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Components of Resistance to Early Blight in Four Potato Cultivars: Effect of Leaf Position

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Abstract

Components of early blight resistance were quantified in leaves of different ages in four potato cultivars. The components of resistance: incubation period (IP), lesion number (LN), early blight severity, lesion expansion rate (LER), latent period (LP) and spore production by lesion area (SPLA), were evaluated separately in the lower, middle and upper leaves of four potato cultivars. Plants of cultivar Aracy (resistant), Delta (moderately resistant), Desirée (susceptible) and Bintje (susceptible) were inoculated with an *Alternaria solani* isolate at the beginning of the flowering stage. Disease severity varied in different plant parts. In all cultivars, regardless of resistance, the smallest values of LN, and severity were recorded on the upper leaves, suggesting that young tissues are less susceptible. In cultivar Aracy, the IP was long, with small values of LN and LER and consequently, low values of early blight severity in all leaf positions were recorded. Although IP was long in cultivar Aracy, no differences between the moderately resistant cultivar Delta and the susceptible cultivars Bintje and Desirée could be detected for this component. The IP was only influenced by leaf position in cultivar Aracy. Clear differences in resistance levels among cultivars could be detected regarding LN, severity and LER. However, neither LP nor SPLA were associated with resistance level of cultivars or with leaf position. Analyses according to plant part suggest that evaluations on leaves of the middle third part are most suitable for screening for early blight resistance in potato.

Introductions

Potato (*Solanum tuberosum*) early blight (EB), caused by *Alternaria solani*, is one of the most destructive fungal foliar diseases in many potato growing regions (Pelletier and Fry, 1989; Shtienberg et al., 1990; Christ, 1991; Van der Waals et al., 2003). Because the high temperatures and humidity during the rainy season are conducive to disease development, EB is the most limiting factor for potato production in Brazil (Reifschneider et al., 1984). Presently, fungicide application is the main control practice adopted worldwide (Stevenson, 1994; Gent and Schwartz, 2003). In Brazil, fungicides are sprayed periodically and more than eight applications are used per season. This practice can represent >10% of the total production costs (Reifschneider et al., 1989). Reducing production costs is of particular importance, mainly in tropical areas because of the high number of resource-poor potato farmers in developing countries. Furthermore, an increase in the frequency of fungicide insensitivity in *A. solani* populations has been reported frequently (Holm et al., 2003; Pasche et al., 2004) and environmental and social concerns require a substantial reduction in fungicide usage.

Cultivar resistance is the most promising strategy for the management of EB, but its use is not widespread: all commercially grown cultivars in Brazil are susceptible to EB (Lopes and Reifschneider, 1999). The adoption of resistance for disease management has been hindered by a lack of resistant cultivars. Resistance is not well characterized and available resistant cultivars do not have desirable agronomic and/or commercial characteristics (Boiteux et al., 1995).

Potato resistance to EB is a quantitative trait, and obtaining commercially successful resistant varieties is not easily achieved (Frank et al., 1979; Herriott et al., 1990; Christ and Haynes, 2001). It has been observed that resistance to EB is age-related: early-maturing cultivars are more susceptible than late-maturing cultivars. Furthermore, older leaves are more susceptible, and the susceptibility increases, as plants grow older (Douglas and Pavek, 1972; Pelletier and Fry, 1990; Rotem, 1994; Boiteux et al., 1995). These characteristics together with the lack of knowledge about mechanisms of resistance, and tetrasomic inheritance, make

genotype discrimination in resistant and susceptible classes complicated.

Quantification of resistance components allows the separation of genotypes into resistance classes and can suggest possible mechanisms involved in resistance (Parlevliet, 1979; Sillero and Rubiales, 2002). Components such as incubation period (IP), lesion number (LN), lesion expansion rate (LER), spore production per lesion area (SPLA), disease severity and infection frequency have already been evaluated in the *S. tuberosum–A. solani* pathosystem (Pelletier and Fry, 1989, 1990; Christ, 1991; Christ and Haynes, 2001). Nevertheless, few studies have been conducted to study the age-related effect on these components.

Although some resistance components were used to support potato breeding programs in Brazil (Reifschneider et al., 1985; Brune et al., 1994), the influence of age-related resistance on these components was not studied in detail. Identifying which components best discriminate EB resistance, and determining the effects of tissue-age on those components, could improve the screening of potato genotypes for EB resistance. Additionally, these studies could generate useful data with which to investigate the mechanism(s) of resistance involved. The objective of this work was to quantify the effect of leaf position on six components of resistance in four potato cultivars with different levels of EB resistance.

Materials and Methods

Plant material and inoculation

Four potato cultivars with different levels of resistance to EB were selected. Cultivar Aracy was used as resistant, Delta as moderately resistant, and Bintje and Desirée as susceptible. The cultivars Aracy and Delta are used as resistant reference cultivars when screening potato genotypes in Brazil (Reifschneider et al., 1985; Brune et al., 1994; Boiteux et al., 1995). Tubers of approximately 50 g were planted in 8L-plastic pots filled with a soil, sand and compost mixture in a 3 : 1 : 1 v/v ratio. All experiments were performed with a single-spore *A. solani* isolate (ASA02) obtained from naturally infected potato plants. For inoculum production, sporulation was induced in 10-day-old *A. solani* colonies grown in potato dextrose agar. Superficial mycelium was removed with 10 ml of sterile distilled water (SDW) and a clean paintbrush and the suspension was discarded. An additional 10 ml of SDW was then poured into the dish, completely covering the mycelia, and the plates were kept under ambient conditions. After 2 days the water was drained and the plates were left without lid under ambient conditions. Abundant sporulation was observed after 2 days. To collect conidia, colonies were scraped with a sterile scalpel and conidia were transferred to a beaker containing SDW.

Before inoculation all the plants were divided (tagged) in three equal sections: lower, middle and upper. The terms plant position or leaves position used along the text correspond to these third plant sections previously delimited. Therefore, although the exact age of leaves assessed was not recorded, we assumed that leaves from lower, middle and upper sections, range from older to younger, respectively.

Twenty plants of each cultivar at the beginning of flowering stage (45–50 days old) were used in the experiments. Fifteen plants of each cultivar were inoculated by spraying to run off with a suspension of 10^3 conidia/ml of isolate ASA02. The other five plants were inoculated by placing a 10 μl-drop of conidial suspension on at least 30 leaflets in each plant section. To enhance drop adherence at the inoculation site, the conidial suspension was supplemented with gelatin (1% w/v) (SigmaG-8150, Sigma Chemical Co., St Louis, MO, USA). For each cultivar, another set of 20 plants were used as controls in which inoculation was simulated in 15 by spraying with SDW, and in five by placing a 10 μl-drop of SDW supplemented with gelatin as describe above. After inoculation, plants were kept for 24 h in a moist chamber at 25°C, 12 h photoperiod. After this time, plants were transferred to greenhouse conditions. All experiments were independently repeated three times using a completely randomized design.

Components of resistance

Components of resistance were evaluated separately in the lower, middle and upper leaves of the inoculated plants. In the 15 plants inoculated by spraying, the following components were evaluated:

Incubation period Defined as the time elapsed between inoculation time and symptom appearance in 100% of the plants, IP was assessed by visual inspection of symptom development every 12 h starting at 12 h postinoculation (HPI).

Lesions number Lesion number was counted at 3, 4, 5, 6 and 7 days postinoculation (DPI). Three leaves on each plant section (lower, middle and upper), of each cultivar were evaluated.

Disease severity Percentage of leaflet area with necrotic tissue was estimated using a diagrammatic scale developed for EB resistance in potato (Reifschneider et al., 1984). Disease severity assessments were taken every 3 days beginning at 5 DPI until 20 DPI. Three marked leaves on each plant section were evaluated. Severity values were plotted against time and the area under the disease progress curve (AUDPC) was calculated (Shaner and Finney, 1977).

In the group of five plants, that were drop-inoculated, the following components of resistance were quantified.

Lesion expansion rate One leaflet from each of the lower, middle and upper thirds of the plant was tagged and the lesion diameter was measured using a ruler. Lesions varied in shape throughout the assessment period and thus measurements were taken consistently at two fixed directions: along (length) and across (width)

the leaflet. Lesion area was estimated using the ellipse area formula ($A = a/2 \cdot b/2 \cdot \pi$, where a is the length, b the width and $\pi = 3.14$).

Latent period We considered LP as days elapsed from inoculation until sporulation of lesions. Detection of conidia was performed as described by Pelletier and Fry (1989) and assessments were taken daily beginning at three DPI until 10 DPI. Three lesions taken from each plant section were assessed in each evaluation. LP was considered completed when sporulation was observed on at least two of the three lesions taken from each position on the plant.

Spore production per lesion area Three lesions from each third of the plants were randomly collected at 10, 15 and 20 DPI. Lesions were photographed with a digital camera, and lesion area was estimated using the ImageTool software (UTHSCSA, University of Texas Health Science Center, San Antonio, TX, USA). The number of spores per lesion was quantified using the procedures described by Pelletier and Fry (1989).

Statistical analysis

The statistical analyses were accomplished using SAS (The SAS Institute Inc., Version 8, Cary, NC, USA). For the LER analysis, the average value of three leaflets was taken and used in the analysis. A linear regression analysis was conducted with values of lesion area by time (lesion area $Y = \alpha + \beta \cdot$time), and β (slope) was used as LER estimator (cm^2/dia). The IP, LN and AUDPC values were submitted to analysis of variance (ANOVA) and treatment means were compared using Tukey's test ($\alpha = 0.05$).

Results and Discussion

Incubation period

Incubation period was significantly longer in cultivar Aracy than in all other cultivars for all leaf positions evaluated (Table 1). Although Pelletier and Fry (1989) reported differences in the IP length between lower (older), and upper (younger) leaves of three potato cultivars, we found this component to be age-related only in the resistant cultivar Aracy. These authors also found differences in IP length among cultivars under field conditions. However, no differences between the susceptible cultivars Bintje and Desirée and the moderately resistant Delta were detected in our study (Table 1). It is possible that under greenhouse conditions the discrimination of cultivar resistance based on IP length may only be effective if large differences in resistance are present in the genotypes being compared. Although other factors such as isolate aggressiveness (Hoffman et al., 2002) or incubation conditions could also influence IP, it seems that detection of moderate resistance using this component in greenhouse studies is less likely to be accomplished.

Lesion number

The values of LN were analysed from data recorded at five DPI. At this time LN did not increase in relation to the previous evaluation and no coalescence of lesions was observed. Regardless of resistance levels, higher LN was observed in the lower than in the upper third of the plant (Table 1). The LN was smaller in Aracy than in all other cultivars at all thirds, except for that of Desirée recorded on leaves in the upper third of the plant. In the lower and middle thirds of the plants, LN was smaller in Delta than in Desirée and Bintje (Table 1). Similar results had been obtained using this component to evaluate EB in whole plants (Douglas and Pavek, 1972; Reifschneider et al., 1981) and in plant sections of potato (Pelletier and Fry, 1989; Christ, 1991). Interestingly, in leaves from the upper third, LN of the susceptible cultivar Desirée did not differ statistically from the resistant Aracy. This trend was consistently observed in all experiments. Although it is speculative, it seems that the upper leaves of Desirée present a differentiated EB resistance that could not be detected by LN.

As previously reported (Reifschneider et al., 1981; Pelletier and Fry, 1989; Christ, 1991), these LN results support the relationship between this component and EB resistance in potato. Additionally, LN evaluations are important to supply evidence of the probable mechanisms of resistance. The small LN in Aracy and on the leaves of the upper position of the plants, suggest that resistance mechanisms are acting at the pre-

Table 1
Incubation period (hours) and lesions number of *Alternaria solani* in leaves of the lower, middle, and upper plant sections of four potato cultivars with different levels of resistance to early blight

	Incubation period (h)[b]			Lesion number[c]		
Cultivar[a]	Lower	Middle	Upper	Lower	Middle	Upper
Aracy (r)	58.8 Aa	61.2 Aab	76.8 Ab	10.2 Aa	8.1 Aa	1.2 Ab
Delta (mr)	46.8 Ba	48.0 Ba	49.2 Ba	22.1 Ba	16.2 Bb	8.2 Bc
Desirée (s)	46.8 Ba	43.2 Ba	51.6 Ba	32.0 Ca	28.2 Ca	4.0 Ab
Bintje (s)	43.2 Ba	45.6 Ba	48.0 Ba	34.8 Ca	24.6 Cb	15.8 Cc

[a]Letters in brackets indicate level of early blight resistance of cultivars: r, resistant; mr, moderately resistant; s, susceptible.
[b]Values correspond to the mean number of hours postinoculation where symptoms were observed in all the plants (15 plants per cultivar).
[c]Values correspond to mean lesion number registered in three leaves per plant position of fifteen plants per cultivar.
Values within a column followed by different uppercase letters and values in the rows followed by different lowercase letters are significantly different at $P = 0.05$, according to Tukey's test.

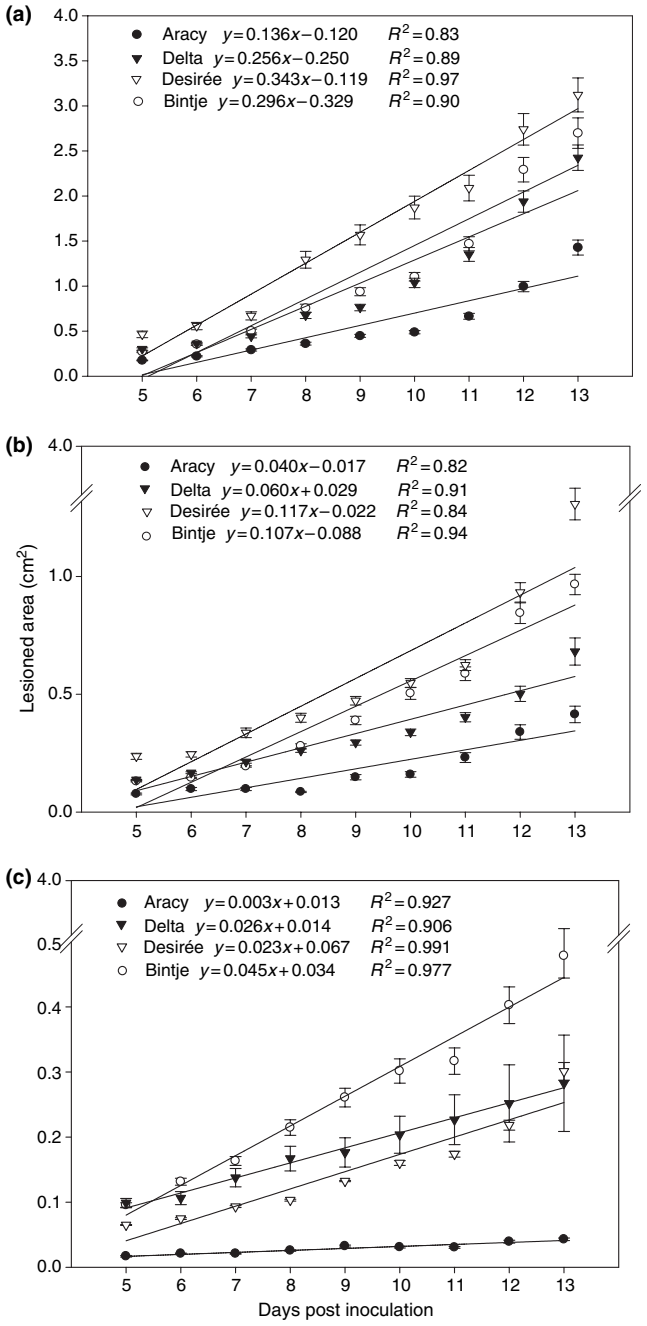

Fig. 1 Average lesion area (cm^2) as a function of days postinoculation with *Alternaria solani* in four potato cultivars with different levels of resistance to early blight. (a) Lower leaves, (b) middle leaves, (c) upper leaves. Values correspond to means of three lesions per plant positions of five plants per cultivar

penetration stages. Histological analyses are required to determine whether this is the case.

Lesion expansion rate

The LER allowed the classification of cultivars according to resistance levels. Smallest LER values were observed in the resistant cultivar Aracy (Fig. 1). In the upper third of Aracy, the lesions were restricted to the infection sites with no expansion throughout the assessment period (Fig. 1c). Regardless of resistance levels, the lowest values of LER were measured in lesions in the upper third of the plants (Fig. 1). These findings confirm previous reports of lesion development in this pathosystem (Douglas and Pavek, 1972; Pelletier and Fry, 1989; Boiteux and Reifschneider, 1993). Contrary to the observations with LN and severity, measurements of LER taken from leaves in the upper third allowed discrimination between the resistant cultivar Aracy and the susceptible Desirée.

Lesion expansion rate reflects the process of host colonization and has been used by plant breeders and plant pathologists to assess cultivar resistance (Berger et al., 1997). Additionally, LER can be a useful component to supply evidence about mechanism of resistance (Parlevliet, 1979). The low values of LER recorded in Aracy and in leaves of the upper third of all cultivars, suggest that resistance events that delay tissue colonization after fungi penetration play a role in *A. solani* – potato pathosystem.

Disease severity

Early blight severity integrated over time as AUDPC varied according to cultivar (Fig. 2). The AUDPC values for the resistant cultivar Aracy were lower than those for the susceptible cultivar Bintje. In the upper third, AUDPC values of Desirée (susceptible) did not differ from that of Aracy (Fig. 2). Regardless of cultivar resistance levels, AUDPC values were smaller in the upper than in the middle and lower thirds (Fig. 2). Better resistance discrimination among cultivars was observed in the middle third. Similar observations were made by Christ (1991) who described the usefulness of quantifying EB severity in the middle third of the potato plant canopy to screen for resistant genotypes.

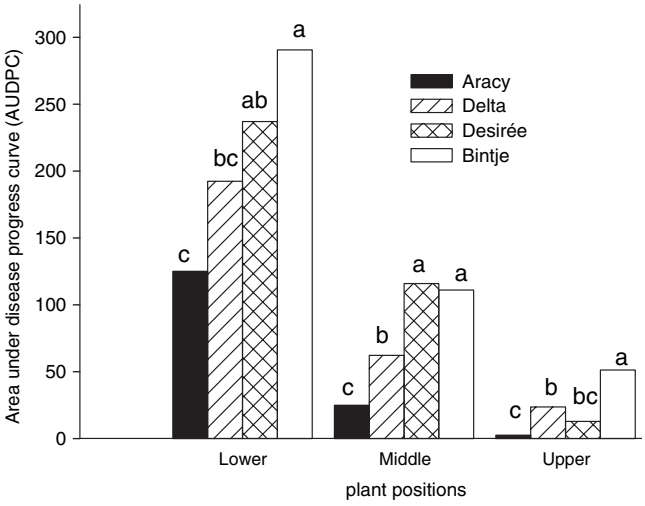

Fig. 2 Area under disease progress curve (AUDPC) in leaves of the lower, middle and upper plant sections of four potato cultivars with different levels of resistance to early blight. Evaluations were performed every 3 days since the 5th until the 20th day postinoculation with *Alternaria solani*. Values correspond to data registered in three leaves per plant section of 15 plants per cultivar. Bars with different letters within the same plant position are significantly different at $P = 0.05$ according to Tukey's test

As with other pathosystems in which resistance is quantitatively determined (Birhman and Singh, 1995; Jeger and Viljanen-Rollinson, 2001), assessment of disease severity allowed the characterization of cultivars according to resistance levels. Using this component, Christ and Haynes (2001) estimated the inheritance of EB resistance in potato and Foolad et al. (2002) identified quantitative trait loci (QTLs) associated with the resistance to this disease in tomato. Disease severity is a useful component for studying resistance to EB once it is a phenotype that summarizes the contribution of several resistance components. Additionally, disease severity can be easily and rapidly evaluated with suitable diagrammatic scale. The scale used in this work (Reifschneider et al., 1984) proved be a practical and accurate tool to discriminate potato genotypes for EB resistance.

Latent period and spore production by lesion area

The LP and SPLA variables did not allow separation of cultivars according to resistance levels. Both components were not related to plant position (thirds). The LP and SPLA components are likely to be influenced by relative humidity and leaf wetness, important microclimatic variables for sporulation of *Alternaria* species (Miller, 1975; Holley et al., 1985; Everts and Lacy, 1990). Proper discrimination of cultivars based on LP and SPLA could have been prevented by unsuitable conditions of humidity under greenhouse conditions. However, the lack of association between SPLA and leaf position in potato plants was also reported in studies carried out under field conditions (Pelletier and Fry, 1989). In the present work, SPLA did not vary among cultivars and was not correlated with lesion area. According to Johnson and Taylor (1976), SPLA is a useful variable to evaluate disease resistance and represents the sum of the effects of all resistance components. The importance of this component has been reported in the Corn-*Bipolaris maydis* pathosystem, where resistance is encoded by the gene *rhm1* and characterized by a striking inhibition of sporulation (Simmons et al., 2001). Whether resistance of the potato cultivars evaluated in the present study is unrelated to LP and/or SPLA or if the methodology of quantification prevented detection of effects remains unknown. Thus, it is important to review the experimental conditions and the quantification methodology in future experiments. Further studies aimed at quantifying whether resistance is associated with a delayed LP or lower SPLA should be carried out, because important information for management schemes can be obtained. Scheduling fungicide sprays according to LP and SPLA integrated with genotype and age-related resistance could potentially reduce the number of applications in a growing season (Shtienberg et al., 1995).

Age-related resistance seems to be characteristic of all pathosystems involving the genus *Alternaria*. This relationship was already reported for *A. macrospora* in cotton (Bashi et al., 1983), for *A. helianthi* in sunflower (Allen et al., 1983), for *A. porri* in onion (Aveling et al., 1994), and for *A. cirsinoxia* in *Cirsium* (Green and Bailey, 2000). Nevertheless, the mechanism involved in this type of resistance still remains unknown. Cultivar Desirée, considered as susceptible, did not differ from Aracy in the upper leaves according to values of LN and AUDPC, neither from Delta when assessing LER. Thus, components that allowed proper discrimination of cultivars in evaluations performed in leaves of the lower or middle positions of the plants failed to discriminate cultivars when assessed in the upper position.

Our results suggest that EB resistance should be evaluated at the middle third of the potato plant, in order to avoid the erroneous selection of genotypes or treatments. The exclusion of the leaves of the lower and upper plant positions could improve screening and save time. When only assessing leaves of the middle position of the plants, the total area for evaluation is reduced and the effect of leaf position in EB resistance can be minimized. This is in agreement with a recently reported study involving potato late blight (Visker et al., 2003). In this study, the authors pointed out that it is important to consider leaf position when testing for late blight resistance, because contrasts in resistance may be ascribed erroneously to different genotypes or treatments, when in fact it can be due to differences in leaf position.

In summary, we have provided quantitative data about the effect of position of leaves on the components of resistance in the potato – *A. solani* pathosystem. These results are useful for screening EB resistant breeding material and management schemes of EB in Brazil. Additionally, these findings have supplied information for guiding future studies about mechanisms involved in the EB resistance in potato.

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