Biochemical Potential of Potato Tubers To Synthesize Blackspot Pigments in Relation to Their Actual Blackspot Susceptibility

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In a study of six potato varieties (*Solanum tuberosum* L.), which were grown under two different fertilization regimes, the biochemical potential of tuber tissue to synthesize blackspot pigments was distinguished from the actual blackspot susceptibility exhibited after impact application. Blackspot susceptibility and biochemical potential for pigment synthesis were not correlated, which supports the hypothesis that the extent to which the blackspot potential is actually being used is mediated by the sensitivity to cell decompartmentation. Quantification of polyphenol oxidase (PPO), soluble protein, and endogenous PPO substrates demonstrated that the content of free tyrosine is the predominant determinant for the biochemical potential for blackspot synthesis. PPO was not a limiting factor for pigment synthesis, even if PPO content was relatively low. It was therefore concluded that manipulation of PPO activity may offer a solution to the problem of blackspot formation only if it leads to a virtually complete elimination of PPO activity.

Keywords: *Solanum tuberosum L.; Solanaceae; potato; blackspot; bruise; melanin; tyrosine; chlorogenic acid; caffeic acid; polyphenol oxidase; enzymic browning*

INTRODUCTION

Blackspot in potato (*Solanum tuberosum* L.) is an internal discoloration of tuber tissue caused by a sequence of biochemical oxidations which is initiated by a mechanical injury which occurs during mechanical harvesting, sorting, transport, and storage. Blackspot is a major quality problem and causes substantial economic damage to the potato breeder and the potato-processing industry worldwide. The susceptibility of potatoes to blackspot damage varies between cultivars and depends also on mineral nutrition and temperature, hydration state, physiological age, and specific gravity of the tuber (Hughes, 1974; Storey and Davies, 1992). The key enzyme in blackspot formation is polyphenol oxidase (PPO), as has been proven by means of antisense inhibition of PPO gene expression (Bachem et al., 1994). PPO of potato catalyzes the oxidation of monophenols (cresolase activity; EC 1.14.18.1) and *o*-diphenols (catecholase activity; EC 1.10.3.2), which leads to the formation of *o*-quinones (Mayer and Harel, 1991). These reactive intermediates may readily be converted nonenzymatically by further oxidations and by reactions with nucleophilic compounds (Peter, 1989).

A recent characterization study of isolated pigments from black-spotted potato tissue showed that, after tissue bruising, PPO reaction products ultimately become covalently linked with protein, resulting in dark brown proteinaceous polymers (Stevens and Davelaar, 1996). These findings indicated that blackspot pigments are random products of nonregulated reactions of PPO generated quinones that take place in disintegrated cells, rather than products of a controlled melanin biosynthesis. The process of blackspot formation may therefore be divided into two stages. The first stage constitutes cell disintegration, resulting in the liberation of PPO from its subcellular compartment. In intact tuber cells the enzyme is exclusively compartmentalized in vesicles inside the amyloplasts (Czaninski and Catesson, 1974). As a result of cell disintegration, PPO can come into contact with its phenolic substrates. This initiates the second stage of blackspot formation, which is the actual synthesis of the pigments via the cascade of biochemical oxidation reactions. The biochemical components that are involved in the synthesis of the pigments, as either substrate, catalyst, or inhibitor, are all considered to contribute to the net biochemical potential for pigment synthesis, here referred to as "blackspot potential". Blackspot susceptibility may thus be determined by two tuber qualities, namely, (1) the sensitivity to subcellular decompartmentation and (2) the biochemical potential to synthesize blackspot pigments. According to this model, the first quality controls the extent to which the second comes to expression after impact application.

Since biosynthetical systems generally break down after cell disintegration, pigment formation is likely to depend on PPO and its substrates that are present during cell damage. The main PPO substrates present in potato tubers are L-tyrosine, chlorogenic acid, and caffeic acid (Baruah and Swain, 1959; Dean et al., 1993). The endogenous concentration of caffeic acid is relatively low, but may be substantially increased by hydrolysis of chlorogenic acid upon cell damage. Although tyrosine is generally assumed to be the principal building block for pigment formation, only chlorogenic acid has ever been demonstrated as a covalently linked constituent of purified blackspot pigments (Stevens and Davelaar, 1996). However, the contribution of covalently bound chlorogenic acid to the final discoloration has not been determined so far. Stark et al. (1985) and Corsini et al. (1992) reported that the amount of free tyrosine correlated well with blackspot susceptibility and observed an inverse relationship between amount of soluble protein and free tyrosine; they suggested that protein biosynthesis affects blackspot susceptibility by reducing the availability of free tyrosine.

This study was confined to two main objectives. The first one was to identify which biochemical component

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S0021-8561(96)00883-7 CCC: $14.00

determines the total blackspot potential of the tuber tissue. We therefore investigated the influence of PPO activity, soluble protein content, and concentration of endogenous PPO substrates on the extent of discoloration in tuber tissue which was completely disintegrated. The second objective was to examine the above presented hypothesis that the blackspot potential does not control blackspot susceptibility, but only determines the extent of pigment synthesis after cell disintegration. Blackspot potential was therefore distinguished from blackspot susceptibility by comparing the amount of pigment formation after complete subcellular decompartmentation of tuber tissue with blackening generated by application of a standardized mechanical impact that simulates authentic bruising. The experiments were performed with six commercial potato cultivars which were grown under two different potassium and nitrogen fertilization regimes in order to obtain batches of tubers that differed in blackspot susceptibility.

MATERIALS AND METHODS

Potatoes. Potato plants of six cultivars were grown in the field in 1994 on sandy soil in Wageningen, The Netherlands. The cultivars were Lady Rosetta, Bintje, Bildtstar, Diamant, Van Gogh, and Agria. Fertilization with or without 200 kg of N and 300 kg of K_2O per ha was applied before planting, which resulted in 12 different batches of potatoes. Twenty-five weeks after planting, the tubers of 80 plants were carefully harvested by hand and stored at 4−6 °C and 70−90% relative humidity. After 19 weeks of storage an aselect sample of 20 tubers was taken for determination of blackspot susceptibility and biochemical analyses.

Assessment of Blackspot Susceptibility. Blackspot susceptibility was determined by means of an impact pendulum in which restrained tubers one by one were precisely subjected to standardized impacts. This method has been developed for accurate laboratory measurements of impact susceptibility for relatively small numbers of tubers, simulating authentic and reproducible bruising (Gall et al., 1967; Skrobracki et al., 1989). Our results agreed well with the relative blackspot susceptibility as registered on the Dutch Recommended List of Varieties of field crops 1996 (compiled by DLO Centre for Plant Breeding and Reproduction Research, Wageningen, The Netherlands). The pendulum apparatus (type MIDAS P 88, manufactured by H. Gall, Gross Lüsewitz, Germany) was equipped with a flat impact head of 7 mm diameter. Two subsequent impacts (140 mJ each) per tuber were applied on a single spot centered between bud end and stolon end. The average energy absorbed by the 12 different potato batches during the first impact ranged between 107 and 112 mJ (n = 10 tubers; maximum standard deviation = 8.5 mJ), and during the second impact, between 98 and 104 mJ (n = 10 tubers; maximum standard deviation = 17.5 mJ). For each determination of blackspot susceptibility a sample of 4 tubers were bruised. After 3 days of incubation at room temperature the depth (D, distance in mm between skin and deepest frontier of the colored patch) and intensity (I) of discoloration were determined. In order to establish the intensity of discoloration, discrete I values were assigned as follows: 0 = no discoloration, 1 = light discoloration, 2 = moderate discoloration, 3 = heavy discoloration. The susceptibility to blackspot was expressed in the blackspot index (B), which is calculated by the formula $B = \Sigma(DI)/4$. The blackspot indices were determined in 5-fold.

Tissue Sampling. Potatoes have been reported to be more susceptible to blackspot formation at the stolon end than at the bud end of the tuber (Corsini et al., 1992); also PPO, tyrosine, and chlorogenic acid are not evenly distributed over the tuber (Brandl and Hermann, 1984; Corsini et al., 1992). The investigations were therefore performed on tissue located between 0 and 10 mm below the skin in a 1 cm broad zone centered between bud end and stolon end of the intact tuber. Three days after standard impact application a cylindrical sample (∅ = 10 mm) was taken from the undamaged side of the tuber, opposite to the inflicted spot, using a cork borer. The samples ranged from the skin to 10 mm below the skin. Per potato batch 20 samples were taken, which were combined, immediately frozen in liquid nitrogen, lyophilized, and after removal of the skin, ground to a fine powder, which was used for determination of potential blackening, PPO activity, protein content, and phenylpropanoids.

Potential Blackening. Pigment formation was allowed to develop to its potential maximum in rehydrated, freeze-dried tissue powder, which has been described as an efficient method for screening enzymic browning (Gubb et al., 1989). Subsequently, the pigments formed were quantified spectrophotometrically after extraction with phenol/acetic acid/water (1:1:1, w/v/v, PAW), which is an excellent solvent for blackspot pigments (Stevens and Davelaar, 1996). To 250 mg of dry powder was added either 750 μL of H_2O or 750 μL of 1.33 mM L-tyrosine, *n*-chlorogenic acid, or caffeic acid. After 16 h of incubation at 20 °C with continuous shaking (150 rpm), 1500 μL of PAW was added and the pigments formed were solubilized by thorough mixing. After clarification by means of centrifugation (3000*g*; 15 min; 20 °C) the absorbance of the extract was read at 405, 450, 540, 600, 630, and 750 nm in a microplate autoreader (Bio-tek EL 310, Bio-tek Instruments), using 250 μL of extract per well and PAW as a reference. Blackspot pigments do not exhibit any characteristic absorption maximum; the dark brown to black discoloration arises from the relatively strong absorption throughout the UV−vis spectrum and gradually declines at higher wavelengths. Extracts of undamaged tuber tissue only absorb light of up to 500 nm, and therefore measurements at higher wavelengths are most appropriate for pigment quantification (Stevens and Davelaar, 1996). Here we express the blackspot potential in absorption units determined at 540 nm, per g fresh weight (FW) or dry weight (DW). Measurements at higher wavelengths (600, 630, and 750 nm) gave the same results, while the absolute absorption values decreased with increasing detection wavelength.

Determination of PPO Activity. Dry, ground tuber tissue (350 mg) was extracted at 4 °C with 3.85 mL of 150 mM tricine pH 8.5 and 50 mM ascorbic acid by gentle end-over-end mixing (5 min; 13 rpm). After centrifugation (800*g*; 15 min; 4 °C) 2.5 mL of supernatant was applied on a size-exclusion chromatography column (PD-10, Sephadex G-25, Pharmacia, bed volume 9.1 mL, void volume 2.5 mL) which was equilibrated with elution buffer (50 mM tricine pH 8.5, 4 °C) in order to separate soluble protein from low molecular weight compounds. The proteins were eluted with elution buffer by gravity flow (ca. 1 mL min^{-1}) at 4 °C and collected in a total volume of 3.5 mL. To 980 μL of aerated 15 mM L-dihydroxyphenylalanine (L-DOPA) in 50 mM NaP_i (pH 6.4; 30 °C) was added 20 μL of protein preparation. Immediately after mixing, the velocity of DOPA oxidation was measured by means of the increase of absorbance at 470 nm over the first 10 s. The activity was calculated using an extinction coefficient of 3313 M^{-1} cm^{-1} (Wichers et al., 1984). Both PPO extraction and PPO assay were performed in duplicate.

Determination of Protein. The amount of soluble protein in the tuber tissues was determined in duplicate using a modified Lowry assay (Lowry et al., 1951) of Bio-Rad (Bio-Rad *DC* protein assay, protocol according to manufacturer) with bovine serum albumin as the standard protein.

Determination of Phenylpropanoids. Since rapid browning was observed when methanol was used for extraction of phenylpropanoids, the extraction procedure was started with the addition of hot H_2O (>90 °C); separate measurements of PPO activity confirmed that this treatment resulted in immediate and complete inactivation of PPO, whereas the extraction mixture remained colorless. To 100 mg of dry tissue powder was added *ca.* 1.5 mL of hot H_2O (>90 °C). The mixture was homogenized and placed in a boiling water bath for 5 min. After cooling, the total volume was adjusted with H_2O to 2.5 mL. Subsequently 2.5 mL of methanol was added. After thorough stirring, the mixture was centrifugated for 15 min at 4000*g*. After extraction with *n*-hexane (1:1 v/v), 1 mL of the polar layer was evaporated to dryness in a vacuum

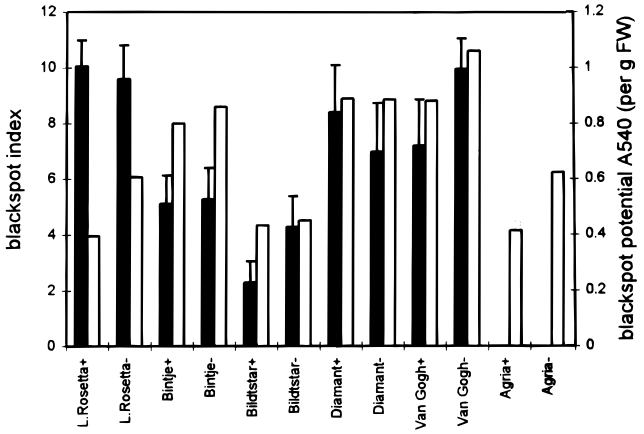

Figure 1. Blackspot indices (■) and potential blackening (□) of six potato cultivars, grown under two fertilization regimes; + and − indicate supplementation and omission of fertilizer, respectively. The vertical bars added to the blackspot indices show the standard error of the mean ($n = 5$).

centrifuge at 20 °C. The residue was dissolved in 1 mL of HPLC eluent (1.179 g of citric acid monohydrate and 1.386 g of $Na_2HPO_4 \cdot 2H_2O$ per L of methanol/H_2O (1:20 v/v) pH 4.2), filtered through an Ultrafree MC 10.000 NMLW filter unit (Millipore), and analyzed by means of isocratic HPLC. The HPLC system consisted of a Waters 510 pump, a Nucleosil C18 column (250 × 4.6 mm; particle size 5 μm; Alltech Associates Inc.) with a C18 μBondapak guard column (Waters), a column oven (40 °C), and a Waters 490 UV−vis detector. The flow rate was 1 mL/min, and the injection volume was 50 μL. Chlorogenic acid and caffeic acid were detected at 310 nm and tyrosine at 275 nm. Reference L-tyrosine, caffeic acid, and *n*-chlorogenic acid were purchased from Sigma, whereas reference cryptochlorogenic acid and neochlorogenic acid were synthesized from *n*-chlorogenic acid as previously described (Brandl and Hermann, 1984).

RESULTS

Blackspot Susceptibility and Blackspot Potential. The susceptibility to blackspot damage is expressed in a blackspot index which in this study was determined by evaluating depth and intensity of the discolored patches that appeared after application of standardized mechanical impacts. The blackspot indices of the six potato cultivars, which were grown with and without potassium and nitrogen fertilization and stored for 19 weeks, showed a large variation and confirmed that blackspot susceptibility is highly cultivar dependent ($P < 0.001$; Figure 1). The most susceptible cultivar was Lady Rosetta, whereas Agria did not exhibit any discoloration at all. Often but not invariably, both N and K fertilization have been shown to reduce the percentage of dry matter, which in turn is associated with reduced susceptibility to blackspot injury (Hughes, 1974). Although N and K fertilization resulted in all cultivars except Van Gogh in a decrease of dry matter content (Figure 2), the influence on blackspot susceptibility was relatively small and ambiguous; only cv. Bildtstar and Van Gogh showed a considerable increase of blackspot susceptibility when fertilization was omitted (Figure 1).

The blackspot potential of the six cultivars is depicted in Figure 1. Bintje, Diamant, and Van Gogh possessed a relatively large capacity for pigment formation, in contrast to Lady Rosetta, Bildtstar, and Agria. In all cases potassium and nitrogen fertilization suppressed potential blackening (Figure 1). The picture makes clear that the blackspot potential and blackspot susceptibility did not correlate; the highly blackspot resistant cv. Agria was capable of producing the same amount of pigments per gram of fresh weight as cv. Lady Rosetta and cv. Bildtstar, which are susceptible and moderately susceptible to blackspot, respectively (Figure 1).

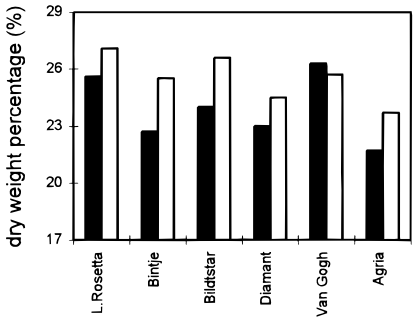

Figure 2. Dry weight percentages of tubers of six potato cultivars, grown with (■) and without (□) N and K fertilization.

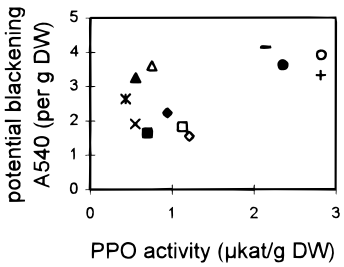

Figure 3. Correlation between potential blackening and PPO content of six potato cultivars, grown under two fertilization regimes. ◇ and ◆ represent Lady Rosetta + and −; △ and ▲, Bintje + and −; □ and ■, Bildtstar + and −; ○ and ●, Diamant + and −; + and −, Van Gogh + and −; × and *, Agria + and −, respectively (+ and − indicate supplementation and omission of fertilizer, respectively).

Blackspot Potential and PPO Activity. The catalytic activity of PPO for the oxidation of monophenols (cresolase activity; EC 1.14.18.1) exhibits a lag phase and is reported to be relatively unstable (Mayer and Harel, 1991). We therefore determined the amount of PPO by measuring the catecholase activity (EC 1.10.3.2) of the enzyme. Blackspot potential and PPO activity (Figure 3) were not correlated well ($r^2 = 0.36$), and neither were blackspot susceptibility and PPO activity ($r^2 = 0.33$). Van Gogh and Diamant exhibited high PPO activities and were capable of producing large amounts of pigment. By contrast, Bintje, which showed an approximately 4-fold lower PPO activity, exhibited about the same blackspot potential (Figure 3). These results indicated that PPO is not a limiting factor for blackspot formation. Interestingly, in all cultivars the combined potassium and nitrogen fertilization consistently led to a relatively small increase of PPO activity in the tuber tissue (Figure 3).

Blackspot Potential and PPO Substrates. The six cultivars all contained three types of chlorogenic acid, namely, 3-*O*-caffeoylquinic acid (*n*-chlorogenic acid), 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), and 5-*O*-caffeoylquinic acid (neochlorogenic acid) in a molar ratio of circa 70:18:13. The total content of chlorogenic acids ranged between 0.37 and 2.12 mmol/kg DW (Figure 4A). No correlation was found between potential blackening and content of chlorogenic acids ($r^2 = 0.03$; Figure 4A), nor between blackspot susceptibility and content of chlorogenic acids ($r^2 = 0.05$; not shown). Caffeic acid in its free form was present in only

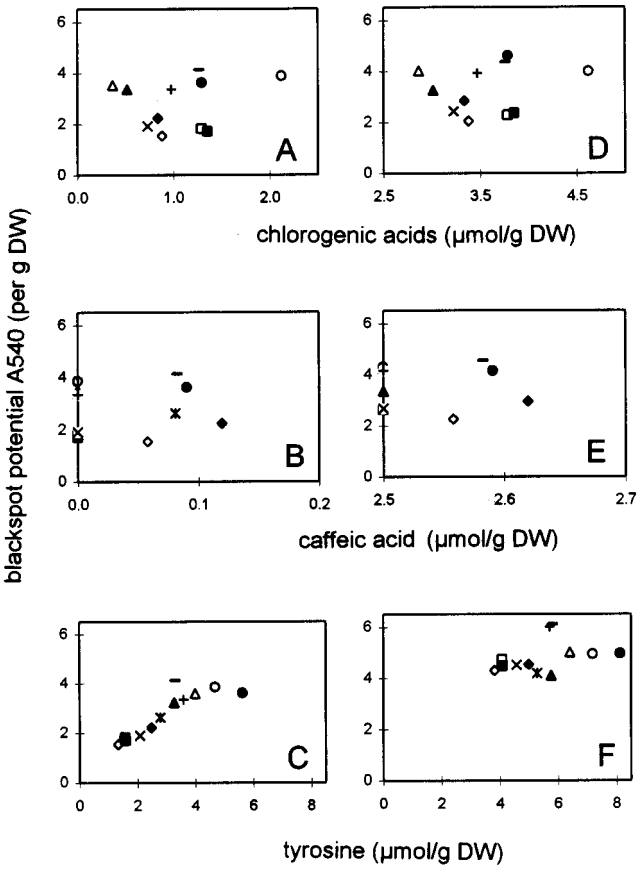

Figure 4. Correlation between potential blackening and amount of endogenous chlorogenic acid (A), caffeic acid (B), and tyrosine (C) and between potential blackening and artificially increased levels of chlorogenic acid (D), caffeic acid (E), and tyrosine (F) of the same tissue samples. The increments of the respective endogenous compounds were realized by supplementation with pure chlorogenic acid, caffeic acid, and tyrosine, respectively. Both potential blackening and amount of substrate are expressed on DW basis. For an explanation of the symbols, see Figure 3.

undetectable or minor quantities (up to 0.12 mmol/kg DW; Figure 4B) and did not show correlation with potential blackening ($r^2 = 0.00$; Figure 4B) or blackspot susceptibility ($r^2 = 0.14$).

The PPO substrate present in the most abundant quantities was L-tyrosine; the endogenous amounts varied between 1.33 and 5.63 mmol/kg DW (Figure 4C). Tyrosine showed good linear correlation with blackspot potential ($r^2 = 0.73$; Figure 4C), but not with blackspot susceptibility ($r^2 = 0.06$; not shown). These results show that tyrosine content was largely cultivar dependent and could be decreased by the combined supplementation of N and K. Only in the case of Bintje the tyrosine content increased after fertilization, and blackspot potential changed proportionally (Figure 4C).

Since the endogenous quantities of tyrosine were considerably higher than those of chlorogenic acid and caffeic acid, the contribution of chlorogenic acid and caffeic acid to the blackspot potential could have been concealed by the predominant presence of tyrosine. This was investigated by determining the blackspot potential after the addition of extra *n*-chlorogenic acid and, in a separate experiment, of extra caffeic acid. We added 2.5 µmol of both substrates/g DW, which largely exceeded the *in vivo* quantities, and which equaled the average quantity of endogenous tyrosine. The results showed that in none of the homogenates did the excess of chlorogenic acid lead to a substantial increase of

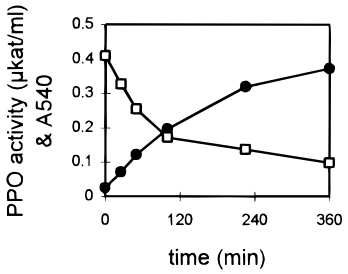

Figure 5. PPO activity (□) in the course of pigment formation (●) in a crude homogenate of Van Gogh tubers.

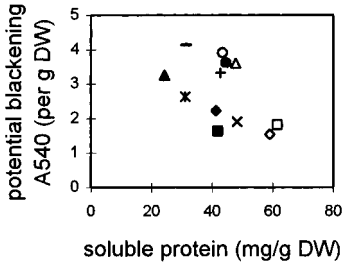

Figure 6. Correlation between potential blackening and soluble protein content of six potato cultivars, grown under two fertilization regimes. For an explanation of the symbols, see Figure 3.

tissue blackening (Figure 4A,D). An increase of absorbance was only restricted to light with wavelengths around 400 nm (not shown) resulting in slightly more orange extracts, which indicated that the added chlorogenic acid was oxidized. The same was found for caffeic acid (Figure 4B,E).

In contrast, a supplement of 2.5 µmol of tyrosine/g DW did lead to a substantial increase of potential blackening (Figure 4C,F). The increase in blackspot potential was lowest in the homogenates of the cultivars that contained relatively high amounts of endogenous tyrosine (Figure 4F). This moderate saturation effect may be due to inhibition of PPO activity by its reaction products. This possibility was investigated by monitoring PPO activity in the course of pigment formation in an incubation mixture of Van Gogh homogenate. The results showed that pigment formation was accompanied by a rapid inactivation of PPO (Figure 5). When the total amount of endogenous PPO substrates is relatively high, PPO may therefore finally become the limiting factor for pigmentation. The critical level of total substrate content presumably depends on PPO level and protein content, since proteins may act as quinone scavengers.

Protein Content and Blackspot Potential. Concentration of soluble protein and blackspot potential of the 12 different batches of potato tubers showed a poor and negative correlation (Figure 6; $r = -0.56$). The correlation between protein content and tyrosine content was even less (not shown; −0.36). All cultivars but Diamant exhibited a reduction of soluble protein content when N and K fertilization was omitted (Figure 6). This reduction was rather large and varied between 26% and 46% as calculated on a DW basis.

DISCUSSION

Blackspot susceptibility of potato tubers may be reduced by lowering the biochemical potential for pigment formation, because this results in reduced amounts of pigments formed per disintegrated cell. This may be achieved either by reducing the level of PPO activity or

by reducing the amount of endogenous PPO substrates. Our results showed that PPO is not a limiting factor for pigment formation, except in cases of extremely high tyrosine levels, probably due to PPO inactivation by the quinones produced. This implies that even largely reduced PPO levels will not result in a substantial reduced blackspot susceptibility; PPO activity should therefore be eliminated to virtual completeness. As has been proven by Bachem et al. (1994) this can be accomplished successfully by means of antisense PPO gene expression, which prevents the synthesis of PPO protein by blocking the mRNA with complementary RNA.

The positive correlation between tyrosine content and blackspot potential strongly indicates that tyrosine is a substrate for pigment formation and is the main determinant for the degree of discoloration of damaged cells. These findings are in agreement with results of earlier studies, in which tyrosine content showed a positive correlation with enzymatic discoloration after abrasive peeling (Stark et al., 1985; Corsini et al., 1992; Dean et al., 1993) and tissue homogenization (Dean et al., 1993; Mondy and Munshi, 1993), which in fact may be considered as different methods to determine blackspot potential. Reduction of free tyrosine levels would therefore be another approach to decrease the blackspot potential; this may possibly be achieved either by inhibition of tyrosine biosynthesis or by stimulating protein synthesis. Our results and previous findings (Mulder, 1949) indicated that N and K fertilization leads to decreased tyrosine concentrations in tubers kept in storage for several months. The common agricultural practice of relatively high N and K supplementation makes a recommendation on this point redundant. Corsini et al. (1992) suggested the suppression of free tyrosine levels by inducing protein synthesis, since they observed that free tyrosine is negatively correlated with soluble protein content. Our results, however, did not supply support for these conclusions.

In a previous study we demonstrated that chlorogenic acid is a covalently linked constituent of blackspot pigments formed in Bildtstar tubers (Stevens and Davelaar, 1996). The data presented here show that the concentration of chlorogenic acids does not influence the final amount of pigmentation. We therefore conclude that chlorogenic acids, and possibly also caffeic acid, are involved in the synthesis of blackspot compounds but do not substantially contribute to the dark color.

The insight that an increased supply of, in particular, potassium nutrition reduces both specific gravity and blackspot susceptibility has become generally accepted (Storey and Davies, 1992). However, in our experiment the application of different potassium and nitrogen fertilization regimes within each cultivar did not result in substantial differences in blackspot susceptibility, although specific gravity was reduced in all cultivars except Van Gogh. Deletion of N and K supplementation invariably led to a decrease of PPO and protein. Since potassium fertilization has been reported to decrease PPO content and nitrogen fertilization to increase PPO content (Matheis, 1987), the here reported effect on PPO levels should probably be accounted to the supply of nitrogen.

The results clearly show that the susceptibility of potato tubers to blackspot damage is not correlated with the biochemical potential for pigment synthesis (Figure 1). Apparently, there must be a variable tuber characteristic which determines to what extent the blackspot potential is actually being used after impact application. It is generally assumed that this regulatory factor is the sensitivity of tuber tissue to cell decompartmentation. If different potato varieties with the same sensitivity for cell disintegration are compared, tyrosine content will consequently appear to be the main determinant for blackspot susceptibility. This may explain the contradicting results previously reported on the relation between tyrosine and blackspot susceptibility (Vertregt, 1968; Corsini et al., 1992; Mondy and Munshi, 1993).

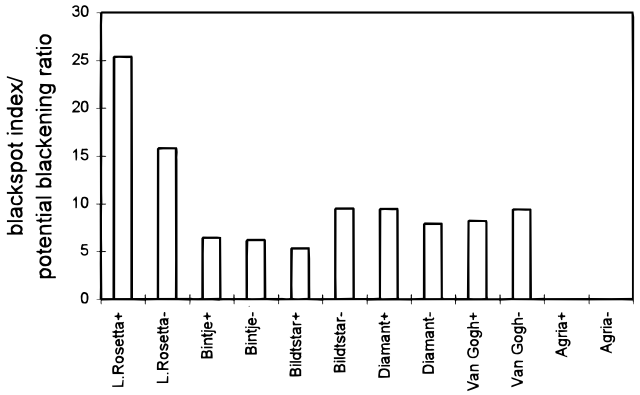

Figure 7. Ratio between blackspot index and potential blackening of six cultivars; + and − indicate supplementation and omission of fertilizer, respectively.

The ratio between blackspot index and blackspot potential may be considered as a crude and relative indicator for the sensitivity for cell disintegration. The blackspot index/blackspot potential ratios of the 12 potato batches investigated here suggest that cell integrity of Lady Rosetta tubers was relatively easily lost, whereas that of Agria tubers was stable; the sensitivity to cell disintegration of the other cultivars seemed to be moderate and more or less the same (Figure 7). Our findings thus indicate that substantial differences between cultivars do exist with respect to their sensitivity to subcellular decompartmentation, and that these differences to a large extent contribute to differences in blackspot susceptibility. Manipulation of this characteristic may therefore be an important tool to change blackspot susceptibility. An advantage of this approach is that, besides pigment formation, also cell necrosis and concomitant suberin formation are decreased, which may be considered as additional aspects of the quality problem of black spots in potato; reduction or even elimination of the biochemical potential for pigment formation will only solve the cosmetic part of the problem. Future research should therefore focus on the process of cell decompartmentation and the regulatory mechanisms involved.

ABBREVIATIONS USED

DOPA, dihydroxyphenylalanine; DW, dry weight; FW, fresh weight; PAW, phenol/acetic acid/water (1:1:1 w/v/v); PPO, polyphenol oxidase.

ACKNOWLEDGMENT

We thank M. C. J. M. Konings and D. Uenk for technical assistance and A. J. Haverkort, J. P. F. G. Helsper, and B. W. Veen for valuable discussion.

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Received for review November 13, 1996. Revised manuscript received August 5, 1997. Accepted August 5, 1997.[⊗] This study was financially supported by the Netherlands Potato Association.

JF9608837

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.