THESIS

POTATO CLONE VARIATION IN BLACKSPOT SUSCEPTIBILITY, EXTRACT DARKENING, AND SOME CHEMICAL FACTORS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MICHAEL KENT THORNTON ENTITLED POTATO CLONE VARIATION IN BLACKSPOT SUSCEPTIBILITY, EXTRACT DARKENING, AND SOME CHEMICAL FACTORS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

POTATO CLONE VARIATION IN BLACKSPOT SUSCEPTIBILITY, EXTRACT DARKENING, AND SOME CHEMICAL FACTORS

Six potato clones were analyzed with respect to tissue extract darkening, phenolic content, dry matter, and potassium content to determine if these properties were related to differences in blackspot susceptibility.

Clones differed significantly in tissue extract darkening, dry matter and potassium content, but not phenolic content. Differences in tissue extract darkening were not significant when cell wall fragments were filtered.

The potential of the tissue to darken, as determined by tissue extract darkening studies, was not consistently related to blackspot susceptibility. Clones with low tissue extract darkening darkened most in response to addition of substrate (catechol). Phenolic content (tyrosine and o-diphenols) were more consistently related to tissue extract darkening than to blackspot susceptibility. Dry matter content was most closely related to blackspot susceptibility, but did not account for all differences. Potassium content was not related to blackspot susceptibility.

Changes in ascorbic acid content of bruised and nonbruised tissue of four potato clones were followed over time to determine if they were related to blackspot susceptibility. Ascorbic acid content of bruised

and nonbruised tissue of three clones decreased over 24 hours. However, the ascorbic acid content of one clone increased during this same period. These changes were not related to blackspot susceptibility. Differences in ascorbic acid content between bruised and nonbruised tissue of the same tuber were significant in 3 of 4 clones but were not related to blackspot susceptibility.

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CHAPTER I

INTRODUCTION

Blackspot bruise is a physiological disorder of potatoes which only develops after mechanical damage to the tuber. Blackspot appears as a general discoloration which develops immediately below the periderm but usually does not penetrate beyond a depth of one centimeter. The color of the blackspot may vary in intensity from light gray to black. The discoloration develops slowly at room temperature, full development requiring 12 to 24 hours at 25°C.

The discoloration results from enzymatic oxidation of phenolic substrates to quinones. Quinones are highly reactive compounds which can polymerize to form melanin or complex with proteins to form melanoprotein pigments.

Studies in some areas have shown that growers may lose up to 20% of their gross income due to damage during mechanical harvesting. Damage results in losses due to rejected tubers, higher labor costs, increased moisture and disease losses in storage, and reduced prices. Nationally, the losses due to blackspot bruise for the 1982 crop may have reached 125 million dollars.

Damage can occur anytime during the harvesting and handling operations. Reduction of blackspot can be achieved by careful operation of machinery and avoidance of low temperatures during these

operations. However, even when treated carefully, some cultivars still sustain high levels of damage.

Differences in cultivar susceptibility to blackspot may be due to several factors. Hughes (30) classified these factors in two broad categories: (a) susceptibility of the tissue to mechanical damage; (b) potential of the tissue to produce colored oxidation products.

In evaluating seedling clones for blackspot susceptibility it was noticed that tubers of certain clones showed no discoloration even when cellular damage was apparent, while those of other clones darkened readily. It was hypothesized that there is a difference in the potential of tuber tissue from different clones to darken and that this potential is related to blackspot susceptibility. In particular, it was believed that there may be inhibitors or reducing substances present in resistant clones which influence the darkening reactions.

CHAPTER II

LITERATURE REVIEW

Susceptibility of tubers to blackspot is usually determined by measuring the amount and intensity of the discolored tissue. The value is often expressed as a "Blackspot Index." Differences between cultivars as determined by this index may be due to differences in the amount of pigment formed per cell or the number of cells damaged. This review will focus on cultivar characteristics accounting for these differences

Cultivar Characteristics Affecting Susceptibility of Tissue to Damage

Blackspot cannot occur without cellular damage. Susceptibility of tissue to damage is dependent upon the mechanical and rheological properties of cells. Attempts to measure directly the rheological properties of cells and relate them to blackspot susceptibility have had mixed results. Finney et al. (23) were able to measure differences between cultivars in various rheological properties such as modulus of elasticity and force to skin rupture, but did not relate them to blackspot susceptibility. Hughes et al. (31) found that within a cultivar the dynamic deformation of the tissue for a given bruising force was directly related to the volume of damaged tissue. In most cases, blackspot susceptible cultivars deformed more at a constant

bruising force. For five of the seven cultivars the volume of damaged tissue was the same for a constant bruising force.

Gray and Hughes (25) suggested that tubers with a strong skin but a weak underlying tissue are most susceptible to blackspot. This theory was not confirmed by Blight and Hamilton (4). They measured the relative strength of the skin versus 2 mm below with a penetrometer. The measurement was not useful in predicting differences in susceptibility between cultivars.

Specific gravity, under-water-weight, and dry matter content are cultivar characteristics which have been related to blackspot susceptibility. These parameters are all linearly related. The relationships have been described by Kushman (39) and Simmonds (62).

In a survey of 10 cultivars, Ophuis et al. (53) found a positive correlation of under-water-weight with blackspot susceptibility (correlation coefficient = 0.77). Killick and Macaurther (35) reported that cultivars with a high specific gravity tended to be more susceptible to blackspot than those with lower specific gravity, but the differences were not significant. Within a cultivar, tubers which expressed blackspot had significantly higher specific gravity than those which did not. They suggested that only 30% of the differences between cultivars in blackspot susceptibility were related to differences in specific gravity. Sawyer and Collin (60) also reported a relationship between high specific gravity and blackspot susceptibility within cultivars. No relationship between specific gravity and cultivar susceptibility was found. Ifenkwe et al. (33) found that specific gravity varies with tuber size within a cultivar. Specific gravity increases with increasing tuber diameter, up to a maximum, then decreases in

very large tubers. Cultivars which produce a high percentage of large tubers may be relatively more susceptible to blackspot.

In contrast, Hudson (29) found that cultivars with low specific gravity, large cells, and extensive intercellular space were the most bruise susceptible.

Miejers (46) attributes the few exceptions of the general relationship between high specific gravity and blackspot susceptibility to differences in cell size and dry matter distribution in the tubers.

Van Es and Hartmans (20) found preliminary indications that susceptibility was related to cell dimensions. Olsson and Fridell (52) described two "sib seedlings" from a breeding program which were similar in tuber size, dry matter content, enzymatic browning rate, and phenolic content, but with significant differences in blackspot susceptibility. The more susceptible clone was characterized by larger cells in the cortex and premedullary regions. A survey of 20 cultivars by the same authors indicated little relationship exists between cell volume and blackspot susceptibility. The maturation characteristics of the cultivar was a better parameter for indicating susceptibility. They state that this indicates that the building of cell walls, which occurs during maturation, is a major factor in resistance to bruise.

Hughes (30) suggested that the relationship between specific gravity and blackspot susceptibility may be either direct or indirect. Specific gravity is linearly related to starch content of the cells (62). It may therefore directly influence cell damage because starch granules may damage membranes during the rapid deformation which results from a bruising force.

Specific gravity may be indirectly related to blackspot susceptibility as it reflects changes in cell size and cell wall properties associated with maturity of the tubers (30).

Specific gravity also reflects the relative water content of the tissue. Kunkel and Gardner (37) showed that the water content of tubers subjected to various dehydrating treatments was related to blackspot susceptibility. Sawyer and Collin (60) found that discs of tissue from extremely blackspot susceptible Pontiac tubers became resistant when bathed in distilled water. Discs of resistant tissue became susceptible when bathed in a solution of 0.8 molar or higher mannitol. They concluded that susceptibility to blackspot is "primarily determined by tissue turgor."

The same authors measured tuber firmness with a durometer and found that cultivars with the firmest tubers were the least susceptible to blackspot. Firmness was not related to specific gravity in all cultivars. They attributed cultivar differences in firmness and blackspot susceptibility to differences in lenticel and skin structure which affect moisture loss from the tuber.

Turgor and water content may affect blackspot susceptibility by influencing rheological properties of the cells. Falk et al. (22) reported that a linear relationship exists between firmness, measured as modulus of elasticity, and turgor pressure of tuber parenchyma cells. But turgor pressure does not always explain the differences in susceptibility between cultivars, or tubers of the same cultivar, especially at extremely low or high turgor levels (36, 38).

Hughes (30) states that the amount of deformation for a given bruising force and the deformation the tissue can withstand before

breakage of the membrane and cell wall occurs will determine the amount of cellular damage. The former will be affected by cultivar cell wall characteristics and turgor pressure. The latter is affected by cultivar cell wall and membrane properties. Properties of the tonoplast would be particularly important because phenolics are stored in the vacuole (8, 28).

Levels of certain nutrients have been shown to influence blackspot susceptibility. Van Es and Hartmans (21) state that tubers with less than 2% potassium in the dry matter are usually susceptible to blackspot, while tubers with more than 2% are resistant. Vertregt (70) found that tubers with at least 2.5% potassium in the dry matter were very resistant to blackspot. This relationship was consistent regardless of the dry matter content. This level of tuber potassium could not be attained by conventional fertilization practices.

Increasing the level of potassium fertilization causes a decrease in specific gravity of the tubers (61). Kunkel and Gardner (37) state that potassium influences both specific gravity and blackspot susceptibility of tissue by affecting the cell water content.

Potassium may directly affect specific gravity of tubers. Haeder et al. (27) reported that potassium influences translocation of photosynthate from leaves to tubers of potato plants. Potassium also affects the conversion of sucrose to starch in the tuber.

Potassium may influence rheological properties of the cell by affecting cell wall extensibility. Hughes et al. (31) found that tubers grown with low levels of potassium fertilization sustained more cellular damage at a constant bruising force, although the dynamic deformation on impact was the same for tubers from all fertilizer levels.

Mondy et al. (48) suggested that potassium may decrease blackspot susceptibility by increasing tuber turgor and maintaining specific gravity of tubers during storage. However, Hudson (29) found that neither fertilization or climatic factors, such as soil moisture or growing temperature, had any effect on specific gravity.

Nitrogen fertilization also has an effect on tuber specific gravity (61). Kunkel et al. (37) found that nitrogen fertilization sometimes increased the susceptibility of tubers to blackspot, although both nitrogen and potassium fertilization decreased specific gravity. They suggested that the effect of these nutrients on water content of the tissue was more important than the effect on specific gravity in influencing blackspot susceptibility.

The interaction of fertilization, specific gravity, and turgor pressure seems to influence the mechanical and rheological properties of cells. This may explain why these properties have not been consistently associated with differences in cultivar susceptibility to blackspot.

Cultivar Characteristics Affecting the Potential of the Tissue to Produce Colored Oxidation Products

Darkening reactions associated with blackspot development involve enzymatic oxidation of phenolic substrates. Enzymes capable of catalyzing these reactions include polyphenol oxidase (M nophenol, dihydroxyphenylalanine : oxygen oxidoreductase, EC 1.14.18.1), peroxidase (Donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7), and catalase (Hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6) (7). Polyphenol oxidase is considered to be the most

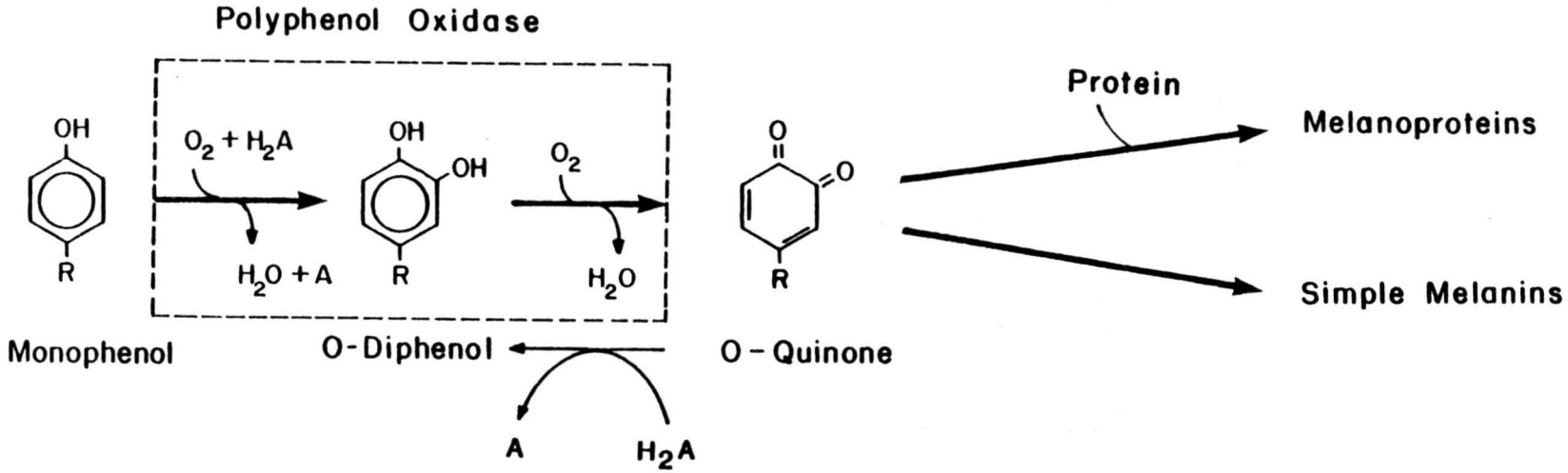
important enzyme system involved in browning of damaged cells (7). Polyphenol oxidase activity increases in tissue following injury (10). But Weaver et al. (75) found that this increase was not related to blackspot susceptibility.

Reactions catalyzed by polyphenol oxidase are illustrated in Figure 1. The enzyme catalyzes both hydroxylation and oxidation-type reactions. The reduced form of the enzyme, which is produced during the subsequent oxidation reactions, is required for the hydroxylase activity. The hydroxylation reaction also requires the presence of a reductant, represented by H_2A in Figure 1. The reductant may reduce the quinones produced by the oxidase activity to o-diphenols. This reaction is also shown in Figure 1.

Clark et al. (12) reported that the blackspot susceptible cultivar Ontario had a significantly higher polyphenol oxidase activity than the resistant cultivar Pontiac. Mondy and Klein (47) confirmed these results and found that the darkening of damaged tissue from both cultivars increased during the six months of storage. Polyphenol oxidase activity decreased during this same period indicating that other factors, such as phenolic content, were influencing the darkening.

Cotter and Sawyer (14) found no relationship between polyphenol oxidase activity and blackspot susceptibility, using tyrosine as substrate. Vertregt (70) noted that cultivars with low enzyme activity would sometimes darken extensively when damaged. Mapson et al. (45) found that polyphenol oxidase activity was not limiting the darkening rate of damaged tissue in any of the eleven cultivars tested. But Weaver et al. (75) found that polyphenol oxidase activity of Russet

Figure 1. Reactions associated with darkening of damaged tissue. Polyphenol oxidase catalyzes the reactions within the dotted lines.


Polyphenol Oxidase
OH
R
$O_2 + H_2A$
$H_2O + A$
OH
OH
R
O_2
H_2O
O
O
R
Protein
Melanoproteins
Simple Melanins
Monophenol
O-Diphenol
O-Quinone
A
H_2A

Burbank tuber tissue was related to blackspot susceptibility when catechol was used as substrate. No relationship was found when tyrosine, p-cresol, or chlorogenic acid were used as substrates.

Patel et al. (55) have shown that peroxidase preparations from plant tissue are capable of oxidizing tyrosine to melanin. Catalase may catalyze peroxidase-type reactions in damaged cells (74). Weaver and Hautala (74) reported that peroxide, peroxidase, and catalase could interact in potato tuber tissue to influence the oxidation of tyrosine and chlorogenic acid. They suggested that this interaction could influence blackspot susceptibility. But Weaver and Hautala (76) found no relationship between peroxidase or catalase activity and blackspot susceptibility. The ratio of the activity of these enzymes with polyphenol oxidase activity was not useful in predicting susceptibility.

The intensity of the color of the blackspot is dependent upon the concentration of phenolic substrates in the tissue (18). Clark et al. (12) reported that the blackspot susceptible cultivar Ontario had a higher phenolic content than the resistant cultivar Pontiac. No qualitative differences in phenolic compounds present were detected by paper chromatography.

The two predominant phenolic substrates in potato tubers are tyrosine and chlorogenic acid (18, 30). Oxidation of tyrosine by polyphenol oxidase to dopaquinone and the subsequent formation of melanin has been described by Muneta (51). Chlorogenic acid is also oxidized by polyphenol oxidase but its oxidation products are not deeply colored (30). Chlorogenic acid increases in bruised tissue (58) while tyrosine decreases as the blackspot develops (70).

Mapson et al. (44) found that tyrosine was the main factor determining the amount of darkening of damaged tissue of seven cultivars. Umaerus and Olsson (66) also found that tyrosine content was important in determining the extent of darkening of damaged tissue. High tyrosine content always resulted in high discoloration upon injury, but some low tyrosine cultivars also darkened extensively. Duncan (18) reported that tyrosine concentration was related to blackspot susceptibility within a cultivar but not between cultivars. Vertregt (70) surveyed 100 cultivars and found that cultivars with low tyrosine could blackspot as severely as those with high tyrosine.

Weaver et al. (75) suggested that chlorogenic acid derived pigments could account for the blackspot if concentrated in a small area. Mapson et al. (44) reported that chlorogenic acid was important in determining the rate of the darkening reactions but did not affect the extent of darkening. The chlorogenic acid concentration in the tubers of the four cultivars was not low enough to limit the darkening rate. Weaver et al. (75) found that chlorogenic acid decreases the lag period for oxidation of tyrosine by polyphenol oxidase. They suggested that the reduction of the lag period could determine the extent of darkening which occurs before the enzyme is inactivated in damaged cells. But Duncan (18) found that chlorogenic acid levels in tubers were not related to blackspot susceptibility in three cultivars.

Quinones produced by the oxidation of o-diphenols are very reactive compounds. Quinones generally have redox potentials more positive than most other naturally occurring classes of compounds in plant cells (45). Quinones may be reduced, polymerize, or combine

with amino and sulfhydryl groups of proteins. The quinone-protein complexes are harmful to cells and under normal conditions their formation is prevented (68). Oxidation and polymerization reactions may be limited in cells by reducing substances or systems, compartmentation of phenolic substrates, and detoxification of phenolics by conversion to more stable glucosides or esters (68).

In cells, phenolic compounds are usually stored in the vacuole as esters (8). Few un-esterified phenolics are detectable in cells (26). But phenolic compounds must be kept in the reduced state during transport to the vacuole from the site of synthesis by either continuous use of reducing power or suppression of polyphenol oxidase activity (69). Quinones do not accumulate in tissue with sufficient concentrations of reducing substances (2). Substances which may nonenzymatically reduce quinones to o-diphenols include NADH, NADPH, ascorbic acid, glutathione, and cysteine. Butt (7) suggests that the rapid rise in oxygen consumption immediately following tissue damage may be due to the mixing of phenolic substrates, polyphenol oxidase, and reducing substances. Tissue browning does not occur until the reducing substances are completely oxidized.

Johnson and Schaal (34) found that ascorbic acid accumulated in cells next to a cut surface in potato tuber tissue, although it was oxidized in the damaged cells. They suggested that this accumulation of reducing substances protects the healthy cells from quinones produced in the damaged cells.

Because systems which reduce ascorbic acid are normally more active than those which oxidize it, dehydroascorbic acid (DHA) constitutes only 0-14% of the total in undamaged tissue (25, 43). Upon

injury, systems which oxidize ascorbic acid become very active with a resulting increase in the percentage of DHA.

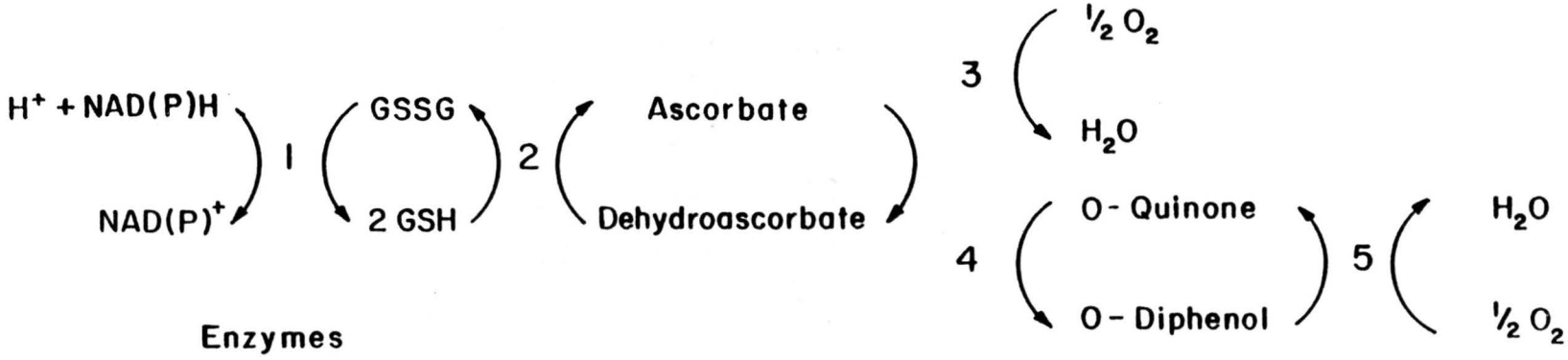
Cotter and Sawyer (14) found a significant negative correlation between ascorbic acid concentration and blackspot susceptibility of three cultivars (correlation coefficient = -0.419). However, Duncan (18) found no relationship between ascorbic acid content and extent of darkening of damaged tissue in three cultivars.

According to Butt (7), hydrogen may be transferred to dehydroascorbic acid from glutathione by glutathione dehydrogenase (Glutathione: dehydroascorbate oxidoreductase, EC 1.8.5.1). Chayen (9) states that no net oxidation of ascorbic acid occurs until all the glutathione is oxidized. Cotter and Sawyer (14) found no relationship between glutathione concentration and blackspot susceptibility of three cultivars.

Glutathione can in turn be reduced by NADPH and NADH produced by pentose phosphate pathway and Krebs cycle dehydrogenases, respectively (5, 79). Systems which transfer hydrogen from NADPH or NADH eventually to oxygen via glutathione and ascorbic acid, illustrated in Figure 2, have been found in plant tissue extracts (44, 79). Also illustrated in Figure 2 is the coupling of this system to phenolic oxidation as suggested by Bonner (5).

The role of such a system in plant cells is unknown. Butt (7) suggested that this system may be involved in regulation of the pentose phosphate pathway. Pentose phosphate pathway activity increases in damaged tissue due to the requirement for nucleotides, phenolics, and NADPH for biosynthesis (67). The NADP/NADPH ratio regulates the pathway by affecting glucose-6-phosphate

Figure 2. Summary of system which transfers hydrogen from NADH or NADPH to oxygen through glutathione and ascorbic acid. System may be coupled to oxidation of phenolics.



Enzymes

1 Glutathione Reductase
2 Glutathione Dehydrogenase
3 Ascorbate Oxidase
4 Non enzymatic
5 Polyphenol Oxidase

dehydrogenase activity, the first step in the pathway (65). The oxidation of NADPH through such a system would allow the production of precursors to continue independent of the cellular demand for NADPH or energy. If the reducing power from this system were used to reduce quinones resulting from phenolic oxidation, then the formation of melanin pigments would be prevented.

Brinkman et al. (6) reported that total NADP(H) increased 900% and NAD(H) 180% within three days following slicing of tuber tissue. Changes were detectable within five hours after wounding. The NAD/NADH ratio decreased during the first 24 hours indicating that systems which reduce NAD were activated. The NADP/NADPH ratio remained constant for 2 days, then decreased.

The enzyme quinone reductase (Reduced-NAD(P): quinone oxidoreductase, EC 1.6.5.1) also uses NADH and NADPH to reduce quinones to phenols (78).

Weaver et al. (72) suggested that there may be factors which modify enzyme activity responsible for determining blackspot susceptibility. Inhibitors which prevent the oxidation of o-diphenols to quinones may be one of these factors.

Both Anderson (1) and Pierpoint (56) reported that reducing substances such as ascorbic acid, cysteine, and thiourea inhibit polyphenol oxidase activity, as well as reduce quinones. The inhibition was reversed when the compounds became oxidized. The sulfhydryl compounds also form colorless complexes with quinones. However, Muneta (51) found no inhibition of polyphenol oxidase activity by ascorbic acid. He determined that sulfhydryl containing compounds such as cysteine, thiourea, and dithiothreitol were strong

inhibitors of enzyme activity. These compounds may modify enzyme activity by chelating copper at the active site of the enzyme. His data indicated that these compounds did not reduce quinones. He theorized that sulfhydryl compounds may control the oxidation of tyrosine in potato tissue.

Muneta (50) also found that the concentration of chlorogenic acid in the tissue would determine the amount of the sulfhydryl compound necessary to inhibit the oxidation of tyrosine. The oxidation of chlorogenic acid was not inhibited as severely by these compounds and the chloroquinones produced complex with the sulfhydryl groups, preventing them from acting as inhibitors.

Some phenolic compounds which inhibit potato polyphenol oxidase were examined by Macrae and Duggleby (41). Several naturally occurring phenolic compounds, including p-coumaric, ferulic, and cinnamic acids were effective inhibitors. The type of inhibition, competitive, noncompetitive or mixed, was dependent on the type of substrate (monophenol or diphenol).

Hyodo and Uritani (32) suggested that p-coumaric acid may regulate polyphenol oxidase activity in sweet potato tissue. They reported that the enzyme had a higher affinity for p-coumaric acid than some of its substrates. Vaughan and Butt (69) found that p-coumaric acid in connection with a tetrahydropteridine reductant suppressed the oxidation of caffeic acid by spinach-beet polyphenol oxidase.

The quinic acid esters of p-coumaric and cinnamic acids have been detected in potato tuber tissue. Levy and Zucker (40) reported that both compounds were precursors of chlorogenic acid, which is continuously metabolized within the tissue at a rate of 50 nmoles per

g fresh weight per hour (17). p-Coumaric and cinnamic acids were not detected and were not utilized as precursors of chlorogenic acid. It is possible there is a small pool of p-coumaric and cinnamic acids in the tissue which may modify enzyme activity, especially in damaged tissue where chlorogenic acid is rapidly synthesized.

Mineral nutrition may affect the biochemical properties of cells which determine the potential of the tissue to darken. Mulder (49) reported in 1949 that potassium deficiency symptoms were associated with extreme blackspot susceptibility. Tubers from plants exhibiting deficiency symptoms contained up to four times more tyrosine than those from normal plants. The increase in tyrosine was not associated with an increase in blackspot susceptibility in all cultivars. Mondy et al. (48) found that potatoes fertilized with 400 pounds K_2O per acre contained significantly lower levels of phenolics than those with 140 pounds K_2O per acre. However, Mapson et al. (44) reported that tubers from plants in a controlled nutrient solution experiment given three times more KCl than the control contained higher phenolic contents, polyphenol oxidase activity and darkened more severely than the control. Dwelle et al. (19) found that potassium fertilization had no effect on phenolic concentration or blackspot susceptibility when potassium levels were initially adequate. In plots where potassium was deficient, fertilization with 250 pounds K_2O per acre decreased the phenolic concentration and blackspot susceptibility only to the same level as the plots with adequate potassium. Increasing application rates from 0 to 250 pounds K_2O per acre resulted in increases in petiole and tuber potassium content. Additional fertilization up to 500 pounds K_2O per acre did not decrease phenolic

concentration, blackspot susceptibility, or increase petiole or tuber potassium content.

Berecki et al. (3) found that increasing potassium fertilization from 60 to 180 pounds K_2O per acre resulted in an increase in polyphenol oxidase activity. But Mondy et al. (48) reported no increase in activity when fertilization was increased from 140 to 400 pounds K_2O per acre.

Mulder (49) also noted that copper deficiency resulted in a decrease in tyrosine concentration. Weaver et al. (72) found a negative correlation between copper concentration of tissue within a cultivar and blackspot susceptibility. They also reported that tissue from the resistant cultivar Red Pontiac contained more than two times as much copper as the susceptible Russet Burbank. Application of chelated copper to the foliage of potato plants increased the tuber copper content up to three times, but decreased the polyphenol oxidase activity.

Nitrogen deficiency has also been associated with lower tyrosine concentration (49). Talley (64) reported that the concentration of all amino acids increase with increasing nitrogen content of the tissue. However, tyrosine increased the least in total concentration of any of the amino acids.

Mapson et al. (44) reported that climatic factors also effect phenolic content and tissue darkening. Their results indicate that the amount of moisture received during the season was related to tyrosine content of the tubers.

Reeve et al. (59) suggested that sampling methods which do not take into account the variability of constituents within the tuber may

explain the lack of correlation of these constituents with susceptibility. They reported a large variation in mineral composition from bud to stem end within the tuber. They also found variability in constituents between the cortical and pith regions of the tuber. Cole (13) found differences in specific gravity between the pith, vascular, and cortical regions of the tuber. These differences were cultivar dependent which suggests that the specific gravity of whole tubers may not be an adequate measurement for relating specific gravity to blackspot susceptibility.

Weaver et al. (77) reported that polyphenol oxidase and catalase activity varies significantly between areas of a tuber and tubers of the same cultivar. Weaver and Hautala (73) state that because of this variability, large numbers of replicates must be used to show differences in enzyme activity.

Phenolic substrate concentration also varies throughout the tuber and between tubers of the same cultivar. Craft et al. (15) found that the chlorogenic acid concentration in the cortex was three times higher than the pith region in both Russet Burbank and Kennebec tubers. Reeve et al. (59) reported that tyrosine was more generally distributed throughout tubers of the same two cultivars. The stem end contained higher concentrations of tyrosine than the bud end.

Summary

Rheological properties of the cells, specific gravity, and biochemical properties which affect the potential of the tissue to darken have been shown to influence the blackspot susceptibility of potato

cultivars. Differences between cultivars in rheological properties such as tissue strength, elasticity, and firmness have been measured but are not always related to differences in blackspot susceptibility. These relationships may be modified by tissue turgor and fertility levels.

Specific gravity and cell size have been related to susceptibility but may reflect differences in tuber maturity, which influences cell rheological properties. Specific gravity is also related to cell water content, which influences blackspot susceptibility.

Development of the blackspot involves the enzymatic oxidation of tyrosine and possibly chlorogenic acid, which results in pigment formation. Polyphenol oxidase is the most important enzyme in this process. Polyphenol oxidase activity has not been shown to be a factor limiting the oxidation of phenolic substrates in damaged tissue. It has been suggested that certain factors may act to modify enzyme activity and influence blackspot susceptibility. Reducing substances or systems and inhibitors are factors which could modify enzyme activity, although this has not been demonstrated in potato tuber tissue.

The levels of certain nutrients may influence blackspot susceptibility. High levels of potassium and copper have been associated with resistance. Potassium may act by influencing tissue phenolic concentration, polyphenol oxidase activity, water content, specific gravity, and rheological properties. Copper may effect enzyme activity.

Mineral and biochemical constituents vary throughout a tuber and between tubers of the same cultivar. This may explain why some

constituents have not been consistently associated with blackspot susceptibility.

CHAPTER III

RELATIONSHIP OF BLACKSPOT SUSCEPTIBILITY TO TISSUE EXTRACT DARKENING, PHENOLIC CONTENT, DRY MATTER, AND POTASSIUM CONTENT OF SIX POTATO CLONES

Objective

Blackspot susceptibility of tissue is determined by the rheological and biochemical properties of the cells. Rheological properties influence the extent of cellular damage which occurs while biochemical properties influence the potential of the tissue to darken when damaged.

This study had two objectives: (a) to determine if the potential of the tissue to darken, without the influence of rheological properties, was related to blackspot susceptibility of the 6 clones; and (b) to determine if the relationships between certain biochemical properties of the tubers and blackspot susceptibility which have been described in the literature were consistent for these clones.

Materials and Methods

Tubers from 6 clones: Centennial, BC 9289-1, Russet Burbank, Lemhi, WC 567-1, and BC 9071-6, grown at the San Luis Valley Research Center in Monte Vista, Colorado, were selected for this study. The tubers were harvested in September of 1982. These clones were chosen based on their susceptibility to blackspot as

determined by two years of evaluations. Lemhi and Russet Burbank were consistently very susceptible, BC 9289-1 and Centennial very resistant. WC 657-1 and BC 9071-6 varied from susceptible to resistant, depending on the year.

Blackspot Evaluation

Ten uniformly sized tubers were selected from each clone. Prior to evaluation, tubers were stored at 3-5°C for 6 months. The tubers were kept at 5°C prior to bruising. Bruising was done by dropping a 150 g weight 45 cm onto 3 previously marked locations. The weight used was 9.5 cm long with a 1.3 cm radius hemi-spherical end. The tubers were incubated at 28°C for 36 hours following bruising.

The blackspot index was calculated by determining the percentage of bruised loci which discolored on the 10 tubers and multiplying by the mean color. The bruise color was evaluated using a scale from 1 to 3. A reading of 1 indicates very little discoloration while 3 indicates an intense black color. The index values are based on both the amount and intensity of the discolored tissue. The index values range from 0 for resistance to 300 for maximum susceptibility.

Tissue Extract Darkening

Procedures used for evaluating tissue extract darkening were modified from Walker (71). Prior to the evaluation, the tubers were stored at 3-5°C for 5 months. Three uniformly sized tubers were washed, air-dried, and placed at 3-5°C. One-half tuber from each clone was used for each observation. Tubers were cut in half lengthwise and the pith region removed, leaving approximately 1 cm of

outer medullary, cortical, and epidermal tissue. The tissue was sliced into small pieces and mixed to ensure a random sample from both stem and bud ends. The sample, which consisted of 40 g of tissue, was placed in a 250 ml square glass container containing 80 ml of cold 0;2 M maleate buffer (pH 6.8). The sample was placed at 3-5°C and nitrogen gas was bubbled through the solution for 3 minutes to displace oxygen. Samples were blended at 3-5°C with a Brinkman Polytron homogenizer (Model PT 10-35) at medium speed for 20 seconds. The resulting extract was divided into three 25 ml aliquots. Each aliquot was poured into a test tube containing either 5 ml of 0.3% sodium thiosulfate, 5 ml of distilled water, or 5 ml of 0.001 M catechol. The solutions were immediately placed into a water bath at 28°C. The solutions were mixed every 15 minutes. After one hour, solutions were filtered through Eaton-Dikeman grade 515 fluted filter paper. A 5 ml aliquot of the filtrate was pipetted into a 1.3 cm diameter spectrophotometer tube. The absorbance of the solutions were measured at 480 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. The wavelength of 480 nm provided the maximum absorbance. The instrument was adjusted to 100% transmission using the solution containing 0.3% sodium thiosulfate, which did not darken.

Walker (71) states that this procedure allows the determination of both the endogenous potential of the tissue to darken and darkening in the presence of excess substrate (catechol).

Tissue from the other half of the tuber was treated in an identical manner, with the exception that the extracts were filtered immediately after being mixed with the reagents. The purpose of this procedure was to provide two extracts from the same tuber in which

darkening occurred with the filterable cell wall fragments either present or absent.

The data were analyzed as a three-way analysis-of-variance.

Phenolic Analysis

Prior to analysis, tubers were stored at 3-5°C for 6 months. Three uniformly sized tubers were selected from each clone. Tubers were washed, air-dried, and sliced to a uniform thickness of 3 mm with a Hobart meat slicer. Tissue was taken from the outer cortical region of the slices using a 1.0 cm diameter cork borer. Cores from all slices of one tuber, except the end sections, were combined to make each sample. Three samples were prepared for each of the 6 clones. The samples were placed on 8-mesh stainless steel screens, rinsed in distilled water, drained, and then frozen at -70°C for 24 hours. The frozen tissue was lyophilized for 48 hours. The freeze-dried tissue was ground through a 40-mesh screen, placed in air-tight vials, and stored at -10°C until analyzed.

Two g of freeze-dried tissue was placed in a 2.3 cm diameter test tube containing 30 ml of 80% ethanol. The solution was mechanically stirred every 15 minutes during extraction. After boiling 3 hours at 85°C, the extract was cooled to room temperature and filtered through Whatman No. 1 filter paper into a 200 ml Erlenmyer flask using a Buchner funnel and a slight vacuum. The powder which remained on the filter was washed once with 80% ethanol. The filtrate was poured into a 25 ml volumetric flask and diluted to volume with 80% ethanol.

Methods used for determination of ascorbic acid, total phenols, and o-diphenols were essentially those of Johnson and Schaal (34). Ascorbic acid content of the extracts was determined by the dichlorophenolindophenol dye method (24). Two ml of the ethanol extract was mixed with 10 ml of dye in a 2.5 cm diameter spectrophotometer tube. The absorbance of the solution was measured after 30 seconds at 515 nm using a Bausch and Lomb Spectronic 20 spectrophotometer. To adjust the spectrophotometer to 100% transmission the dye was replaced by 10 ml of distilled water. A standard curve determined for ascorbic acid with the dye was used for calculating ascorbic acid content of the samples (Figure A-1).

Total phenols were determined with Folin-Denis reagent. One ml of the ethanol extract was pipetted into a 100 ml volumetric flask containing 70 ml of distilled water. Two ml of Folin-Denis reagent was added and the solution allowed to stand for 2 minutes. Twenty ml of a 10% sodium carbonate solution was added and the solution diluted to volume with distilled water. The solutions were kept at room temperature for 1.5 hours to allow the development of the blue color. Twenty-five ml of the solution was pipetted into a 2.5 cm diameter spectrophotometer tube and the absorbance measured at 660 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. A blank sample was prepared in which the Folin-Denis reagent was replaced by 2 ml of distilled water. A standard curve for Folin-Denis reagent was determined using chlorogenic acid (Figure A-2). This curve was used to calculate total phenol content.

A standard curve was also determined for ascorbic acid with Folin-Denis reagent (Figure A-3). The purpose of this procedure

was to determine the correction factor for ascorbic acid to subtract from the total phenol determination (Figure A-2). Ascorbic acid and all phenols react with Folin-Denis reagent to produce a blue color. The total phenol measurement was adjusted by using the value for ascorbic acid calculated from Figure A-1 to determine the correction factor from Figure A-3.

The o-diphenol content was determined using Arnow reagent. One ml of the ethanol extract was pipetted into a 2.5 cm diameter spectrophotometer tube. One ml of 0.5 N HCL, 1 ml of Arnow reagent, 10 ml of distilled water, and 2 ml of 1.0 N NaOH were added and the contents mixed. The absorbance was measured after 30 seconds at 515 nm with a Bausch and Lomb Spectronic 20 spectrophotometer. In the blank sample, the Arnow reagent was replaced by 1 ml of distilled water. A standard curve for Arnow reagent was determined using chlorogenic acid (Figure A-4). This curve was used to calculate the o-diphenol content. Arnow reagent does not react with monophenols or ascorbic acid (34).

The tyrosine content of the sample was obtained by subtracting the o-diphenol content from the corrected total phenol content. This value would include all monophenols, including tyrosine. Because the content of other monophenols is low compared to tyrosine, the value is a good estimate of tyrosine content (34).

All of the data were calculated on a mg per g dry weight basis. One sample from each clone was analyzed in each of the three replications. The data for each constituent were analyzed as a two-way analysis-of-variance.

Dry Matter and Potassium Content

The tubers had been stored at 3-5°C for 6 months prior to analysis. Three uniformly sized tubers from each clone were washed and air-dried. The tubers were sliced in half lengthwise and the pith region removed and discarded. This left approximately 1 cm of the outer medullary, cortical, and epidermal tissue. Tissue from both halves was sliced into small pieces and composited. A sample of 20 g was placed in an aluminium weighing pan and dried to a constant weight at 70°C. The percentage dry matter was calculated by dividing the sample dry weight by the fresh weight. The dried samples were ground through a 40-mesh screen and taken to the CSU soil testing lab for nutrient analysis. Sample potassium content was determined as percentage of dry matter from a 2% acetic acid extract.

The data for each constituent were analyzed as a one-way analysis-of-variance.

Results and Discussion

The 6 clones differed considerably in blackspot susceptibility. The index values ranged from 30.3 to 175.0 (Table 1). WC 567-1, BC 9289-1, and Centennial were the most resistant clones. Lemhi was the most susceptible, while Russet Burbank was slightly less susceptible. BC 9071-6 was moderately susceptible but exhibited a high proportion of shatter bruise. In contrast to blackspot, shatter bruise does not discolor as intensely and appears as a series of cracks which break the skin of the tuber. The discoloration occurs

Table 1

Comparison of 6 Clones with Respect to Blackspot Susceptibility. Values Represent Means of 10 Tubers Bruised at 3 Locations Each

Clone	Mean % Loci Discolored	Mean Color[a]	Blackspot Index[b]
Centennial	30.0	1.9	57.0
BC 9289-1	33.3	1.1	36.7
Russet Burbank	66.6	2.0	133.3
Lemhi	70.0	2.5	175.0
WC 567-1	23.3	1.3	30.3
BC 9071-6	70.0	1.4	98.0
Mean	48.9	1.7	88.4

[a]Darkness increases with increasing value.

[b]Index = mean % loci discolored x mean color; 0 = resistant, 300 = maximum severity.

along the edges of the cracks. The blackspot index does not separate these two types of bruise very well.

The absorbance of the tissue extracts after 1 hour at 28°C is given in Table 2. Over all experimental conditions, the differences between clones were highly significant. Statistical analysis of the data is given in Appendix Table A-1. The purpose for the procedure used for tissue extraction was to measure the potential of the tissue to darken without the influence of rheological properties. The results indicate that the potential to darken is not always related to blackspot susceptibility. BC 9289-1, a very resistant clone, had a very low potential to darken. But Centennial and WC 567-1, both resistant clones, darkened to about the same extent as Lemhi, the most susceptible clone. The potential is only expressed when extensive cellular disruption occurs, as during the extraction procedure. Centennial and WC 567-1 have an intermediate to high potential to darken when cells were ruptured, but the potential is not expressed in intact tubers when bruised. Apparently, these clones have rheological properties which prevent extensive cellular damage from occurring.

The presence of cell wall fragments resulted in a highly significant increase in tissue darkening (Table 3). The interaction between clones and cell wall fragments was also significant. The differences between clones in tissue extract darkening were not significant when cell wall fragments were absent (Table 3, Figures 3 and 4). The differences were significant when the cell wall fragments were present. The effect of the presence or absence of cell wall fragments on tissue extract darkening was not the same for all clones. Tissue extracts of

Table 2

Absorbance (480 nm) of Tissue Extracts from 6 Clones after 1 Hour at 28°C
Values are Means of 3 Observations

Clone	Without Added Substrate		With Added Substrate		Mean
	Without Cell Wall Fragments	With Cell Wall Fragments	Without Cell Wall Fragments	With Cell Wall Fragments	
Centennial	0.477	0.612	0.946	1.004	0.760
BC 9289-1	0.285	0.246	0.697	0.722	0.488
Russet Burbank	0.469	0.820	0.909	1.420	0.905
Lemhi	0.444	0.524	0.837	0.989	0.689
WC 567-1	0.367	0.445	0.789	0.915	0.631
BC 9071-6	0.230	0.266	0.566	0.689	0.438
Mean	0.379	0.486	0.791	0.957	
Overall Mean	0.433		0.874		

Table 3

Absorbance (480 nm) of Tissue Extracts from 6 Clones After 1 Hour at 28°C in the Absence or Presence of Cell Wall Fragments. Values are Means of 6 Observations Fisher's LSD at the 5% Probability Level

Clone	Without Cell Wall Fragments[a]	With Cell Wall Fragments[a]	Mean
Centennial	0.712	0.809	0.760
BC 9289-1	0.491	0.484	0.488
Russet Burbank	0.689	1.120	0.905
Lemhi	0.641	0.757	0.698
WC 567-1	0.578	0.680	0.631
BC 9071-6	0.398	0.478	0.438
Mean	0.585	0.722	

Clone FLSD = 0.126

[a]Includes observations with and without added substrate.

Figure 3. Absorbance of tissue extracts without added substrate after 1 hour at 28°C, in the absence or presence of cell wall fragments. Values are means of 3 observations. Vertical bars represent LSD at 5% probability level. Bars not overlapping are significantly different. Clones:

1. Centennial
2. BC 9289-1
3. Russet Burbank
4. Lemhi
5. WC 567-1
6. BC 9071-6

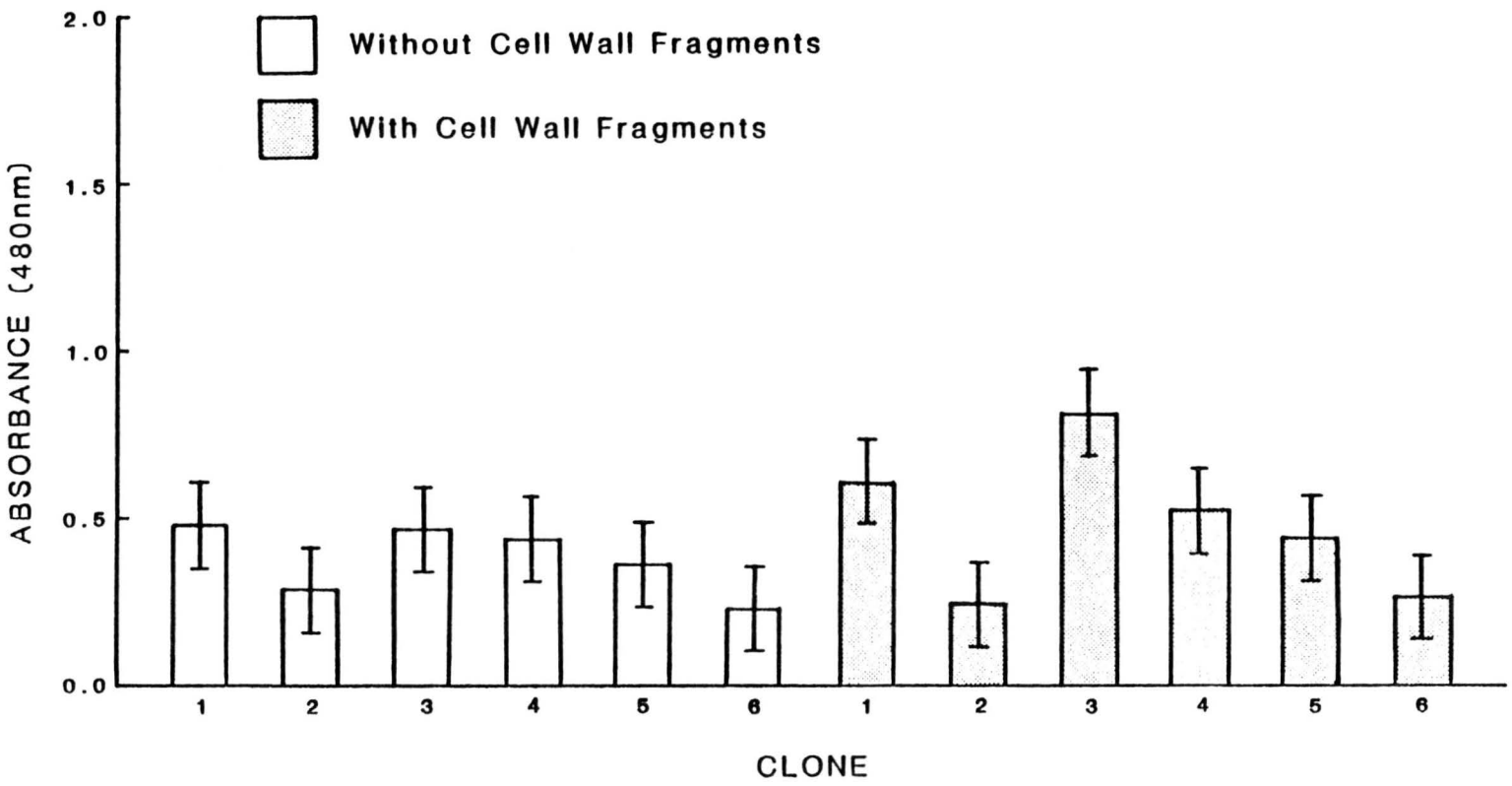
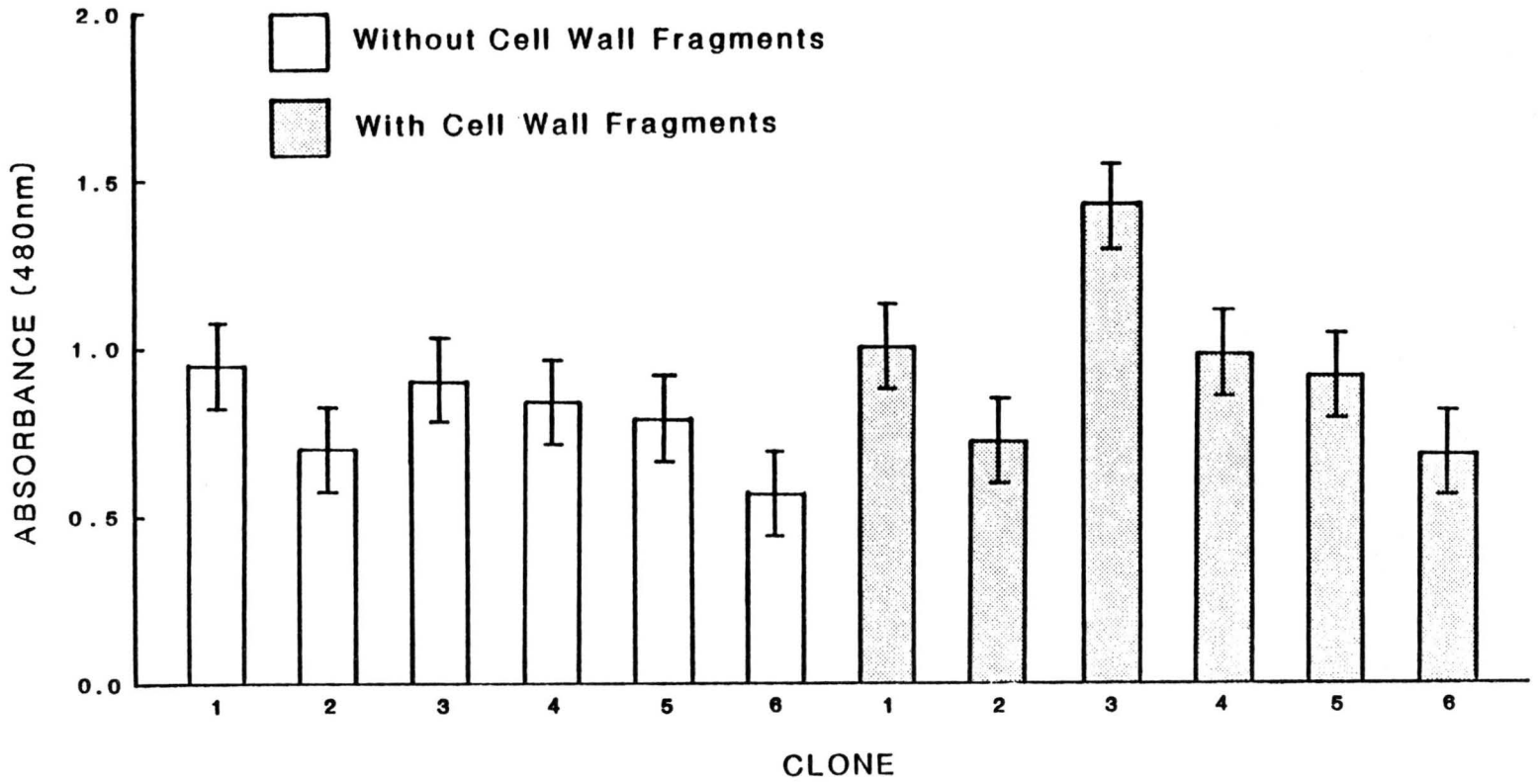

Without Cell Wall Fragments
With Cell Wall Fragments
ABSORBANCE (480nm)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
1
2
3
4
5
6
CLONE

Figure 4. Absorbance of tissue extracts with added substrate after 1 hour at 28°C, in the absence or presence of cell wall fragments. Values are means of 3 observations. Vertical bars represent LSD at 5% probability level. Bars not overlapping are significantly different. Clones:

1. Centennial
2. BC 9289-1
3. Russet Burbank
4. Lemhi
5. WC 567-1
6. BC 9071-6


Without Cell Wall Fragments
With Cell Wall Fragments
ABSORBANCE (480nm)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
1
2
3
4
5
6
CLONE

all clones, with the exception of BC 9289-1 without added substrate, darkened more when cell wall fragments were present (Figures 5 and 6). However, only Russet Burbank showed a statistically significant increase in tissue extract darkening due to the presence of cell wall fragments.

To further examine the effect of cell wall fragments, the ratio of the absorbance of the extracts with cell wall fragments to the absorbance without cell wall fragments was calculated. Results are given in Table 4. Regardless of whether or not substrate was added, the differences in response to cell wall fragments between clones were significant. Statistical analysis of the data is given in Appendix Table A-2.

The differences in tissue extract darkening due to the absence or presence of cell wall fragments were not related to blackspot susceptibility. WC 567-1, BC 9071-6, Centennial, and Lemhi all responded to about the same extent. Cell wall fragments of BC 9289-1 had the least effect on tissue extract darkening and those of Russet Burbank the greatest effect. The differences may be due to the absence or presence of oxidizing enzymes or the absence or presence of factors which modify enzyme activity. Some peroxidase and polyphenol oxidase activity has been found associated with the cell wall fraction (16).

In clones where the potential to darken was low, such as BC 9289-1 and BC 9071-6, factors other than rheological properties may be important in determining resistance. Polyphenol oxidase activity and phenolic content concentration are biochemical properties which influence the potential of the tissue to darken. The purpose of adding substrate to the extracts was to determine if enzyme

Figure 5. Comparison of darkening of tissue extracts in the absence and presence of cell wall fragments after 1 hour at 28°C, without added substrate. Values are means of 3 observations. Vertical bars represent LSD at 5% probability level. Bars not overlapping are significantly different. Clones:

1. Centennial
2. BC 9289-1
3. Russet Burbank
4. Lemhi
5. WC 567-1
6. BC 9071-6

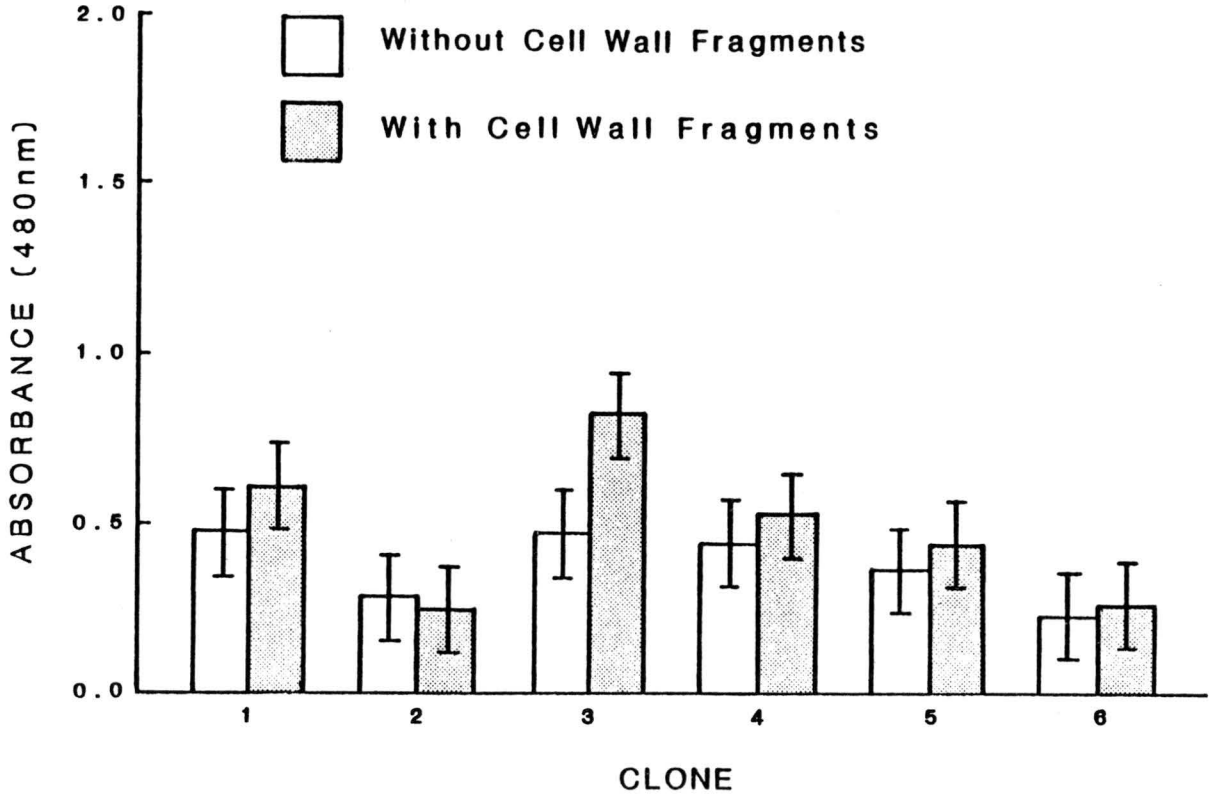

Without Cell Wall Fragments
With Cell Wall Fragments
ABSORBANCE (480nm)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
CLONE

Figure 6. Comparison of darkening of tissue extracts in the absence and presence of cell wall fragments after 1 hour at 28°C, with added substrate. Values are means of 3 observations. Vertical bars represent LSD at 5% probability level. Bars not overlapping are significantly different. Clones:

1. Centennial
2. BC 9289-1
3. Russet Burbank
4. Lemhi
5. WC 567-1
6. BC 9071-6

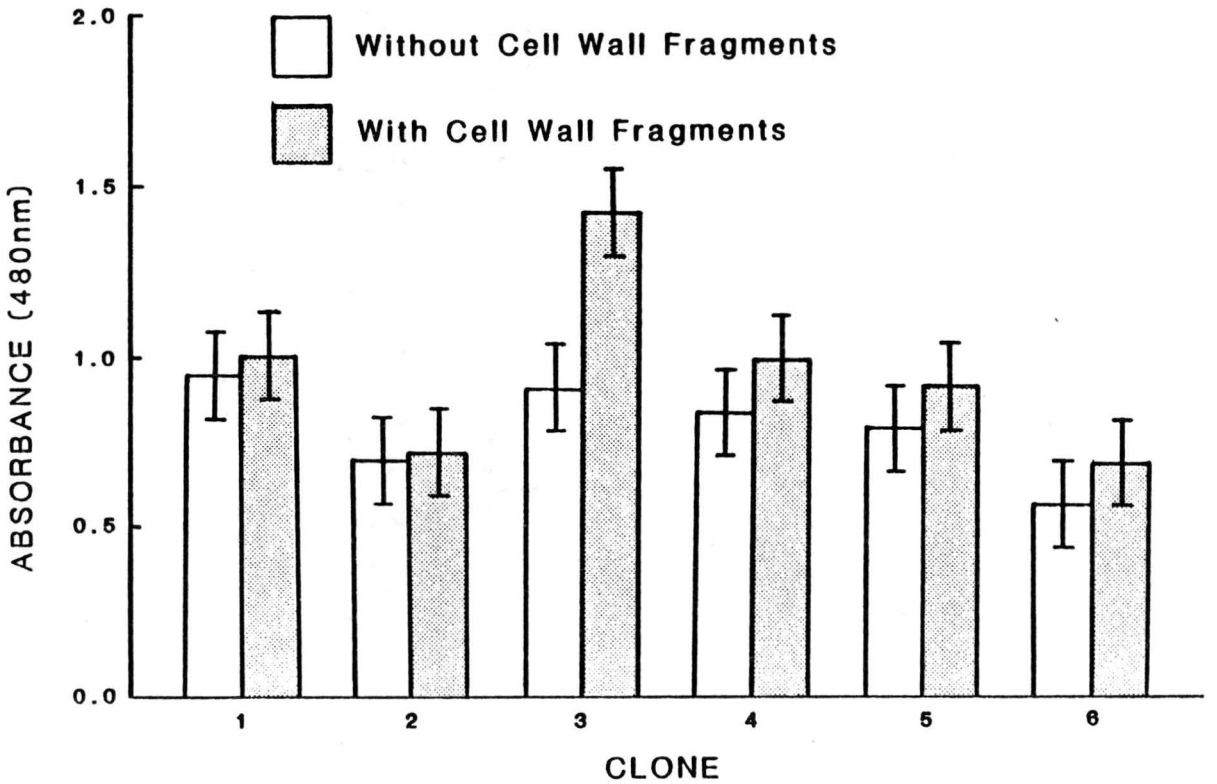

Without Cell Wall Fragments
With Cell Wall Fragments
ABSORBANCE (480nm)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
CLONE

Table 4

Ratio of the Absorbance (480 nm) of Tissue Extracts from 6 Clones with Cell Wall Fragments to Without Cell Wall Fragments after 1 Hour at 28°C. Values are Means of 3 Observations
Fischer's LSD at the 5% Probability Level

Clone	Without Added Substrate	With Added Substrate	Mean
Centennial	1.28	1.07	1.18
BC 9289-1	0.89	1.09	0.99
Russet Burbank	1.77	1.55	1.66
Lemhi	1.19	1.19	1.19
WC 567-1	1.26	1.18	1.22
BC 9071-6	1.16	1.22	1.19
Mean	1.25	1.22	

Clone FLSD = 0.56

activity was limiting tissue extract darkening. Overall, the effect of substrate addition was highly significant. Addition of substrate resulted in a doubling of tissue extract darkening (Table 5). All clones responded to addition of substrate, but the increases were not consistent.

To further examine the effect of substrate, the ratio of the absorbance of the extracts with added substrate to the absorbance without added substrate was calculated. Results are given in Table 6. Regardless of whether or not cell wall fragments were present, the differences in response to substrate between clones were significant. Statistical analysis of the data is given in Appendix Table A-3. Tissue extract darkening of BC 9289-1 and BC 9071-6 increased over two and one-half times in response to substrate. The response was significantly less in Lemhi, Russet Burbank and Centennial.

If enzyme activity was limiting tissue extract darkening, the extracts would not respond to the addition of substrate. The results indicate that the enzyme activity was not the limiting factor. However, even with addition of substrate, the darkening of the tissue extracts was significantly different (Table 5). This suggests that there may be other factors present which modify enzyme activity. Factors which affect oxidation of phenols to quinones by polyphenol oxidase include reducing substances or systems and inhibitors (1,5,41,51,56).

Reducing substances within cells which may reduce quinones to phenols include ascorbic acid, glutathione, cysteine, NADH, and NADPH. However, it is unlikely that reducing substances are responsible for the lack of darkening in some tissue extracts. Ponting and

Table 5

Absorbance (480 nm) of Tissue Extracts from 6 Clones after 1 Hour at 28°C in the Presence or Absence of Added Substrate
Values are Means of 6 Observations

Clone	Without Added[a] Substrate	With Added[a] Substrate	Mean
Centennial	0.545	0.975	0.760
BC 9289-1	0.266	0.710	0.488
Russet Burbank	0.645	1.165	0.905
Lemhi	0.484	0.913	0.698
WC 567-1	0.406	0.852	0.631
BC 9071-6	0.248	0.628	0.438
Mean	0.433	0.874	

[a]Includes observations with and without cell wall fragments.

Clone FLSD = 0.126

Josyln (57) reported that ascorbic acid was completely oxidized within 5 seconds in apple tissue extracts due to polyphenol oxidase activity. In the extract polyphenol oxidase may function at 95% of maximum activity (1). The extraction procedures may also disrupt systems which produce reducing power within cells. It is more likely that reducing substances would effect blackspot development in intact tubers where oxidation occurs much more slowly and cells are not completely disrupted.

Hyodo and Uritani (32) suggested that phenolic inhibitors may regulate polyphenol oxidase activity in sweet potato tissue. Several naturally occurring phenolic compounds, including p-coumaric, ferulic, and cinnamic acids are inhibitors of polyphenol oxidase purified from potato tuber tissue (41). The quinic acid esters of p-coumaric and cinnamic acids have been detected in potato tuber tissue but do not inhibit the enzyme (17). These compounds are precursors of chlorogenic acid. p-Coumaric and cinnamic acids were not detected. It is possible that these compounds are present in low concentrations in damaged tissue where chlorogenic acid is rapidly synthesized. If the enzyme does have a high affinity for the inhibitors, as reported by Hyodo and Uritani (32), then low concentrations may be adequate to effect enzyme activity.

Clones which exhibited the lowest tissue extract darkening: BC 9289-1, BC 9071-6, and WC 567-1, darkened more in response to addition of substrate than the other clones (Table 6). This suggests that the phenolic substrate concentration may be limiting tissue extract darkening. The predominant phenolic substrates in potato tissue are tyrosine and chlorogenic acid (18, 44).

Table 6

Ratio of the Absorbance (480 nm) of Tissue Extracts from 6 Clones with Added Substrate to Without Added Substrate after 1 Hour at 28°C
Values are Means of 3 Observations
Fischer's LSD at the 5% Probability Level

Clone	Without Cell Wall Fragments	With Cell Wall Fragments	Mean
Centennial	1.99	1.67	1.83
BC 9289-1	2.51	2.91	2.71
Russet Burbank	1.94	1.77	1.86
Lemhi	1.88	1.89	1.89
WC 567-1	2.17	2.06	2.12
BC 9071-6	2.47	2.60	2.54
Mean	2.16	2.15	

Clone FLSD = 0.48

Results of determinations of the ascorbic acid, total phenols, o-diphenols, and tyrosine are given in Table 7. The clones did not vary significantly in any of the four constituents. Statistical analysis of the data is given in Appendix Table A-4. The levels of tyrosine measured are within the range of values reported in the literature (63). The o-diphenol content accounted for approximately 20% of total phenols in the tissue.

The tyrosine content was not closely related to either tissue extract darkening or response to addition of substrate. Russet Burbank and WC 567-1 tended to have high tyrosine contents, but WC 567-1 darkened significantly less than Russet Burbank. WC 567-1 also responded more to addition of substrate than other clones which had lower tyrosine contents, such as Centennial and Lemhi.

The o-diphenol content was closely related to tissue extract darkening and response to addition of substrate. Clones which had the highest potential to darken: Russet Burbank, Lemhi, and Centennial, tended to have high o-diphenols. However, Centennial darkened less than Russet Burbank but tended to have slightly higher o-diphenol content. Clones which responded the most to addition of substrate tended to have low o-diphenol contents.

The predominant o-diphenol in potato tissue is chlorogenic acid (34). Mapson et al. (44) found that chlorogenic acid content can effect the rate of tissue darkening. None of the cultivars studied had a chlorogenic acid content low enough to limit the darkening rate. The results indicate that addition of an o-diphenol substrate (catechol) to tissue extracts increased darkening more in clones with low o-diphenol

Table 7

Comparison of 6 Clones with Respect to Ascorbic Acid, Total Phenol, o-diphenol, and Tyrosine Content. Values are Means of 3 Observations. Expressed as mg per g Dry Weight

Clone	Ascorbic[a] Acid	Total Phenols	o-Diphenols	Tyrosine[c]
Centennial	0.202	1.892	0.379	1.450
BC 9289-1	0.233	1.442	0.250	1.192
BC 9289-1	0.233	1.442	0.250	1.192
Russet Burbank	0.153	2.534	0.353	2.171
Lemhi	0.243	1.859	0.317	1.542
WC 567-1	0.314	2.355	0.300	2.054
BC 9071-6	0.261	1.425	0.284	1.142
Mean	0.234	1.907	0.316	1.592

[a]In ethanol extract.

[b]Corrected for ascorbic acid.

[c]Tyrosine content = total phenols - o-diphenols.

contents. It is possible that o-diphenols are limiting the darkening rate to some extent in these clones.

The tyrosine content was not consistently related to blackspot susceptibility WC 567-1, a resistant clone, tended to have a tyrosine content higher than Lemhi, the most susceptible clone.

The o-diphenol content was also not closely related to blackspot susceptibility. Centennial, a resistant clone, tended to have the highest o-diphenol content.

Results of dry matter and potassium determinations are given in Table 8. The clones differed significantly in both dry matter and potassium content. Statistical analysis of the data is given in Appendix Table A-5. Dry matter content was closely related to blackspot susceptibility. In general, resistant clones had lower dry matter content than susceptible clones. However, Centennial had a dry matter content almost as high as Lemhi. If dry matter content does reflect changes in cell wall properties, starch content, and water content associated with blackspot susceptibility (30), then these relationships are not consistent in all clones. Cell volume may interact with dry matter to affect these relationships (46).

Potassium content may influence blackspot susceptibility by affecting dry matter content, phenolic content, rheological properties, and polyphenol oxidase activity (3,31,37,48). Van Es and Hartmans (21) state that tubers with less than 2% potassium in the dry matter are generally blackspot susceptible. The results indicate that this relationship was not consistent for all the clones. WC 567-1 and Centennial contained less than 2% potassium but were very resistant

Table 8

Comparison of 6 Clones with Respect to Dry Matter and Potassium Content. Values are Means of 3 Observations. Fisher's LSD at the 5% Probability Level

Clone	% Dry Matter	% K in Dry Matter
Centennial	24.5	1.90
BC 9289-1	23.9	2.20
Russet Burbank	26.5	1.78
Lemhi	25.7	1.99
WC 567-1	23.4	1.89
BC 9071-6	24.0	2.18
Mean	24.7	1.78

Dry matter FLSD = 1.7

% K FLSD = 0.26

to blackspot. Lemhi, the most susceptible clone, had a potassium content slightly below 2%.

Summary

No one factor studied was consistently related to differences in blackspot susceptibility. It seems that biochemical and rheological properties interact to determine susceptibility. Of the factors studied, dry matter content was the most closely related to blackspot susceptibility. The o-diphenol content was closely related to the extent of tissue extract darkening and response to addition of substrate.

WC 567-1 had an intermediate potential to darken, but had a high substrate level. This indicates that there may be other factors present which modify enzyme activity. The low dry matter content of this clone may indicate that extensive cellular damage does not occur when tubers are bruised. BC 9289-1 had a low potential to darken. This was probably due to the low level of substrate present in the tissue. This clone also had a low dry matter content. Centennial had a high potential to darken, but this potential is not expressed when tubers are bruised. This clone apparently has rheological properties which prevent severe cellular damage from occurring. Centennial does not have low dry matter or high potassium content which have been associated with blackspot resistance. Lemhi is very similar to Centennial in almost all characteristics studied. The results do not explain why Lemhi is so susceptible while Centennial is resistant to blackspot.

The differences between clones in tissue extract darkening were not significant when cell wall fragments were filtered, but were significant when cell wall fragments were present. Cell wall fragments of BC 9289-1 had the least effect on tissue extract darkening and those of Russet Burbank the greatest effect. These differences were not related to blackspot susceptibility over all clones.

CHAPTER IV

CHANGES IN ASCORBIC ACID CONTENT OF BLACKSPOT RESISTANT AND SUSCEPTIBLE CLONES FOLLOWING BRUISING

Objective

Previous workers have not found a consistent relationship between the ascorbic acid content of a cultivar and blackspot susceptibility (18, 14). However, only the initial level of ascorbic acid was determined and not the changes which occur following bruising.

The objective of this experiment was to determine if there was a relationship between changes in ascorbic acid content following bruising and blackspot susceptibility. It was hypothesized that the ascorbic acid content of resistant clones would remain relatively constant following bruising. This reducing power could be used to prevent polymerization of quinones associated with darkening reactions. The ascorbic acid content of susceptible clones was expected to decrease as darkening proceeded.

Materials and Methods

Tubers from four clones: Lemhi, Russet Burbank, Centennial, and BC 9829-1, grown at the San Luis Valley Research Center in Monte Vista, Colorado, were selected for this study. The tubers

were harvested in September of 1981 and then stored at 3-5°C until the analyses were begun in February, 1982. Distinct differences in blackspot susceptibility of the four clones had been observed over the past two years. Russet Burbank and Lemhi were very susceptible to blackspot while Centennial and BC 9289-1 were resistant.

Before being bruised, 25 uniformly sized tubers from each clone were washed, placed in bags, identified, and stored at 5°C. The tubers were bruised on one side by dropping a 150 g weight 45 cm onto 3 previously marked locations. The weight used was 9.5 cm long with a 1.3 cm radius hemispherical end. The tubers were incubated at 28°C following bruising. At time intervals of 0, 3, 6, 12, and 24 hours after bruising, the tubers were analyzed for ascorbic acid content. Bruised and nonbruised tissue were obtained from each tuber. A 1.5 cm diameter cork borer was centered over each bruised location and run transversely through the tuber. One centimeter of tissue was removed from each end of the resulting core to provide bruised and nonbruised tissue for analysis. The three core sections of bruised or nonbruised tissue were composited and adjusted to a total weight of 9 g. This sample, which consisted of mostly cortical and epidermal tissue, was immediately placed in 90 ml of 0.25% oxalic acid in a Waring Blender. Blender speed was controlled by a Powerstat Variable Autotransformer which was set at 100 volts. Samples were blended four minutes then filtered through Eaton-Dikeman grade 515 fluted filter paper. The first 10 ml of filtrate was discarded, while the remainder was collected in a 125 ml Erlenmyer flask.

Reduced ascorbic acid content was determined by the 2,6-dichlorophenolindophenol method (24). Samples were prepared for

analysis by pipetting 2 ml of filtrate and 10 ml of dye solution into a 2.3 cm diameter colorimeter tube and mixing with a Bronwill vortex mixer. The color of the solution was read after 10 seconds with an Evelyn Photoelectric Colorimeter using a 515 nm filter. In the blank sample the dye was replaced by 10 ml of distilled water. A 0.015 mg per ml ascorbic acid solution was used as a standard for calculating ascorbic acid content. Ascorbic acid was calculated as mg per 100 g fresh weight. Five tubers from each clone were analyzed at each of the 5 time intervals. The data were analyzed as a three-way analysis-of-variance. Additionally, the data for each clone were analyzed separately as a paired t-test to determine differences between bruised and nonbruised tissue.

Results and Discussion

The ascorbic acid content of each clone for each treatment is given in Table 9. The differences between clones in ascorbic acid content over all treatments were significant. Statistical analysis of the data is given in Appendix Table A-6.

Ponting and Joslyn (57) showed that plant tissue darkening was closely related to ascorbic acid oxidation. Ascorbic acid was oxidized in connection with the reduction of quinones produced by polyphenol oxidase activity.

The initial level of ascorbic acid in nonbruised tissue ranged from 8.71 to 17.45 mg per 100 g fresh weight. The initial level was not related to blackspot susceptibility. Lemhi, a very susceptible clone, had the highest ascorbic acid content. BC 9289-1, a resistant clone, contained about 40% less ascorbic acid than Lemhi.

Table 9

Changes in Ascorbic Acid Content (mg/100 g fresh weight) with Time in Bruised and Nonbruised Tissue of Potato Clones Differing in Blackspot Susceptibility. Values are Means of 5 Tubers

Clone	Treatment	Time After Treatment (hrs) 0	3	6	12	24	Treatment Mean
Lemhi	Bruised	15.73	16.67	16.17	13.84	12.05	14.89
	Nonbruised	17.45	18.80	16.39	15.20	15.59	16.69
BC 9289-1	Bruised	10.78	9.82	9.99	10.84	8.89	10.06
	Nonbruised	10.56	11.07	11.09	10.60	9.51	10.66
Russet Burbank	Bruised	7.67	8.78	8.80	8.37	6.67	8.06
	Nonbruised	8.71	8.61	8.67	7.95	7.57	8.30
Centennial	Bruised	14.86	17.40	16.85	16.19	16.62	16.39
	Nonbruised	14.52	16.62	15.61	14.96	14.67	15.20
Mean	Bruised	12.26	12.81	12.95	12.31	11.05	12.35
	Nonbruised	12.81	13.69	12.94	12.17	11.83	12.71

Over all clones and times there was no significant difference in ascorbic acid content between bruised and nonbruised tissue. However, there was a significant interaction between clones and bruised versus nonbruised tissue (Table 10). Bruised tissue of Lemhi, BC 9289-1, and Russet Burbank tended to have lower ascorbic acid contents than nonbruised tissue. But Centennial contained higher ascorbic acid in bruised tissue. When the data from each clone were analyzed separately as a paired t-test, the differences were significant in Lemhi, Centennial, and BC 9289-1. The difference was not significant in Russet Burbank.

It was expected that ascorbic acid would be oxidized to dehydroascorbic acid (DHA) in bruised tissue as the blackspot developed. Even after blackspot developed in Lemhi tissue, between 6 and 12 hours after bruising, the differences in ascorbic acid content were not great. The mean ascorbic acid content of bruised Lemhi tissue was only 23% less than nonbruised tissue after 24 hours. This was the largest difference observed in any of the clones.

Butt (7) states that darkening of damaged tissue does not occur until all reducing substances have been oxidized. The small changes in ascorbic acid content in bruised potato tuber tissue may indicate that ascorbic acid oxidation is not directly involved in phenolic oxidation in these tissues.

Johnson and Schaal (34) reported that ascorbic acid content doubled in sound cells next to a cut surface within 48 hours after injury. Ascorbic acid was rapidly oxidized in damaged cells. Core sections used to obtain bruised tissue in this experiment would contain both damaged and undamaged cells. The techniques used would

Table 10

Ascorbic Acid Content (mg/100 g fresh weight) of Bruised and Nonbruised Tissue of Potato Clones Differing in Blackspot Susceptibility. Values are Means of 25 Measurements taken 0, 3, 6, 12 and 24 Hours After Treatment
Fisher's LSD at 5% Probability Level

	Treatment		
Clone	Bruised	Nonbruised	Means
Lemhi	14.89	16.69	15.79
BC 9289-1	10.06	10.66	10.36
Russet Burbank	8.06	8.30	8.18
Centennial	16.39	15.20	15.79
Mean	12.33	12.69	

Clone FLSD = 0.69

not differentiate between changes in ascorbic acid content in undamaged cells next to a bruise and the oxidation of ascorbic acid in damaged cells. Vertregt (70) reported that only 5 to 15% of the cells in a blackspot area are damaged and discolor. He suggested that chemical analysis may therefore not be representative of changes occurring in those areas.

Development of pigments associated with blackspot is a slow process, requiring 12 to 24 hours at room temperature. Presumably, the ascorbic acid in bruised tissue would be slowly oxidized during this time. In tissue expressing blackspot, the oxidation would be complete when the pigments begin to develop. In tissue which does not darken when damaged, the ascorbic acid may not be completely oxidized, preventing the development of pigments.

Over all clones, the ascorbic acid content decreased with time after treatment (Table 11). Slight changes in ascorbic acid content occurred in both bruised and nonbruised tissue during incubation at 28°C (Figures 7 and 8). Both Lemhi and Centennial exhibited a slight increase in ascorbic acid content in bruised and nonbruised tissue during the first three hours. Therefore, this increase was a response to handling conditions rather than bruising. The ascorbic acid content of Lemhi, BC 9289-1, and Russet Burbank tended to decrease with time after treatment. Bruised tissue from Lemhi exhibited a 28% decrease in ascorbic acid content between 3 and 24 hours after bruising. But the ascorbic acid content of nonbruised tissue decreased 17% during this same period. These results indicate that the decrease in ascorbic acid was not related to blackspot development. The ascorbic acid content of Centennial tended to

Table 11

Changes in Ascorbic Acid Content (mg/100 g fresh weight) with Time of Potato Clones Differing in Blackspot Susceptibility. Values are Means of 10 Measurements of both Bruised and Nonbruised Tissue. Fisher's LSD at 5% Probability Level

	Time After Treatment (hrs)					
Clone	0	3	6	12	24	Mean
Lemhi	16.59	17.74	16.28	14.52	13.82	15.79
BC 9289-1	10.67	10.45	10.54	10.72	9.20	10.36
Russet Burbank	8.19	8.70	8.74	8.16	7.12	8.18
Centennial	14.69	16.83	16.23	15.58	15.65	15.79
Mean	12.54	13.43	12.94	12.24	11.50	

Clone FLSD = 0.69

Time FLSD = 0.71

Figure 7. Comparison of Lemhi and Russet Burbank with respect to changes in ascorbic acid content with time at 28°C in bruised and nonbruised tissue.

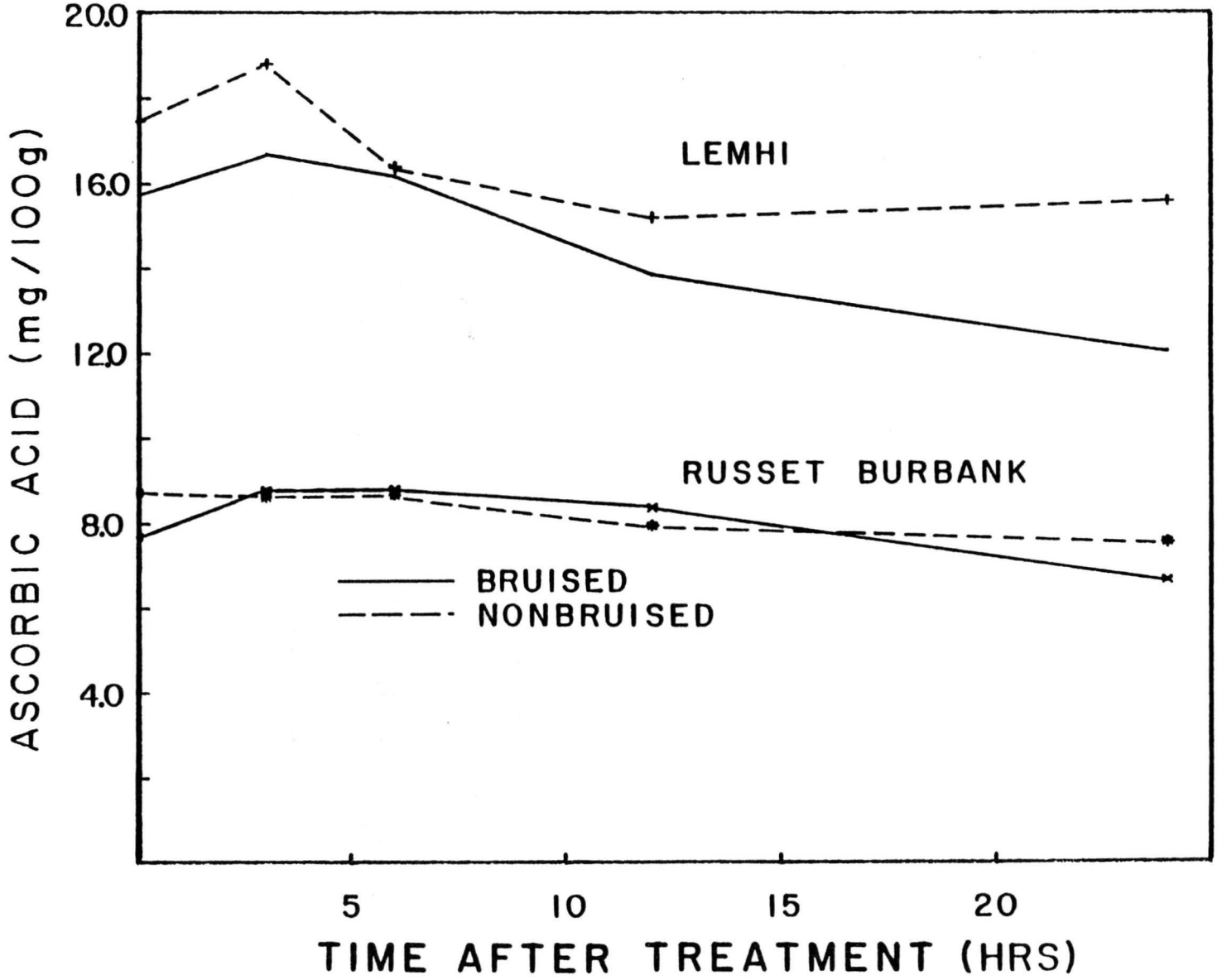
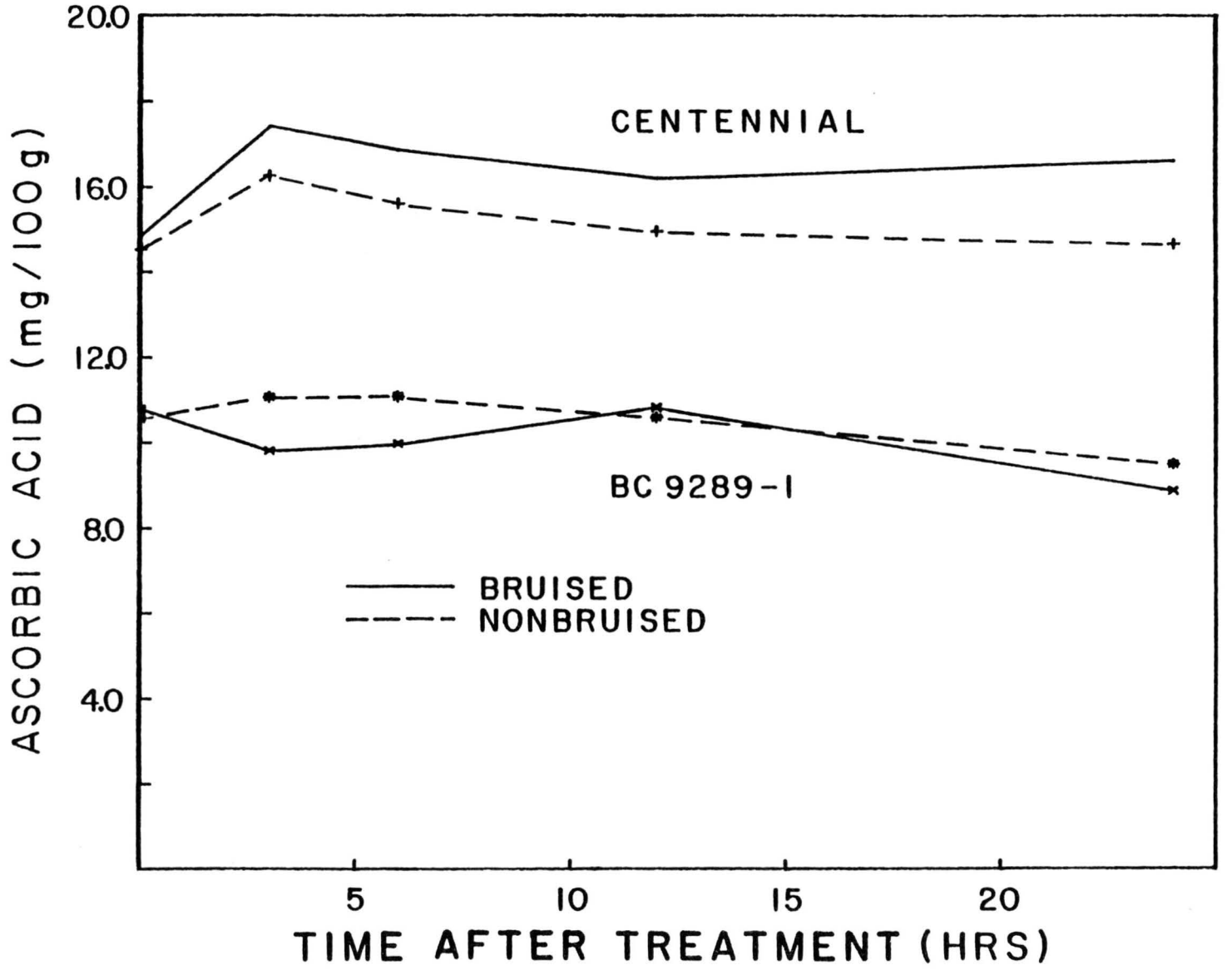

20.0
16.0
12.0
8.0
4.0
ASCORBIC ACID (mg/100g)
LEMHI
RUSSET BURBANK
BRUISED
NONBRUISED
5
10
15
20
TIME AFTER TREATMENT (HRS)

Figure 8. Comparison of Centennial and BC 9289-1 with respect to changes in ascorbic acid content with time at 28°C in bruised and nonbruised tissue.


20.0
16.0
12.0
8.0
4.0
ASCORBIC ACID (mg/100g)
CENTENNIAL
BC 9289-1
BRUISED
NONBRUISED
5
10
15
20
TIME AFTER TREATMENT (HRS)

increase slightly with time after treatment, resulting in a significant interaction between clones and time after treatment (Table 11).

It is difficult to determine from these results the extent of ascorbic acid oxidation in bruised tissue. In undamaged tissue, the ratio of DHA to ascorbic acid is very low (43). Upon injury, ascorbic acid is oxidized and the ratio increases. Ascorbic acid may have been synthesized in bruised tissue, but then oxidized to DHA. Determination of ascorbic acid alone would not detect this. A determination of changes in the ratio of DHA to ascorbic acid might give a better indication of the use of reducing power in damaged tissue.

The oxidation of ascorbic acid is affected by other reducing substances (9). Systems which utilize NAD(P)H from dehydrogenase reactions to continuously reduce dehydroascorbic acid have been demonstrated in plant tissue extracts (43, 79). Such a system could be coupled to phenolic oxidation (5). The fact that changes in ascorbic acid content were not associated with blackspot development in this experiment may indicate that other reducing substances are involved in preventing tissue darkening. NADH, NADPH, glutathione, and cysteine all reduce quinones to phenols.

Reducing power is also utilized in other processes in cells. NADH and NADPH are utilized in production of phenolic precursors for suberin synthesis. Reeve (58) reported extensive suberization of cells in large blackspot areas. Small bruises showed very little evidence of suberization. Clones which exhibit extensive cell damage may utilize reducing power for suberin synthesis. Resistant clones which exhibit limited cellular damage might utilize the reducing power for reduction of quinones.

Summary

Changes in ascorbic acid content of bruised and nonbruised tissue were followed over time to determine if they were related to blackspot susceptibility of four clones. The clones differed significantly in their endogenous level of ascorbic acid, but the differences were not related to blackspot susceptibility.

Differences in ascorbic acid content of bruised and nonbruised tissue of the same tuber were significant in 3 of 4 clones, but were not related to blackspot susceptibility. BC 9289-1, a resistant clone, had slightly lower ascorbic acid in bruised tissue. Centennial, also resistant, had slightly higher ascorbic acid in bruised tissue. The magnitude of the differences in ascorbic acid content between bruised and nonbruised tissue was much less than would be expected if ascorbic acid oxidation was associated with phenolic oxidation.

The ascorbic acid content increased slightly for 3 to 6 hours after tubers were placed at 28°C following storage at 5°C. Lemhi, Russet Burbank, and BC 9289-1 tissue decreased in ascorbic acid content over 24 hours. Centennial tissue increased slightly in ascorbic acid content during the same period. The changes occurred in both bruised and nonbruised tissue and were not related to blackspot susceptibility.

The hypothesis tested in this study was that blackspot resistant clones would exhibit a different pattern of change in ascorbic acid content following bruising than the susceptible clones. The results of this study do not support this hypothesis. Because bruised tissue contains both damaged and undamaged cells, the measurements

may not represent changes which occur in the cells which eventually discolor. More precise techniques are required to determine what changes are occurring in those cells in which the pigments develop.

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APPENDICES

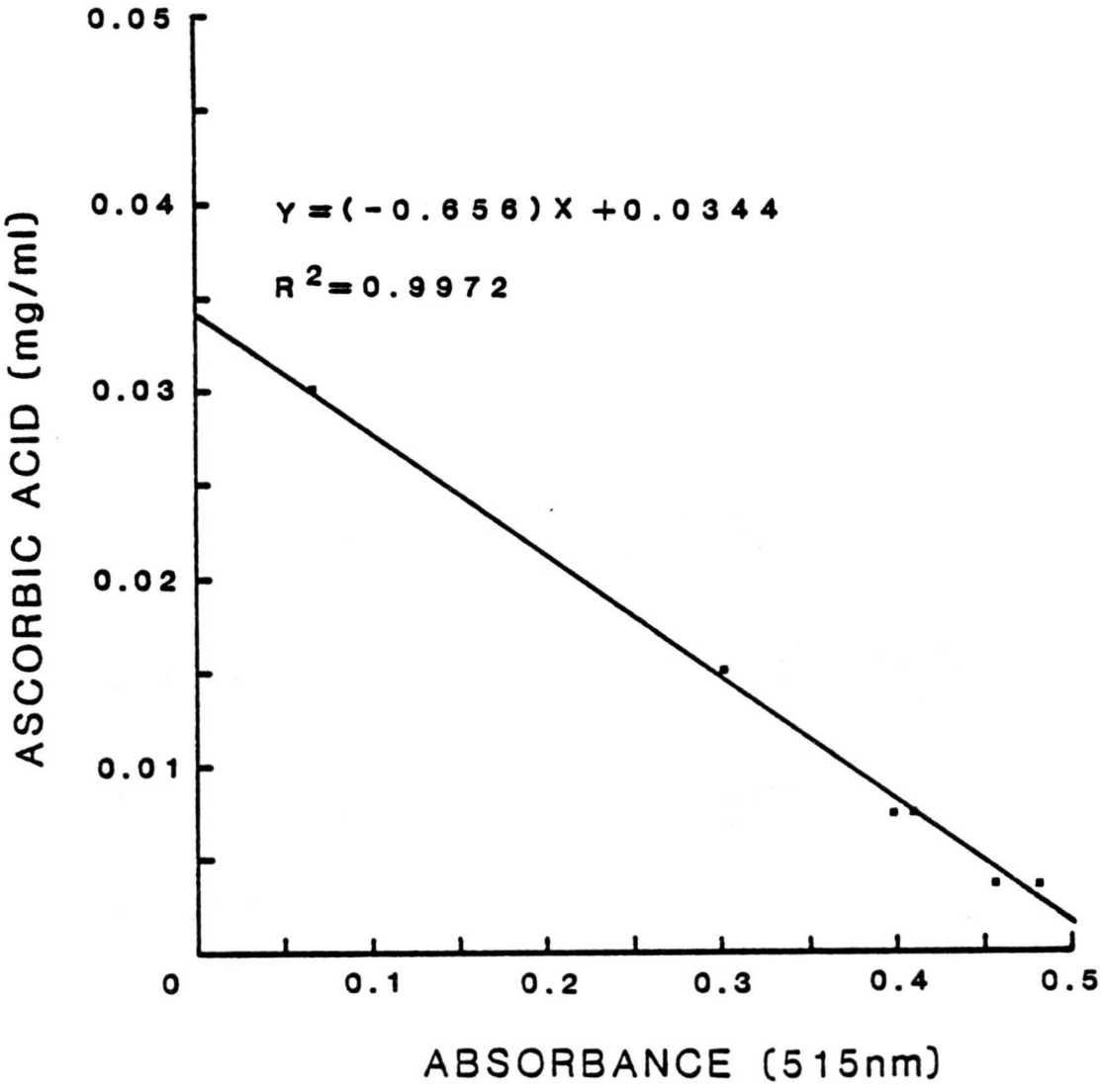

Figure A-1. Standard curve for ascorbic acid with 2,6-dichlorophenol-indophenol dye used for calculating ascorbic acid content of ethanol extracts.

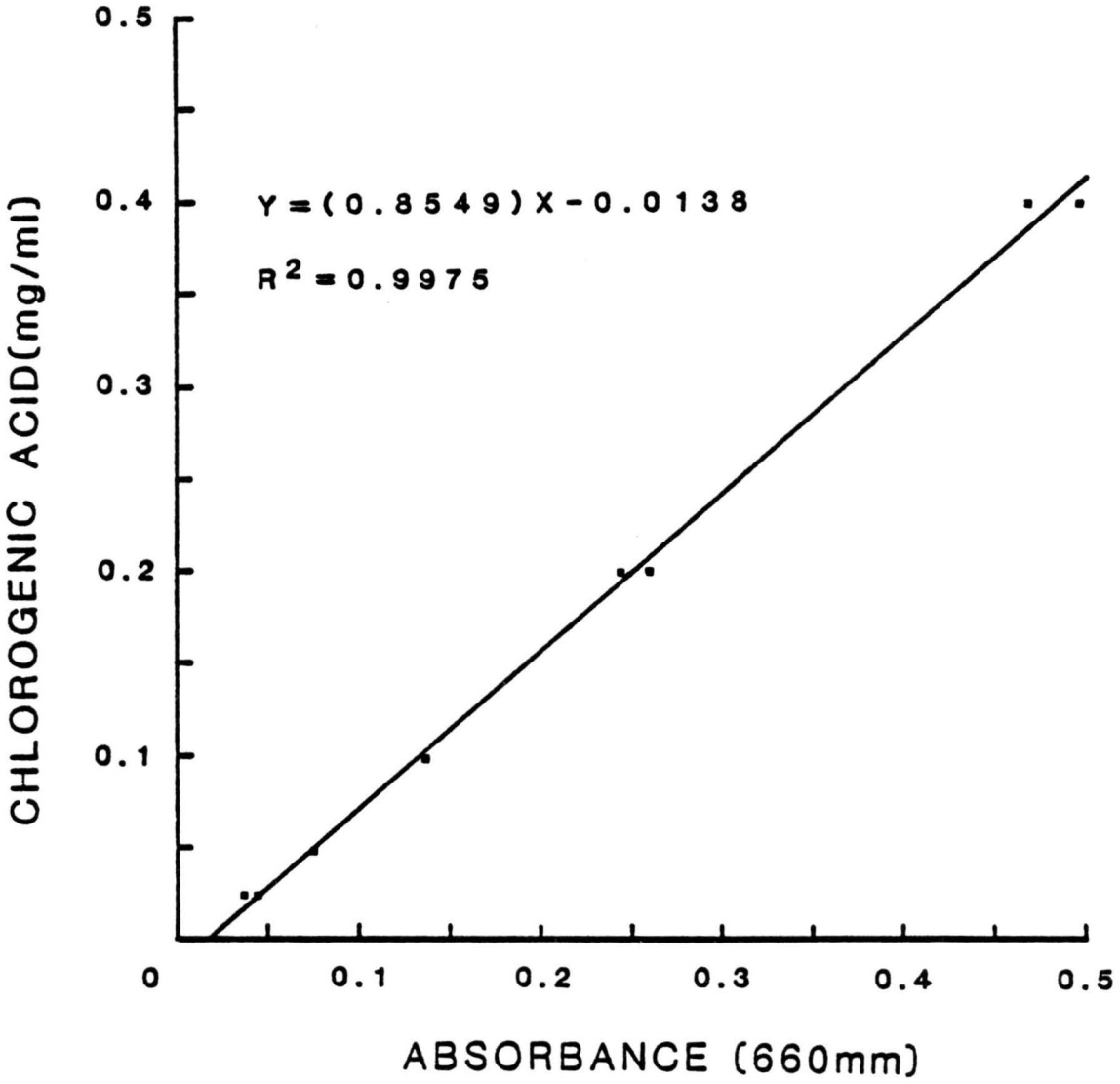

Figure A-2. Standard curve for chlorogenic acid with Folin-Denis reagent used for calculating total phenol content of ethanol extracts.

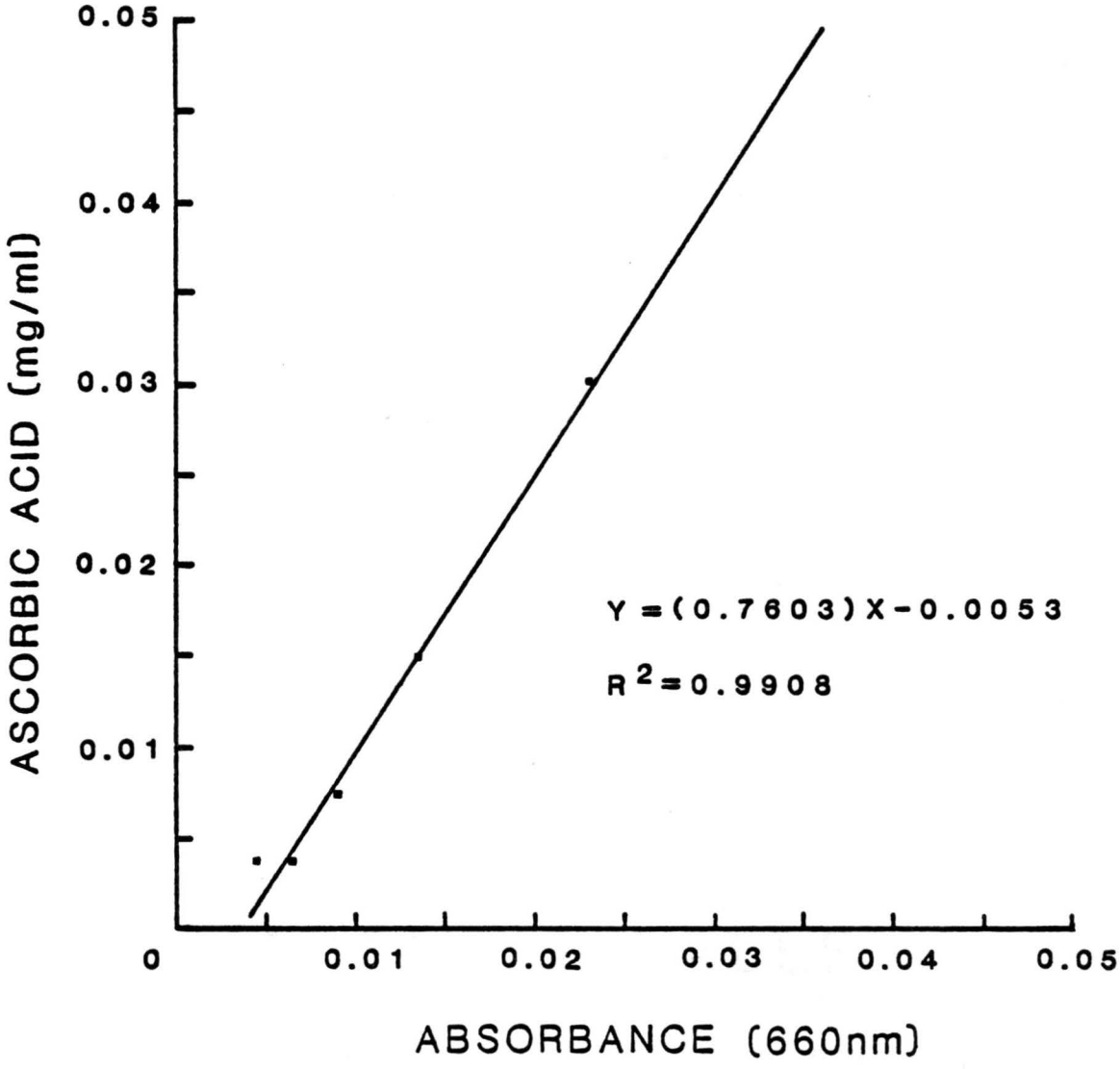

Figure A-3. Standard curve of ascorbic acid with Folin-Denis reagent used for calculating a correction factor for total phenol determination.

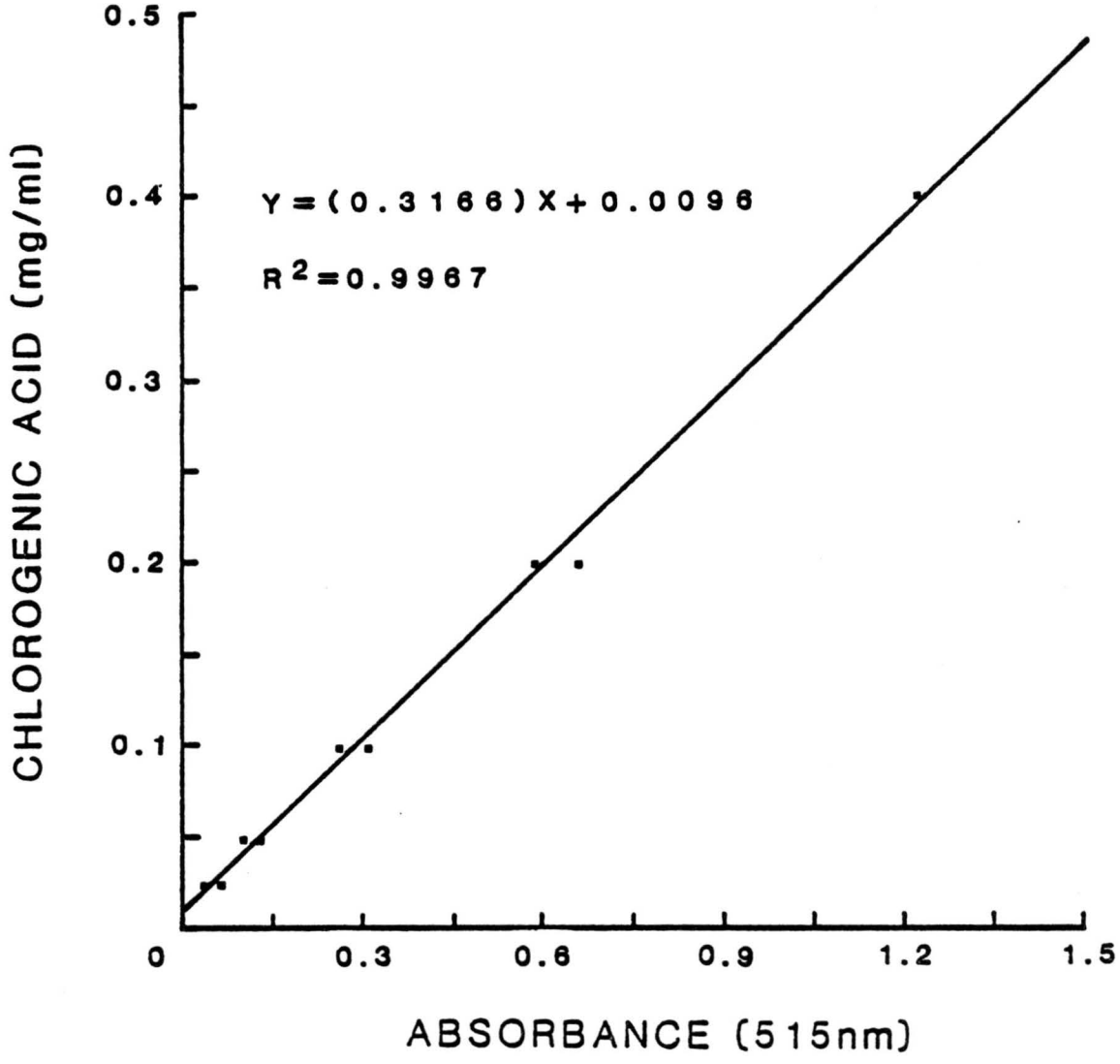

Figure A-4. Standard curve for chlorogenic acid with Arnow reagent used for calculating o-diphenol content of ethanol extracts.

Table A-1

Statistical Analysis of Tissue Extract Darkening
Data in Table 2

	Analysis-of-Variance			
Source	DF	SS	F	Sig.
Clone (C)	5	1.81	15.34	0.01
Substrate (S)	1	3.50	149.01	0.01
Cell Wall (CW) Fragments	1	0.33	14.12	0.01
C X S	5	0.03	0.26	N.S.
C X CW	5	0.34	2.92	0.05
S X CW	1	0.17	0.70	N.S.
C X S X CW	5	0.22	0.19	N.S.
Error	48	1.13		

Table A-2

Statistical Analysis of Data in Table 4

Source	Analysis-of-Variance DF	SS	F	Sig.
Clone (C)	5	1.50	2.64	0.05
Substrate (S)	1	0.02	0.14	N.S.
C X S	5	0.19	0.34	N.S.
Error	24	2.72		

Table A-3

Statistical Analysis of Data in Table 6

Source	Analysis-of-Variance DF	SS	F	Sig.
Clone (C)	5	4.36	10.35	0.01
Cell Wall (CW) Fragments	1	0.02	0.02	N.S.
C X CW	5	0.48	1.15	N.S.
Error	24	2.02		

Table A-4

Statistical Analysis of Phenolic Content Data in Table 7

		Analysis-of-Variance											
		Ascorbic Acid			Total Phenols			O-diphenols			Tyrosine		
Source	DF	SS	F	Sig.	SS	F	Sig.	SS	F	Sig.	SS	F	Sig.
Clone	5	0.04	2.67	N.S.	3.15	1.67	N.S.	0.04	0.70	N.S.	2.80	1.47	N.S.
Rep.	2	0.02	3.33	N.S.	0.25	0.23	N.S.	0.02	0.90	N.S.	0.14	0.19	N.S.
Error	10	0.03			3.78			0.10			3.81		

Table A-5

Statistical Analysis of Dry Matter and Potassium Content Data in Table 8

		Analysis-of-Variance					
		% Dry Matter			% K		
Source	DF	SS	F	Sig.	SS	F	Sig.
Clone	5	21.93	4.89	0.05	0.43	4.19	0.05
Error	12	10.77			0.25		

Table A-6

Statistical Analysis of Ascorbic Acid
Data in Table 9

	Analysis-of-Variance			
Source	DF	SS	F	Sig.
Clone (C)	3	2244.77	280.94	0.01
Bruised vs (B) Nonbruised	1	6.71	2.51	N.S.
Time After (T) Treatment	4	84.18	7.90	0.01
C X B	3	56.38	7.06	0.01
C X T	12	70.92	2.22	0.05
B X T	4	7.38	0.69	N.S.
C X B X T	12	20.78	0.65	N.S.
Error	160	426.15		

The Effect of Foliage Applied Chemicals on Blackspot Susceptibility of Russet Burbank and WC 521-12 Tubers

Objective

Presently it is possible to effect metabolic processes in plants with applications of chemicals to the foliage. One example of this is the prevention of potato tuber sprouting with Maleic Hydrazide. Darkening reactions associated with blackspot development involve enzymatic oxidation of phenols to quinones by polyphenol oxidase. Because this is an enzymatic process, it might also be influenced by application of chemicals to the foliage.

The purpose of this study was to determine the effect of foliar sprays on the blackspot susceptibility of two potato clones. Both Russet Burbank and WC 521-12 were susceptible in previous evaluations.

The chemicals applied all have some effect on the darkening reactions associated with blackspot development. Reducing substances such as ascorbic acid, cysteine, glutathione, and sodium thiosulfate prevent darkening by reducing quinones to phenols (1, 56). These compounds also inhibit polyphenol oxidase activity (51, 56). Phenolic compounds such as p-coumaric acid, ferulic acid, and 2,3-dihydroxynapthalene act as competitive, noncompetitive, or mixed-type inhibitors (41). Compounds containing sulfhydryl groups, such as cysteine, glutathione, thiourea, and sodium

diethyldithiocarbamate (NaDIECA) prevent darkening by chelating copper at the active site of the enzyme or forming colorless complexes with quinones (1, 50).

It was hoped that this study would identify some substances which could be applied as foliar sprays before harvest to reduce blackspot expression of the tubers.

Materials and Methods

Russet Burbank plants grown at the San Luis Valley Research Center and WC 521-12 grown at Lucerne, Colorado, were selected for the study. Each experimental unit consisted of four individual plants. Prior to spraying, several plants were removed from between units to facilitate spraying and harvesting operations. The plots were arranged in a randomized complete block design. The Russet Burbank plot contained four replications while the WC 521-12 plot contained two. The 10 treatments listed in Table A-7 were randomized in each replication.

Ascorbic acid, cysteine, glutathione, sodium thiosulfate, and NaDIECA were each dissolved in 1000 ml of distilled water. Sodium dioctyl sulfosuccinate (0.5 ml) was added as a wetting agent.

p-Coumaric acid, ferulic acid, and 2,3-dihydroxynapthalene were each dissolved in 100 ml of 95% ethanol, then mixed with 900 ml of distilled water with 0.5 ml sodium dioctyl sulfosuccinate added as a wetting agent.

The treatments were applied with a small hand-pump sprayer. A separate sprayer was used for each treatment. The spray was

Table A-7

Chemical Treatments Applied to the Foliage of Russet Burbank and WC 521-12 Plants

Chemical	Concentration
Water (control)	--
Ascorbic acid	0.05 M
Cysteine	0.05 M
Glutathione	0.01 M
Sodium thiosulfate	0.05 M
p-Coumaric acid	0.01 M
Ferulic acid	0.01 M
2,3-Dihydroxynapthalene	0.01 M
Thiourea	0.05 M
Sodium diethyldithiocarbamate	0.05 M

concentrated on the young terminal leaflets. The spray volume was adequate to cover all four plants to dripping.

Russet Burbank plants were sprayed with 1000 ml of the designated treatment solutions on August 24 and September 9, 1982. WC 521-12 plants were sprayed on August 27, September 9, and September 21, 1982. The first spray was applied when tubers were at least 2 inches in diameter.

Tubers from the Russet Burbank plots were harvested by hand on September 23, 1982. All tubers from each experimental unit were placed in bags, identified, and stored at 3-5°C. WC 521-12 tubers were hand harvested on October 2, 1982, and handled in an identical manner.

On January 24, 1983, the tubers were prepared for blackspot evaluation. Four tubers of uniform size were selected from each treatment in each replication. The tubers were kept at 5°C until bruised at 3 locations on each tuber. Bruising was done by dropping a 150 g weight 45 cm onto 3 previously marked locations. The weight used was 9.5 cm long with a 1.3 cm radius hemi-spherical end. Tubers were incubated at 28°C for 36 hours following bruising.

The parameters measured were bruise volume, bruise color, and blackspot index. The bruise volume was determined by cutting tubers transversely through the bruised locations, measuring the width and depth of any resulting bruises, and calculating the volume using the formula for calculating the volume of a cylinder. The mean volume was determined from only those locations which expressed bruise symptoms. Bruise color was determined on the same locations. The color was subjectively evaluated using a scale from 1 to 3. A

reading of 1 indicates very little discoloration while 3 indicates an intense black color. The blackspot index was calculated by determining the percentage of bruised loci which discolored and multiplying by the mean color. Results for each parameter and clone were analyzed separately as a one-way analysis-of-variance.

Results and Discussion

There was variation between the treatments in all three parameters measured. Overall, none of the differences between treatments were significant. The mean volume of bruised tissue of Russet Burbank tubers ranged from 679 to 1071 mm^3 (Table A-8). None of the treatments significantly decreased the bruise volume when compared with the control. The mean bruise volume of WC 521-12 varied from 382 to 926 mm^3. These values were not significantly different than the control.

These substances have not been reported to affect cell rheological properties. These properties determine the extent of cellular damage which results from a bruising force (30). It was not expected that these substances would effect the volume of bruised tissue. The results obtained in this study are in agreement with this. These substances influence the darkening reactions of damaged tissue and could effect the bruise color and blackspot index.

The mean color of bruised tissue of Russet Burbank varied from 1.9 to 2.2 while the values ranged from 1.0 to 1.3 for WC 521-12. There were no significant color differences among treatments or in comparison with the control (Table A-8). It should be noted that the scale used for estimating bruise color is not very precise or objective.

Table A-8

Effect of Foliage Applied Chemicals on Blackspot Susceptibility of Russet Burbank and WC 521-12 Tubers. LSD at 5% Probability Level

Chemical	Russet Burbank (Means of 4 reps)			WC 521-12 (Means of 2 reps)		
	Mean Volume[a]	Mean Color[b]	Blackspot[c] Index	Mean Volume[a]	Mean Color[b]	Blackspot[c] Index
Water	839	2.0	198	451	1.1	78
Ascorbic Acid	838	2.2	213	799	1.0	71
Cysteine	684	1.9	180	643	1.3	110
Glutathione	729	2.1	199	675	1.3	113
Sodium Thiosulfate	759	2.1	206	577	1.2	110
p-Coumaric Acid	679	1.9	179	926	1.1	100
Ferulic Acid	870	2.0	188	547	1.0	76
2,3-Dihydroxy-napthalene	706	2.1	203	484	1.1	96
Thiourea	1071	2.1	194	686	1.1	91
NaDIECA	769	2.1	208	382	1.1	96
Overall Means	794	2.1	197	617	1.1	94
LSD	260	0.3	25	334	0.3	33

[a]Value in cubic millimeters.

[b]Darkness increases with increasing value.

[c]Index = Mean % loci showing color x mean color; 0 = resistant, 300 = maximum severity.

The measurements might not have detected slight differences in discoloration. But to have any significant effect on blackspot development the compounds would have to greatly decrease the mean color.

The blackspot index is a relative value based on both the amount and intensity of discolored tissue. The value ranges from 0 for resistance to 300 for maximum susceptibility. The index varied from 179 to 213 in Russet Burbank (Table A-8). None of the treatments were significantly different than the control. The index varied from 71 to 113 in WC 521-12 and no treatment differed significantly from the control.

These chemicals affect the darkening reactions by inhibiting polyphenol oxidase activity, reducing quinones to phenols, or forming colorless complexes with quinones (1, 41, 51). If these substances were absorbed into leaves and translocated to the cells where the damage occurred, they should have influenced blackspot development. It cannot be determined from this study if the lack of effectiveness of these spray treatments in reducing blackspot is due to lack of absorption, translocation, or other factors. Pate (54) reported that ascorbic acid and glutathione have been detected in the phloem of plants. No information was found on absorption or translocation of the other compounds.

If absorbed into leaves, some of these compounds could be degraded. Both ascorbic acid and certain phenolics are constantly synthesized and degraded in plant cells (11, 17). Absorption of the naturally occurring compounds into the plant would result in an

increase in their concentration. This would probably result in an inhibition of synthesis of these compounds by the plant.

Over all treatments, Russet Burbank tended to have higher values for all three parameters than WC 521-12 (Table A-8). This indicates that under the conditions of this experiment, Russet Burbank is the more susceptible clone.

Preliminary results from experiments on tissue darkening (unpublished) indicated that several of these compounds were very effective inhibitors of the darkening reactions at the concentrations used in the spray applications. Cysteine and glutathione were the most effective inhibitors. This may indicate that sulfhydryl compounds are important in the regulation of polyphenol oxidase activity as suggested by Muneta (51).

Before more work on spray applications is done, it is essential to determine the concentration of these compounds which will effectively inhibit darkening of damaged tissue. The tissue extract procedure described in Chapter III would be a good method for these determinations. Further work would then have to be done to determine if the most effective inhibitors are absorbed and translocated in the plant.

Most commercial chemical sprays used to effect metabolic processes are classified as growth regulators. These compounds influence the translocation, synthesis, or activity of specific plant hormones. The hormones effect metabolic processes in the plant associated with the response. In contrast, the chemicals applied in this study directly effect a specific reaction. Application of

growth regulators may be a more practical approach. Both ethylene and abscisic acid (ABA) are plant hormones which affect enzymes involved in phenolic synthesis. Application of a growth regulator which blocks ethylene synthesis, such as aminoethoxyvinylglycine (AVG), may result in a decrease in the concentration of phenolic substrates. But this could have an adverse effect on other metabolic processes such as wound healing.