phytoplasmal concentration of these tubers was high as detected by Q-PCR. There were also phytoplasmas detected in non-symptomatic tubers although the phytoplasmal concentration in tubers with severe symptoms was considerably higher compared to non-symptomatic tubers. Variations in phytoplasmal concentrations between individual samples were notable. Also, Christensen et al. (2004) and Crosslin et al. (2006) reported large differences in phytoplasmal concentrations within one plant and between plants of the same cultivar and variety. Differences in phytoplasmal concentrations between non-symptomatic tubers and tubers with severe symptoms within cultivars were statistically not significant. The reason for that seems to be the small number of single data points and the high variance in individual cultivars. Perhaps significant differences could be established if the average values of all cultivars were compared. This means that even though for both tuber categories (severely infected, non-symptomatic) phytoplasmal infections were detected the two categories differ significantly on the basis of the considerable differences in phytoplasmal concentration detected in the Q-PCR. Tubers with mild symptoms were considered to have medium infection intensity.

In the 2007 and 2008 field experiments, sucrose concentration of non-symptomatic potato tubers of all tested potato cultivars was relatively homogeneous in the range between 2,340 and 3,670 mg kg^{-1} FW. This sucrose content was similar to that measured by others (Haase and Weber 2007; Bervald et al. 2010). In comparison to severely diseased tubers, the considerably lower phytoplasmal concentration present in non-symptomatic tubers did not affect sugar metabolism significantly. Rather, the sucrose levels increased two- to threefold in potato tubers with severe symptoms in 2007 and three- to sixfold in 2008, respectively. Bethke et al. (2009) found that a decreased water potential in potato tubers due to heat and drought caused dramatic declines in the tuber pressure potential. Tuber sucrose content increased with the severity of stress. Consequently, the dry and hot weather during the growing seasons of 2007 and 2008 in Romania suggests an influence on the metabolism of the potato. Under the described conditions, however, no detrimental effects were exhibited in non-symptomatic tubers, whereas heat and drought together with high infection intensity potentially led to high sucrose levels in severely diseased tubers.

Physiological and biochemical analyses have shown that infections with phytoplasmas cause an increase in soluble carbohydrate and starch content and a decrease in net photosynthesis rate in source leaves and a reduced supply of sugars from source leaves to the roots (Hren et al. 2009b; Ji et al. 2009). In phytoplasmal-infected seedlings of elm (*Ulmus americana*), accumulation of ^{14}C assimilate was significantly lower in roots than in healthy plants, but in source leaves, it was vice versa (Braun and Sinclair 1978). Lepka et al. (1999) detected significantly increased sucrose content in phytoplasmal-infected leaves of periwinkle (*Catharanthus roseus*) and tobacco (*Nicotiana tabacum*), whereas in the roots, sucrose levels were similar to those of healthy plants or even lower. In our experiments, sugar concentration in the metabolic sink organ, i.e. the potato tuber, of stolbur-infected potatoes, was

analysed. Contrary to expectations, tubers with severe stolbur symptoms showed much higher sucrose values compared with non-symptomatic ones. Gao et al. (2009) also found a significant increase of sucrose concentration in potato tubers of Zebra chip-diseased potato plants when infected at an early stage (4-week growth stage). Also, the glucose concentration of Zebra chip-diseased potato tubers increased significantly with early infection. Glucose and fructose concentration in stolbur-infected tubers were comparable to that in non-symptomatic tubers or also slightly higher, but there were no significant differences.

It can be assumed that a portion of total tuber sucrose decomposes and enters the Maillard reaction during frying. Clegg and Chapman (1962) have pointed out a principal implication of sucrose in Maillard-related discolouration under some circumstances. De Vleeschouwer et al. (2009) have identified a thermal breakdown of sucrose into its two monomers at temperatures above 140 °C with respect to acrylamide formation, which is a principal reaction between asparagine and reducing sugars. Our frying experiment with the cv. 'Lady Rosetta' confirmed this observation. Tubers with severe stolbur symptoms, a very high sucrose level, and a slightly increased amount of reducing sugars resulted in dark-coloured crisps, whereas non-symptomatic tubers with a low sucrose level had only some crisp slices with little discolouration.

Within a single tuber, concentration of reducing sugars differed widely. Thompson et al. (2008) pointed out that heat and drought stress at specific growth stages of the potato plant affect the content of reducing sugars in stem and bud end parts of the potato tuber. Concentration of reducing sugars in stolbur-infected tubers was highest in the stem-end region. On the other hand, sucrose distribution was similar in all tuber regions in non- and stolbur-symptomatic tubers (Iritani et al. 1973; Weaver et al. 1978).

Currently, management of phytoplasmal diseases relies on herbicide applications against potential host plants and insecticide applications targeted against insect vectors. However, insecticide applications are of low effectiveness (Mori et al. 2008). The erratic feeding behaviour and rapidity in transmitting the stolbur phytoplasma to facultative host plants by *Hyalesthes obsoletus* seems to be the reason why insecticide treatments have no significant effect on stolbur disease incidence (Bressan et al. 2007). One of the favoured strategies for controlling phytoplasmal diseases is based on selecting resistant or tolerant plants. In resistance tests of the 1960s and 1970s, the number of potato plants with withering habits and spindly sprouts was used to evaluate stolbur resistance (Zadina and Nováček 1966; Volchev 1970). In these early tests, differences in susceptibility were demonstrated, but high levels of resistance could not be identified among potato cultivars. Munyaneza et al. (2009) carried out field experiments to assess susceptibility of potato cultivars to the phytoplasma-associated purple top disease. Disease incidence based on purple top symptoms suggested that cultivars appear resistant to or tolerant of this disease. Cadena-Hinojosa et al. (2003) also evaluated potato cultivars for their resistance to purple top disease. Criteria in their evaluation were severity of internal browning and abnormal sprouting. There are also data on molecular traits that may be suitable for identifying resistance. Hren et al. (2009b) found that strong signals (gene expression) of certain genes could be used as markers of phytoplasmal disease status. The authors pointed out that this method could support field-conducted

resistance experiments in the future. Our cultivar ranking of resistance was based on sucrose levels predicated on different phytoplasmal concentrations in potato tubers having different tuber textures. Potato plants were cultivated during a vegetative period in field experiments when they were exposed to more or less intensive stolbur infection pressure. In the experiments, potato cultivars with high susceptibility to stolbur disease like 'Courage' and 'Lady Rosetta', or low susceptibility like 'Lady Claire', were identified. But there were no fully resistant cultivars as described for woody plants such as elm, apple and pear or herbaceous plants such as sesame (Sinclair et al. 2000; Rajeswari et al. 2010; Bisognin et al. 2009; Seemüller et al. 2009). In order to be able to offer suitable potato material for breeding projects, a larger number of genotypes than tested in our study should be screened for stolbur resistance.

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