

HENRIQUE DA SILVA SILVEIRA DUARTE

**REQUEIMA E PINTA PRETA EM BATATEIRA: RESISTÊNCIA  
E EPIDEMIOLOGIA COMPARATIVA**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

VIÇOSA  
MINAS GERAIS - BRASIL  
2012

**Ficha catalográfica preparada pela Seção de Catalogação e  
Classificação da Biblioteca Central da UFV**

T

D812r  
2012

Duarte, Henrique da Silva Silveira, 1983-  
Requeima e pinta preta em batateira : resistência e  
epidemiologia comparativa / Henrique da Silva Silveira  
Duarte. – Viçosa, MG, 2012.  
xii, 105f. : il. ; (algumas color.) ; 29cm.

Texto em inglês.

Orientador: Lércio Zambolim

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Batata - Resistência a doenças e pragas. 2. Requeima.  
3. Pinta-preta. 4. Epidemiologia. I. Universidade Federal de  
Viçosa. Departamento de Fitopatologia. Programa de  
Pós-Graduação em Fitopatologia. II. Título.

CDD 22. ed. 635.2194

HENRIQUE DA SILVA SILVEIRA DUARTE

**REQUEIMA E PINTA PRETA EM BATATEIRA: RESISTÊNCIA  
E EPIDEMIOLOGIA COMPARATIVA**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Doctor Scientiae*.

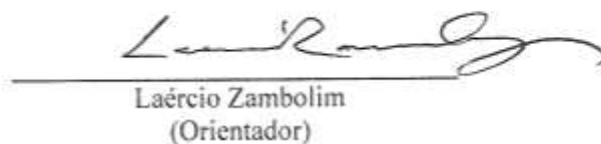
APROVADA: 17 de dezembro de 2012

  
Fabricio de Ávila Rodrigues

  
Olinto Liparini Pereira

  
Alexandre Sandri Capucho

  
Antônio Alves Pereira

  
Laércio Zambolim  
(Orientador)

Aos meus pais, Maria Auxiliadora e Áureo

Ao meu irmão, Diogo

A minha namorada, Raíssa

Aos meus familiares e amigos,

que sempre acreditaram em mim,

**dedico.**

## AGRADECIMENTOS

Primeiramente a Deus por ter me dado a vida e por ter colocado pessoas tão especiais na minha caminhada.

A minha mãe Maria Auxiliadora, que me deu muita força para continuar lutando e alcançar meus objetivos. Ao meu pai Áureo, ao meu irmão, Diogo, pelo carinho e apoio.

À minha namorada Raíssa pelo carinho, companheirismo, incentivo e compreensão em todos os momentos que passamos juntos e naqueles em que estive ausente.

A toda minha família, que sempre estiveram presentes em todos os momentos da minha caminhada me dando muito carinho e força para que alcançasse meus objetivos. Agradeço também a compreensão pelos momentos em que estive ausente das reuniões familiares e pelo orgulho das minhas conquistas.

À Universidade Federal de Viçosa, pela oportunidade em realizar este curso.

Ao CNPq e à CAPES pelo apoio financeiro e pela concessão de bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) pelo apoio financeiro do projeto.

Ao Prof. Laércio Zambolim, pela orientação, confiança e disponibilização de recursos para que fosse desenvolvido o trabalho.

Aos meus coorientadores Prof. Eduardo S. G. Mizubuti e Prof. José Ivo Ribeiro Júnior pelas sugestões e ensinamentos para a melhoria do trabalho.

Ao professor Pierce Anderson Paul pela ajuda nas análises estatísticas dos trabalhos e pela oportunidade de ter feito o doutorado sanduíche pela Ohio State University (OARDC).

Ao pesquisador da EPAMIG Dr. Joaquim Gonçalves de Pádua, que muito contribuiu para a obtenção das cultivares e ensinamentos sobre a cultura da batata.

Aos professores da Universidade Federal de Viçosa, pelos valiosos conhecimentos transmitidos durante o curso.

Aos amigos do laboratório, pelo convívio agradável. Em especial a Sérgio Milagres, José Cláudio, Bira, Pedro Nery, Jonas, Toninho, Hitor, Gabriel, Deco, Toim, Franklin, Aline 1, Aline 2, Danival, Priscila, Dani e Rodrigo.

Aos meus amigos Alexandre Capucho, Daniel Lage, Ueder Lopes, Nadson Pontes, Alessandro Nicole, Douglas Parreira, Paulo Ferreira, Hélvio Ferraz e Fabrício Lanza pela amizade sincera, apoio, conselhos e discussões científicas.

Ao meu amigo Eder Novaes Moreira pela companhia, paciência, discussões científicas, ensinamentos durante o doutorado sanduíche.

Aos familiares Liliam, Jason, Ethan, Andréia, Aninha e Guilherme pela atenção, ajuda e carinho no período que estivesse nos EUA.

Aos vários amigos de Wooster e Smithville que muito me ajudaram no período de Doutorado Sanduíche. Em especial a Celeste, Alan, Barbara, Noemia, Deborah, Tomaz, Daniela, Matheus, Misses Maasz, Phil, Janet Calhoun, Jhony Mera, David Salgado, Elizabeth, Maurício, Luciano, Liz e Edmilson.

A todos os meus companheiros de república, pela amizade, compreensão e pelos momentos de descontração.

Aos meus amigos de minha cidade natal que mesmo em minha ausência sempre estiveram torcendo por mim.

Às pessoas que me acompanharam no decorrer dos anos e que, de alguma forma, contribuíram para o desenvolvimento deste e deixaram lições que me acompanharão por toda a vida.

**Muito Obrigado!**

## **BIOGRAFIA**

HENRIQUE DA SILVA SILVEIRA DUARTE, filho de Maria Auxiliadora da Silva Silveira Duarte e Áureo Silveira Duarte, nasceu em Florestal, Minas Gerais, em 11 de agosto de 1983.

Em fevereiro de 1999, ingressou no curso Técnico em Agropecuária na Central de Ensino e Desenvolvimento Agrário de Florestal (CEDAF), formando em fevereiro de 2002.

Em maio de 2002, ingressou no curso de Agronomia na Universidade Federal de Viçosa, graduando-se em 16 de março de 2007.

No período de 2004 a 2007, foi bolsista de iniciação científica no Departamento de Fitopatologia da UFV, onde desenvolveu atividades de pesquisa em manejo integrado de doenças de plantas.

Em março de 2007, iniciou no Programa de Pós-graduação, ao nível de Mestrado em Fitopatologia, na Universidade Federal de Viçosa, obtendo o título de *Master Scientiae* em fevereiro de 2009.

Em março de 2009, iniciou o curso de Doutorado em Fitopatologia, pela Universidade Federal de Viçosa. Foi contemplado com uma bolsa de doutorado sanduíche para realizar atividades de pesquisa na Ohio State University (OARDC), Wooster-OHIO, no período de fevereiro a agosto de 2012. Em dezembro de 2012, obteve o título de *Doctor Scientiae*.

## SUMÁRIO

<b>RESUMO</b> .....	<b>ix</b>
<b>ABSTRACT</b> .....	<b>xi</b>
<b>General Introduction</b> .....	<b>1</b>
<b>References</b> .....	<b>3</b>
<b>ARTICLE 1</b> .....	<b>4</b>
<b>The field resistance of potato cultivars to foliar late blight and its relationship with foliage maturity type and skin type in Brazil</b> .....	<b>4</b>
<b>Abstract</b> .....	<b>4</b>
<b>1 Introduction</b> .....	<b>4</b>
<b>2 Material and Methods</b> .....	<b>6</b>
2.1 General aspects .....	6
2.2 Experiment 1 .....	7
2.3 Experiment 2 .....	7
2.4 Determination of mating type .....	7
2.5 Disease assessment .....	8
2.6 Data analysis .....	8
2.7 Disease progress curves .....	10
2.8 Relationships between resistance to foliar late blight and foliage maturity type and between resistance to foliar late blight and the skin type of the potato .....	10
<b>3 Results</b> .....	<b>10</b>
3.1 Mating type .....	10
3.2 Meteorological data .....	10
3.3 Disease progress .....	11
3.4 Resistance levels of in the cultivars of experiment 1 to foliar late blight .....	11
3.5 The level of resistance to foliar late blight in the cultivars of experiment 2 .....	12
3.6 The relationship between the resistance to foliar late blight and the foliage maturity type of potato .....	13
3.7 The relationship between resistance to foliar late blight and the skin type of the potato .....	13
<b>4 Discussion</b> .....	<b>13</b>
<b>5 Acknowledgements</b> .....	<b>16</b>
<b>6 References</b> .....	<b>17</b>
<b>7 Figures</b> .....	<b>21</b>
<b>8 Tables</b> .....	<b>25</b>
<b>ARTICLE 2</b> .....	<b>30</b>
<b>Development and validation of a set of standard area diagrams and field key to estimate severity of potato early blight</b> .....	<b>30</b>
<b>Abstract</b> .....	<b>30</b>
<b>1 Introduction</b> .....	<b>31</b>
<b>2 Material and Methods</b> .....	<b>33</b>
2.1 Development of the standard area diagram (SAD) set .....	33
2.2 Validation of the SADs to determine potato early blight severity on leaves .....	34
2.3 Development of the field keys (FK) .....	35

2.4 Validation of the FK and SAD for the assessment of potato early blight severity in experimental plots .....	35
<b>3 Results .....</b>	<b>36</b>
3.1 The use of the SAD to determine potato early blight severity on leaves .....	36
3.2 The FK and SAD for the assessment of potato early blight severity in experimental plots .....	38
<b>4 Discussion.....</b>	<b>39</b>
<b>5 Acknowledgements.....</b>	<b>41</b>
<b>6 References.....</b>	<b>41</b>
<b>7 Figures .....</b>	<b>45</b>
<b>8 Tables.....</b>	<b>50</b>
<b>ARTICLE 3.....</b>	<b>53</b>
<b>Field resistance of potato cultivars to early blight and its relationship with foliage maturity type and skin type in Brazil.....</b>	<b>53</b>
<b>Abstract.....</b>	<b>53</b>
<b>1 Introduction .....</b>	<b>53</b>
<b>2 Material and Methods.....</b>	<b>56</b>
2.1 General Aspects.....	56
2.2 Experiment 1.....	56
2.3 Experiment 2.....	57
2.4 Experiment 3.....	57
2.5 Disease assessment .....	57
2.6 Data analysis .....	58
2.6.1 Experiment 1 .....	58
2.6.2 Experiment 2 and 3 .....	59
2.7 Disease progress curves .....	60
2.8 Relationships between resistance to early blight and foliage maturity type and between resistance to early blight and the skin type of the potato .....	60
<b>3 Results .....</b>	<b>60</b>
3.1 Meteorological data .....	60
3.2 Disease progress .....	61
3.3 The level of resistance to early blight in the cultivars of potato.....	61
3.3.1 Experiment 1 .....	61
3.3.2 Experiment 2 .....	62
3.3.3 Experiment 3 .....	63
3.4 The relationship between the resistance to early blight and the foliage maturity type of potato .....	64
3.5 The relationship between resistance to early blight and the skin type of the potato .....	64
<b>4 Discussion.....</b>	<b>65</b>
<b>5 Acknowledgements.....</b>	<b>68</b>
<b>6 References.....</b>	<b>68</b>
<b>7 Figures .....</b>	<b>72</b>
<b>8 Tables.....</b>	<b>77</b>
<b>ARTICLE 4.....</b>	<b>83</b>
<b>Comparative epidemiology of late blight and early blight of potatoes under different environmental conditions and fungicide applications in Brazil.....</b>	<b>83</b>

<b>Abstract.....</b>	<b>83</b>
<b>1 Introduction .....</b>	<b>83</b>
<b>2 Material and Methods.....</b>	<b>85</b>
2.1 General aspects .....	85
2.2 Experiment 1.....	86
2.3 Experiment 2 and 3.....	88
2.5 Disease assessments and yield determination .....	88
2.6 Data analysis .....	88
<b>3 Results .....</b>	<b>89</b>
3.1 Meteorological data .....	89
3.2 Disease progress .....	89
3.3 Area under the disease progress curve (AUDPC) and yield for late blight and early blight under different environmental conditions and different fungicide applications programs.....	90
<b>4 Discussion.....</b>	<b>92</b>
<b>5 Acknowledgements .....</b>	<b>96</b>
<b>6 References.....</b>	<b>96</b>
<b>7 Figures .....</b>	<b>100</b>
<b>8 Tables.....</b>	<b>102</b>
<b>GENERAL CONCLUSIONS.....</b>	<b>104</b>

## RESUMO

DUARTE, Henrique da Silva Silveira, D.Sc., Universidade Federal de Viçosa, dezembro de 2012. **Requeima e pinta preta em batateira: resistência e epidemiologia comparativa.** Orientador: Laércio Zambolim. Coorientadores: Eduardo Seiti Gomide Mizubuti e José Ivo Ribeiro Júnior.

A produção de batata (*Solanum tuberosum* L.) é limitada por vários fatores, dentre estes, as doenças de plantas. A requeima causada por *Phytophthora infestans* e a pinta preta causada por *Alternaria grandis* são consideradas de grande importância. Estudos sobre a epidemiologia e resistência da requeima e pinta preta da batateira são muito importantes para melhor entender estes patossistemas. Para avaliar a resistência de cultivares à requeima em folhas de batata e verificar como essa resistência a requeima relaciona como o tipo de maturação foliar e o tipo de pele em cultivares de batata plantadas no Brasil, 2 experimentos de campo foram conduzidos em diferentes condições ambientais na Universidade Federal de Viçosa (UFV). Para o primeiro experimento foi usado 34 cultivares (tratamentos) e para o segundo experimento foi usado 17 cultivares. A maioria dos cultivares de batata foram suscetíveis a requeima. Os cultivares com os maiores níveis de resistência a requeima (resistente e moderadamente resistente) são de ciclo mais tardios (meio tardio e tardio). A maioria dos cultivares que foram classificados como moderadamente suscetível e suscetível foram mais precoces (precoce e meio precoce). No geral, cultivares que foram mais resistentes a requeima possuem pele áspera, enquanto que a pele das cultivares mais suscetíveis foram lisas. Para estimar a severidade da requeima e da pinta preta da batateira em folhas e em plantas de parcelas experimentais é necessário o uso de uma escala diagramática (EDG) e uma escala descritiva (EDS), respectivamente. Atualmente, existem essas duas escalas somente para a requeima. Para a pinta preta há apenas a EDG, que compreende diagramas de folhas de batata com a severidade da pinta preta variando de 0 a 50%, a qual possui limitações. Sendo assim, foi desenvolvida e validada uma EDG e uma EDS. A EDG proposta contém ilustrações de folhas com doze níveis de severidade da doença (0,05; 0,5; 2; 4; 8; 16; 32; 48; 62; 82; 96 e 100%). A EDS foi uma adaptação da EDS desenvolvida para avaliar a severidade da requeima da batateira. Ambas EDG e EDS melhoraram a acurácia, precisão e reprodutibilidade das estimativas de severidade da pinta preta da batateira. Estas escalas podem ser utilizadas para avaliar a severidade para o melhoramento de plantas visando à resistência, screening de fungicida e

caracterização de patótipo. Para avaliar a resistência de cultivares à pinta preta em folhas de batata e verificar como essa resistência se relaciona com o tipo de maturação foliar e o tipo de pele em cultivares de batata plantadas no Brasil, 3 experimentos de campo foram conduzidos em diferentes condições ambientais na UFV. Para o primeiro e o segundo experimento foram usadas 26 cultivares (tratamentos) e para o terceiro experimento foi usado 24 cultivares. A maioria dos cultivares de batata foram suscetíveis e moderadamente suscetíveis à pinta preta da batateira. O nível de resistência de alguns cultivares a pinta preta mudou de acordo com as condições ambientais. Os cultivares resistentes a pinta preta são de meia estação, meio tardio e tardio. Não foi observado nenhum cultivar suscetível a pinta preta com ciclo mais tardio (meio tardio e tardio). Na maioria dos casos, os cultivares suscetíveis a pinta preta são de ciclo mais precoce (precoce e meio precoce). Cultivares resistentes a pinta preta tiveram pele áspera, meio áspera e lisa. Cultivares suscetíveis à pinta preta com pele áspera não foram observados. Na maioria dos casos, os cultivares que foram suscetíveis e moderadamente suscetíveis à pinta preta possuem pele lisa. Objetivando comparar epidemias de requeima e pinta preta da batateira sobre diferentes condições ambientais e programas de aplicação de fungicidas, três experimentos foram conduzidos usando a cultivar Ágata. Cada experimento consistiu de dois ensaios lado a lado e cada ensaio foi conduzido no delineamento em blocos casualizados com 5 tratamentos e 5 repetições. Aos 30 dias após o plantio, as plantas de batata do ensaio 1 foram inoculadas artificialmente com um isolado do grupo de compatibilidade A2 de *P. infestans* (200 esporângios/mL) e as plantas do ensaio 2 foram inoculadas com isolados de *A. grandis* (200 conídios/mL). As aplicações de fungicidas iniciaram aos 7 dias após a inoculação. A severidade de ambas doenças foram quantificadas a cada dois dias e os dados de severidade foram usados para calcular a área abaixo da curva de progresso (AACPD). A produtividade e os danos foram também obtidos. A requeima foi mais agressiva do que a pinta preta sobre as diferentes condições climáticas, e isso refletiu na produtividade. A requeima causou danos de 82,4%, enquanto que a pinta preta causou danos de 44,9%. A eficiência dos fungicidas mudou sobre as diferentes condições ambientais, mostrando a importância do uso de ferramentas que monitoram o clima, como o uso de sistema de previsão, para o controle eficiente das doenças.

## ABSTRACT

DUARTE, Henrique da Silva Silveira, D.Sc., Universidade Federal de Viçosa, December, 2012. **Late blight and early blight on potato: Resistance and comparative epidemiology.** Adviser: Laércio Zambolim. Co-advisers: Eduardo Seiti Gomide Mizubuti and José Ivo Ribeiro Júnior

Potato (*Solanum tuberosum* L.) production is limited by several factors, including diseases. Among the diseases that affect potatoes, late blight caused by *Phytophthora infestans* and early blight caused by *Alternaria grandis* are considered of great importance. Studies about epidemiology and resistance of potato to late blight and early blight are very important to better understand these pathosystems. To assess the resistance of potato cultivars to foliar late blight and examine how resistance to foliar late blight relates to foliage maturity type and skin type in potato cultivars in Brazil, two field experiments were carried out under different environmental conditions at the Universidade Federal de Viçosa (UFV) in Viçosa-MG. The first experiment used 34 cultivars (treatments). The second experiment used 17 cultivars. The majority of potato cultivars were susceptible to foliar late blight. The cultivars with the highest levels of resistance to foliar late blight (resistant and moderately resistant) were later maturity (mid-late and late maturity). Most cultivars that have been classified as moderately susceptible or susceptible were earlier maturity (early or mid-early maturity). In general, cultivars that are more resistant to foliar late blight had a rougher skin, whereas the skins of the more susceptible cultivars had smoother. To estimate the severity of late blight and early blight of the potato on the leaves and experimental plots, it is necessary to use a standard area diagram (SAD) and a field key (FK), respectively. Currently, there are these two scales to late blight only. For early blight, there is only a SAD, comprising diagrams of potato leaves with early blight severity ranging from 0 to 50%. However, this scale has limitations. For this, were developed and validated a SAD and FK. The proposed SAD contains illustrations of leaves with twelve disease severity levels (0.05, 0.5, 2, 4, 8, 16, 32, 48, 62, 82, 96 and 100%). The proposed FK was an adaptation of a FK developed to assess the severity of potato late blight. Both the SAD and FK improved raters' ability to accurately, precisely and reliably estimate potato early blight severity, and as such these scale can be used for assessing severity for many purposes, including plant breeding for resistance,

fungicide screening, and pathotype characterization. To assess the resistance of potato cultivars to foliar early blight and examine how resistance to foliar early blight relates to foliage maturity type and skin type in potato cultivars in Brazil, three field experiments were carried out under different environmental conditions at the UFV in Viçosa-MG. The first and second experiments used 26 cultivars (treatments). The third experiment used 24 cultivars. The majority of potato cultivars were susceptible and moderately susceptible to foliar early blight. The resistance levels of some cultivars to foliar early blight changed according to the environmental conditions. The resistant cultivars to foliar early blight had mid-season, mid-late or late maturity. No cultivars susceptible to foliar early blight were observed to have later maturity (mid-late or late maturity). In most cases, the cultivars found to be susceptible to foliar early blight had earlier maturity (early or mid-early maturity). The cultivars found to be resistant had a rough, mid-rough or smooth skin. Cultivars that were susceptible to foliar early blight and had rough skin were not observed. In most cases, the cultivars that were found to be susceptible or moderately susceptible to foliar early blight had smooth skin. Aiming to compare late blight and early blight potato epidemics under different environmental conditions and fungicide applications programs, three experiments were carried out using the 'Ágata' cultivar. Each experiment consisted of two side-by-side trials, and each trial was set in a randomised block design with 5 treatments and 5 replications. At 30 days after inoculation, the potato plants of trial 1 were artificially inoculated with the A2 mating type isolate (200 sporangia/mL) of *P. infestans* and the potato plants of trial 2 were artificially inoculated with isolates (200 conidia/mL) of *A. grandis*. Fungicide applications started seven days after inoculation. The late blight severity and the early blight severity were quantified every two days, and the disease severity data were used to calculate the area under the disease progress curve (AUDPC). The yield and its loss were also measured. The late blight was more aggressive than the early blight under different environmental conditions, and this was reflected in the yields. The late blight reached yield losses of 82.4%, while the early blight reached yield losses of 44.9%. Fungicide efficiency changed under the different environmental conditions, showing the importance of using tools that monitor the weather, such as forecast systems, for effective disease control.

## General Introduction

The potato (*Solanum tuberosum* L.) ranks fourth among the foods produced worldwide, and it is cultivated in more than one hundred countries. Potato production is limited by several factors, including diseases. Among the diseases that affect potatoes, late blight caused by *Phytophthora infestans* and early blight caused by *Alternaria grandis* are considered of great importance due to the losses they cause in potato crops.

Among late blight and early blight control measures, the regular application of fungicides is the most important and is considered to be effective (Aylor et al. 2001; De Souza and Iamauti 2005; Guenther et al. 1999, Mizubuti 2001; Töfoli et al. 2003, Shuman and Christ 2005). However, regular use of expensive fungicides throughout the entire crop cycle significantly increases production costs. Fungicides also pose risks to human health, cause environmental contamination by run-off or leaching of excessively sprayed products, leave residues in tubers, and their use increases the risk of selection for fungicide-resistant isolates, especially those with more specific mechanisms of action. All of these factors are sources of concern and have led to an increase in research aimed at developing practices and management strategies that are rational, efficient and economically viable (Costa et al. 2002). Among these strategies is the use of cultivars with higher levels of resistance to late blight and early blight to assist the chemical control, this because fungicides cannot yet be completely removed from the disease management program. The use of resistant cultivars is the preferred control method for plant diseases due to its high efficacy, low cost, and ease of implementation. However, the main cultivars planted in Brazil are still unclassified in terms of their resistance to foliar late blight and to foliar early blight.

In addition to disease resistance, the foliage maturity type and the skin type of the potato tuber are factors that growers may consider when they select a cultivar to plant. Their selection will also depend on the culinary attractiveness of the cultivar in the consumer market. The current preference is to use earlier maturity cultivars that also have a smooth skin and are resistant to disease resistance. Thus, it is important to know whether it is possible to obtain all three characteristics in the same cultivar.

To estimate the severity of late blight and early blight of the potato on the leaves and experimental plots, it is necessary to use a standard area diagram (SAD)

and a field key (FK), respectively. Currently, there are these two scales to late blight only. For early blight, there is only a SAD comprising diagrams of potato leaves with early blight severity ranging from 0 to 50%. However, this scale has two limitations: It has not been validated for higher levels of severity, and it is unclear whether the raters are precise and accurate when using this scale. Additionally, using only the SAD to assess early blight at an experimental plot is not an easy task; therefore, the accuracy and precision of measures of disease intensity can be improved with a combination of the SAD and a FK.

In addition to the works cited above, comparative epidemiological studies could provide a better understanding of the epidemiology of these pathosystems and offer new insights on how to manage these diseases in the field. Comparative epidemiology looks at similarities and differences among epidemics (Kranz 1988).

Despite the impact of late blight and early blight on potato crops, there have been no comparative epidemiological analyses of these diseases under different environmental conditions. Another area worth exploring is the effect of different fungicides for the control of late blight and early blight of potatoes in these conditions.

The objectives of this study were:

- 1- To assess the resistance of potato cultivars to foliar late blight and examine how resistance to foliar late blight relates to foliage maturity type and skin type in potato cultivars in Brazil.
- 2- To develop and validate a SAD to quantify the severity of early blight on potato leaves and providing assistance in the use of the FK developed and validated in this work to quantify the severity of early blight on potato plants in experimental plots.
- 3- To assess the resistance of potato cultivars to foliar early blight and examine how resistance to foliar early blight relates to foliage maturity type and skin type in potato cultivars in Brazil.
- 4- To compare epidemics of LB and EB of potatoes under different environmental conditions and fungicide application programs in Brazil.

## References

- Aylor DE, Fry WE, Mayton H, Andrade Piedra, JL (2001) Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology* 91:1189-1196
- Costa RV, Zambolim L, Vale FXR, Mizubuti ESG (2002) Previsão da requeima da batateira. *Fitopatol Bras* 27: 349-354
- De Souza JAC, Iamauti MT (2005) Doenças da batateira. In: Kimati H, Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (Eds) *Manual de Fitopatologia - Doenças das Plantas Cultivadas*. São Paulo, pp.119-149
- Guenther JF, Wiese MV, Pavlista A D, Sieczka JB, Wyman J (1999) Assessment of pesticide use in the US potato industry. *Am J Pot Res* 76: 25-29
- Kranz, J (1988) The methodology of comparative epidemiology. In: Kranz J, Rotem J (Eds) *Experimental Techniques in Plant Disease Epidemiology*. Heidelberg. Springer-Verlag, pp.279-290
- Mizubuti ESG (2001) Requeima ou mela da batata e do tomate. In: Luz EDN, Santos AF, Matsuoka, K, Bezerra JL (Eds) *Doenças causadas por Phytophthora no Brasil*. Campinas, BR: Livraria Editora Rural, pp 100-174
- Reifschneider FJB, Furumoto O, Filgueira FAR (1984) Illustrated key for the evaluation of early blight of potatoes. *FAO Plant Protect B* 32:91-94
- Shuman JL, Christ BJ (2005) Integrating a host-resistance factor into the Fast system to forecast early blight of potato. *Am J Pot Res* 82: 9-19.
- Töfoli JG, Domingues RJ, Garcia Junior O, Kurozawa C (2003) Controle da pinta preta do tomateiro por fungicidas e seus impactos na produção. *Summa Phytopathol* 29: 225-233

## ARTICLE 1

### **The field resistance of potato cultivars to foliar late blight and its relationship with foliage maturity type and skin type in Brazil**

#### **Abstract**

The use of resistant cultivars is the preferred control method for plant diseases. This approach is attractive owing to its high efficacy, low cost, and ease of implementation. This work aimed to assess the resistance of potato cultivars to foliar late blight (*Phytophthora infestans*) under different environmental conditions. It further aimed to assess the relationships between resistance to foliar late blight and foliage maturity type and between resistance to foliar late blight and the skin type of the potato. Two field experiments were carried out under different environmental conditions at the Universidade Federal de Viçosa in Viçosa. The first experiment used 34 cultivars (treatments). The second experiment used 17 cultivars. The severity of foliar late blight in each plot was quantified every two days using a field key. The values of the epidemiological variables were then calculated. The proposed levels of resistance to foliar late blight were defined as: resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). The majority of potato cultivars were susceptible to foliar late blight. The cultivars with the highest levels of resistance to foliar late blight (R and MR) were later maturity (mid-late and late). Most cultivars that have been classified as MS or S were earlier maturity. In general, cultivars that are more resistant to foliar late blight had a rougher skin, while for the more susceptible it was smoother.

**Keywords:** *Phytophthora infestans*; potato disease; *Solanum tuberosum* L..

#### **1 Introduction**

The potato (*Solanum tuberosum* L.) crop is the fourth most produced food worldwide and is cultivated in more than hundred countries. Potato production is limited by several factors, including diseases. From planting to harvest, the potato crop is subject to a large number of diseases. For this reason, the commercial production is dependent on fungicide application. Among the diseases, late blight caused by *Phytophthora infestans* (Mont.) de Bary is considered one of the most destructive and

the major concern for potato cultivation not only in Brazil but all around the world (Aylor et al. 2001; Flier et al. 2001; Flier et al. 2003; Mizubuti and Fry 2006). When control measures fail, late blight epidemics may destroy an entire production field in a few days.

Late blight occurs in all Brazilian regions that cultivate potatoes. High humidity and temperatures between 12 and 20°C (Fohner et al. 1984) favor disease development. When the temperature is over 30°C disease development is slow or even stopped. Because the pathogen may remain alive in the host's tissues, it may spur and cause damage as soon as climatic conditions become favorable (Lima et al. 2009).

Potatoes are cultivated in Brazil in regions and seasons in which the conditions are highly favorable to development of late blight. The planting of susceptible cultivars can result in severe epidemics (Costa et al. 2002). Among late blight control measures, the regular application of fungicides is the most important and is considered to be effective (Aylor et al. 2001; Mizubuti 2001). It should occur as soon as the first leaves emerge and until the end of the cropping cycle. Applications occur at three to five-day intervals (Nazareno et al. 1999); this regular use of expensive fungicides throughout the entire crop cycle significantly increases production costs. It is also responsible for greater risks to human health, environmental contamination caused by run-off, leaching of excessively sprayed products, fungicide residues in tubers and the risk of selection for fungicide-resistant isolates, especially those with more specific mechanisms of action. All of these factors are elements of concern and have led to an increase in research related to practices and management strategies that are rational, efficient and economically viable (Costa et al. 2002). Among these strategies is the use of cultivars with higher levels of resistance to late blight to assist the chemical control, this because the fungicides cannot yet be completely removed from the disease management program.

The use of resistant cultivars is the preferred control method for plant diseases due to its high efficacy, low cost, and simplicity to implement. In disease management programs, host resistance may be combined and/or complemented with the use of fungicides to improve late blight control under highly favorable conditions (Fry et al. 1983; Mizubuti and Fry 2006). Potato cultivars with higher levels of resistance demand less fungicide than cultivars with lower levels of resistance (Fry 1978). Furthermore, the use of cultivars with higher resistance levels may reduce yield losses and dependence on chemical control (Garrett et al. 2001). For this purpose, it is vital to evaluate the

resistance of the cultivars. However, the main cultivars planted in Brazil are still unclassified in terms of their resistance to foliar late blight.

In addition to disease resistance, the foliage maturity type and the skin type of the potato tuber are factors that growers may consider when they select a cultivar to plant. Their selection will also depend on the culinary attractiveness of the cultivar in the consumer market. The current ideal trend is to use earlier maturity cultivars that also have smooth skin and disease resistance. For this reason, it is important to know whether it is possible to obtain all three characteristics in the same cultivar.

This work aimed to assess the resistance of potato cultivars to foliar late blight. Likewise, it aimed to assess the relationships between resistance to foliar late blight and foliage maturity type and between resistance to foliar late blight and skin type in potato cultivars in Brazil.

## **2 Material and Methods**

### **2.1 General aspects**

Two field experiments were carried out under different environmental conditions to study the resistance of potato cultivars to foliar late blight. Each plot consisted of two rows measuring 2.5 m x 1.6 m. Each cultivar had 15 stems/m<sup>2</sup>, and the plots were separated by 2.0 m unplanted row.

The climate variables measured included: precipitation; maximum, average, and minimum relative humidity; and maximum, average and minimum temperature.

Plants were fertilized according to the soil analyses and local recommendations. Sprinkle irrigation was used over the entire experimental area to favor foliar late blight epidemic. Dimetomorph + chlorothalonil (239.7 + 1198.5 g a.i./ha) fungicides were sprayed to control foliar late blight every five days after plant emergence, and the use of these fungicides was suspended when the plants of all cultivars were at least 25 cm tall. Cultivars differed regarding emergence time; the fungicides, therefore, were intensively used so that plants that emerged first were not infected by *P. infestans* until the natural or artificial inoculation.

Specific fungicides were applied to control potato early blight (*Alternaria* spp.), allowing only the establishment of late blight in the field. Fungicides were applied with a backpack sprayer with a pressure-regulating valve to obtain a 30 lb/inch<sup>2</sup> working pressure. A conical tip was used and fungicide mix was applied at a rate of 600 L/ha.

The experiments were performed in a completely randomized block design with three replications, and each treatment was represented by one cultivar.

## **2.2 Experiment 1**

This trial was carried out in an experimental field at the Universidade Federal de Viçosa in Viçosa, which is in the southeast of the state of Minas Gerais, at 20°44'44"S, 42°50'59"W and in at 661 m above sea level. The experiment was conducted from April 13th to July 12th 2009 using 34 potato cultivars. The cultivars, origins, maturity type and skin type are in Table 1. Plots were set in a red-yellow latosol with 58% of clay, low level of organic matter (pH= 6.2; K = 154 mg/dm<sup>3</sup> and P =16.2 mg/dm<sup>3</sup>).

The leaves of potato plants were artificially inoculated with a backpack sprayer 38 days after planting (DAP) with one A2 mating type isolate of *P. infestans* collected from each potato region of Minas Gerais state (Maria da Fé, Perdizes and São Gotardo) to be used in this study. The three A2 mating type isolates were mixed to obtain 500 sporangia/mL.

## **2.3 Experiment 2**

This trial was carried out at the Universidade Federal de Viçosa from May 18<sup>th</sup> to September 1<sup>st</sup> 2009 using 17 potato cultivars. Plots were set in a red-yellow latosol with 58% of clay, low level of organic matter (pH= 6.1; K = 181 mg/dm<sup>3</sup> and P =22.2 mg/dm<sup>3</sup>). The origins, foliage maturity type and skin type of potato cultivars evaluated in experiment 2 are in Table 2.

The cultivars, origins, maturity type and skin type are in Table 2. The leaves of potato plants were artificially inoculated with a backpack sprayer 56 DAP with one A2 mating type isolate of *P. infestans* collected from each potato region of Minas Gerais state (Maria da Fé, Perdizes and São Gotardo) to be used in this study. The three A2 mating type isolates were mixed to obtain 500 sporangia/mL.

## **2.4 Determination of mating type**

For experiments 1 and 2, in which the potato plants were artificially inoculated, determination of the isolates mating type took place before inoculation. Isolates of the

A2 mating type were used because are more aggressive to potato than isolates of the A1 mating type (Suassuna *et al.* 2004). To confirm the mating type, the three isolates were paired with two standard mating types: A1 (US-1) and A2 (BR-1) from the Laboratório de Biologia de Populações de Fitopatógenos (Universidade Federal de Viçosa). The pairing took place in a 90 mm-diameter Petri dish in which a portion of the culture medium, approximately 0.5 cm in width and 3 cm in length containing mycelia of *P. infestans* from a standard isolate, was cut and placed on one end of the plate. A disk of the unknown mating type isolate to be tested was cut from the culture medium and placed on the other end of the dish. The Petri dish culture medium containing the paired isolates was kept in an incubator at 18°C in the dark. After two weeks of incubation, each Petri dish was observed under a light microscope to evaluate oospore formation. If the unknown isolate formed oospores when paired with the A1 standard isolate, it was classified as A2, and if oospores were formed when paired with the A2 standard isolate, it was classified as A1.

## **2.5 Disease assessment**

The foliar late blight severity of each plot was quantified every two days from the onset of the first disease symptoms using a descriptive key (James, 1971) modified by Fry *et al.* (1977).

## **2.6 Data analysis**

The epidemiological variables used were the same variables selected by Duarte *et al.* (2010). These variables discriminate effectively among the different levels of resistance to potato late blight. From the severity data of each cultivar, the following variables were obtained:

- 1- Severity in the middle of the epidemic ( $Y_{50}$ ). An epidemic in the experiment was considered from the onset of the first symptoms in any cultivar until all of them reached 100% severity.
- 2- Area under the disease progress curve (AUDPC) (as proposed by Shaner & Finney, 1977). An epidemic in the experiment was considered from the onset of the first symptoms in any cultivar until all of them reached 100% severity. Then the AUDPC was divided by the total area of the graph (=duration of the epidemic in days x 100) to

obtain the relative area under the disease progress curve (RAUDPC) as proposed by Fry (1978).

3- Time in days from the onset of the first symptoms in the experiment until 0.5% severity ( $T_{0.5}$ ). The time for each cultivars was considered from the onset of the first symptoms among all cultivars until a given cultivars to reach 0.5% severity.

4- Disease progress rate ( $r$ ). From the disease severity progression data, the exponential, monomolecular, Gompertz and logistic models were adjusted with linear and non-linear regressions (Madden *et al.*, 2007). The best model was selected using criteria such as the lower value of the residual mean square, independence and homogeneity, and highest determination coefficient ( $R^2$ ). Analysis was carried out with the SAS (Statistical Analysis System) program, version 9.0.

The average values of the epidemiologic variable were obtained for use in principal component analysis.

To determine the potato cultivars' levels of resistance to foliar late blight, principal component analysis (PCA) was used. This analysis was carried out using a data correlation matrix and the estimated eigenvectors and eigenvalues were obtained for each principal component (PC). The scores of all potato cultivars were estimated through eigenvectors of each response variable for PCs presenting eigenvalues greater than the overall mean (Kaiser 1958). PC1 scores were obtained from PCA to experiment 1 and 2. The analysis was carried out with the MINITAB program, version 14. To allow the scores to have the same sign (standardized value), the PC1 scores of each cultivar were added to the value with the opposite sign from the PC1 score of Ibituaçu cultivar, which was used as a resistant standard. After standardization, the percentage corresponding to the standardized value of each cultivar was obtained by dividing the cultivar standardized value by standardized value of the Elodie cultivar considered to be susceptible standard, and multiplying by 100. After obtaining the percentages, each cultivar was classified according to its level of resistance to foliar late blight based on the value range defined by Duarte et al, 2010, where cultivars with percentage values between <0.00 and 9.99 were considered as resistant; between 10.00 and 49.99 as moderately resistant; between 50.00 and 75.99 as moderately susceptible and >76.00 as susceptible. With percentages values, too was possible to obtain a ranking of the cultivars resistance.

After the classification of the cultivars based on their foliar late blight resistance, the average values were obtained for epidemiological variables of experiment 1 and 2 within each resistance level.

## **2.7 Disease progress curves**

The disease progress curves of the different resistance levels for all two experiments were obtained after classification of the cultivars according to their level of resistance to foliar late blight based on the average of the severity data over time within each resistance level.

## **2.8 Relationships between resistance to foliar late blight and foliage maturity type and between resistance to foliar late blight and the skin type of the potato**

The levels of resistance to foliar late blight for all the potato cultivars were determined under different environmental conditions. Graphical analysis and Chi-square test of independence at 5% level were then used to investigate the relationships between resistance to foliar late blight and foliage maturity type and between resistance to foliar late blight and the skin type of the potato.

## **3 Results**

### **3.1 Mating type**

After comparing three isolates collected from each potato region of Minas Gerais state (Maria da Fé, Perdizes and São Gotardo) to the two standard isolates (one belonging to the A1 mating type and the other to the A2 mating type), it was observed that oospores formed only when isolates were paired to the standard isolate of the A1 mating type. Therefore, the isolates infecting plants in experiment 1 and 2 belong to the A2 mating type.

### **3.2 Meteorological data**

In experiment 1, the temperature ranged from 6.5 to 27.8°C, and the average temperature during the epidemic was 16.6°C (Fig. 1a). Relative humidity ranged from

35 to 100% (data not shown), and average relative humidity was 83.9% (Fig. 1a). Pluviometric precipitation during the experiment was 81.0 mm (Fig. 1a).

In experiment 2, the temperature ranged from 8.7 to 29.3°C, and the average temperature during the epidemic was 17.5°C (Fig. 1b). Relative humidity ranged from 36 to 100% (data not shown), and average relative humidity was 78.1% (Fig. 1b). Pluviometric precipitation during the experiment was 31.0 mm (Fig. 1b).

### **3.3 Disease progress**

In experiment 1, the foliar late blight epidemic started 42 DAP. At 46 DAP, all cultivars presented symptoms of the disease. Only at 90 DAP the Ibituaçu cultivar presented 100% disease severity. Therefore, the epidemic for experiment 1 lasted 49 days.

In experiment 2, the foliar late blight epidemic started 60 DAP. At 62 DAP, all cultivars presented symptoms of the disease. Only at 104 DAP the Ibituaçu cultivar presented 100% disease severity. Therefore, the epidemic for experiment 2 lasted 45 days.

The model that fit the severity data best for all cultivars in all the three experiments over time was the logistic model with non-linear regression. Therefore, data on the disease progression values ( $r$ ) were compared among all cultivars.

### **3.4 Resistance levels of in the cultivars of experiment 1 to foliar late blight**

With the data from the four epidemiological variables for each cultivar, it was possible to obtain PC scores. The average of the eigenvalues obtained from all PCs was 1.0. Therefore, only PC1 was interpreted because it was the only one that presented an eigenvalue higher than the average. PC1 explained 80.5% of the total variation of the data (data not shown).

According to the percentage values of the cultivar standardized values in relation to the Elodie cultivar standardized value and in line with the range of values defined by Duarte et al, 2010, the Ibituaçu cultivar was classified as resistant; the Colorado cultivar was classified as moderately resistant; the Aracy, Aracy Ruiva, Baraka, Baronesa, BRS Ana, BRS Elisa, Caesar, Catucha, Florice, and IAPAR Cristina cultivars were classified as moderately susceptible; and the Ágata, Almera, Asterix,

Atlantic, Canelle, Chipie, Cupido, Éden, Elodie, Emeraude, Eole, Fontane, Gourmandine, Gredine, Itararé, Markies, Melody, Monalisa, Naturella, Opaline, Soléia and Voyager cultivars were classified as susceptible (Table 3). The percentages of resistant, moderately resistant, moderately susceptible and susceptible cultivars were 2.9, 2.9, 29.5 and 64.7%, respectively.

Estimating PC1 scores using the four selected response variables and obtaining the percentage corresponding to the standardized value of each cultivar, a ranking of the cultivars was obtained. It was then possible to identify, which cultivars were more resistant or more susceptible to foliar late blight. The resistance of potato cultivar decreased as the percentage value increased. Conversely, the resistance of potato cultivar increased as the percentage value decreased (Table 3).

### **3.5 The level of resistance to foliar late blight in the cultivars of experiment 2**

Using the data of the four epidemiological variables for each cultivar, PC scores were obtained. The average eigenvalue obtained from all PCs was 1.0. Therefore, only PC1 was interpreted because it was the only one that presented an eigenvalue higher than the average. PC1 explained 89.3% of the total variation of the data (data not shown).

According to the percentage values of the cultivar standardized values in relation to the Elodie cultivar standardized value and in line with the range of values defined by Duarte et al, 2010, the Ibituaçu cultivar was ranked as resistant; the Innovator, Victoria and Vivaldi cultivars were ranked as moderately susceptible; and the Annabelle, Ambra, Amorosa, Armada, Elodie, Fabula, Madeleine, Maranca, Marlen, Matador, Mozart, Rodeo and Sinora cultivars were ranked as susceptible. The percentages of resistant, moderately resistant, moderately susceptible and susceptible cultivars to foliar late blight were 5.9, 0.0, 17.6 and 76.5%, respectively.

Estimating PC1 scores using the four selected response variables and obtaining the percentage corresponding to the standardized value of each cultivar, a ranking of the cultivars was obtained. It was then possible to identify, which cultivars were more resistant or more susceptible to foliar late blight. The resistance of potato cultivar decreased as the percentage value increased. Conversely, the resistance of potato cultivar increased as the percentage value decreased (Table 3).

### **3.6 The relationship between the resistance to foliar late blight and the foliage maturity type of potato**

A graphical representation of the relationship between the level of resistance to foliar late blight and the foliage maturity type of each cultivar, based on the two experiments, is shown in Fig. 3. It is observed that resistant and moderately resistant cultivars to foliar late blight had later maturity (mid-late and late maturity), whereas in most cases, the cultivars found to be susceptible and moderately susceptible to foliar late blight had earlier maturity (early or mid-early maturity). This relationship observed between the level of resistance to foliar late blight and the foliage maturity type by graphical representation was confirmed by Chi-square test of independence because there was a relationship significant between the level of resistance to foliar late blight and the foliage maturity type at 5% level ( $P=0.002$ ).

### **3.7 The relationship between resistance to foliar late blight and the skin type of the potato**

A graphical representation of the relationship between the level of resistance of the cultivars to foliar late blight and the skin type of each cultivar, based on the two experiments, is shown in Fig. 4. The cultivars found to be resistant or moderately resistant had a rougher skin, except the Ibituaçu cultivar that had smooth skin. In most cases, the cultivars that were found to be susceptible or moderately susceptible to foliar late blight had smooth skin. This relationship observed between the level of resistance to foliar late blight and the skin type by graphical representation was confirmed by Chi-square test of independence because there was a relationship significant between the level of resistance to foliar late blight and the skin type at 5% level ( $P=0.000$ ).

## **4 Discussion**

In the present research, we evaluated the resistance to foliar late blight for potato cultivars in Brazil in different climatic conditions. We did not assess the resistance to late blight in tuber because we did not find symptoms of late blight on tubers in the two experiments. We believe that was due several reasons: a) oospores of *P. infestans* were not detected in Brazil (Reis et al. 2006), b) the sporangia were not able to cause

symptoms on tubers due to low survival in the soil, c) precipitation during the late blight epidemic in the three experiments was very low and perhaps the only irrigation was not sufficient to lead large amount of inoculum to the ground, d) the experiments were carried in clay soil (58% of clay for experiments 1 and 2) which are unfavorable conditions to the development of symptoms of late blight on tubers, and e) the experiments were not done in commercial potato fields, where there are many crops by year. Late blight in tuber is not important for Brazil in conditions and potato tuber is not considered as an important inoculum source of *P. infestans*.

This study confirms that most cultivars planted in Brazil are susceptible to foliar late blight. The same results have been observed in Western Europe (Colon et al. 1995) and in North America (Platt and Tai 1998) where most of the cultivars were also observed to be susceptible.

No potato cultivar was immune to foliar late blight. For this reason, the disease will occur in the field whenever the climatic conditions are favourable and the *P. infestans* inoculum is present. However, the intensity of the disease will depend on each cultivar's resistance level.

The classification of the cultivars according to their level of resistance to foliar late blight in experiment 1 and 2 was representative for Zona da Mata of the State of Minas Gerais. To obtain a more comprehensive classification of the cultivars concerning their level of resistance to foliar late blight, studies in other regions should be carried out to verify the stability of cultivars' resistance to foliar late blight.

The occurrence of the *P. infestans* A2 mating type in potato plants in experiment 1 is consistent with the literature. The first report of the occurrence of *P. infestans* isolates in Brazil occurred in 1986, and these isolates were also of the A2 mating type (Brommonschenkel 1988). In a survey carried out in Brazil, 82% of 184 potato isolates of *P. infestans* were of the A2 mating type (Reis et al. 2003). The population of *P. infestans* in Brazil consists of two clonal lineages, US-1 associated with tomatoes (A1 mating type) and BR-1 (A2 mating type) associated with potatoes. Isolates of BR-1 clonal lineages are more aggressive to potato cultivars than to tomato cultivars, indicating significant host-specificity (Suassuna et al. 2004).

The results obtained in these experiments are consistent with those in the literature in terms of climatic conditions for the development of foliar late blight epidemics. The average temperature during the epidemic favored the occurrence of sporangia germination, increasing the potential of *P. infestans* to cause infection and

severe disease. The relative humidity in this study also favored the disease epidemic. A trial was conducted in the winter in Viçosa-MG, and this revealed that relative humidity of 80% was enough to generate leaf wetness (Costa et al. 2002) because dew tends to form at lower relative humidity in the winter than in the summer (Jones 1986). It can be concluded that leaf wetness in this study was enough to develop foliar late blight epidemics in the experiments. Foliar late blight-favorable climatic conditions associated with a more aggressive isolate of *P. infestans* in potato cultivars explained the rapid development of the disease under field conditions.

The variables used to identify potato cultivar resistance to foliar late blight in the present trials are the same as those used by a number of other authors, including the area under the disease progress curve (Cadena-Hinojosa et al. 2007; Flier et al. 2003; Forbes et al. 2005; Fry 1978; Grünwald et al. 2000; Hansen et al. 2005; Haynes and Weingartner 2004; Ojiambo et al. 2000), the rate of disease progress (Fry 1978; Ojiambo et al. 2000), the disease severity (Cadena-Hinojosa et al. 2007, Fry 1978;) and the number of epidemic days (Hansen et al. 2005). In these papers, variables were analyzed individually over time, but in the present work the variables were simultaneously analyzed to obtain a more global and simplified interpretation.

The cultivars with the highest levels of foliar late blight resistance (resistant or moderately resistant) exhibited later maturity (mid-late and late maturity). Similar results have been found by Visker et al. (2004), who showed that resistance against *P. infestans* coincides with later maturity. Most cultivars that have been classified as moderately susceptible or susceptible exhibit earlier maturity (early and mid-early maturity). The relationship between resistance and foliage maturity type is well known (Toxopeus 1958). It is rare to find cultivars exhibiting later maturity that are very susceptible to foliar late blight, whereas cultivars exhibiting earlier maturity and having high resistance levels do not occur (Swiezynski 1990; Toxopeus 1958). This relationship was also observed in the current study. Some cultivars exhibiting mid-late maturity were susceptible to foliar late blight. However, cultivars with the highest levels of foliar late blight resistance (resistant or moderately resistant) and exhibiting earlier maturity were not observed (Fig. 3). The mechanism underlying this phenomenon might be either physiological or genetic (Toxopeus 1958). Physiological linkage is plausible in view of the observation that both foliar late blight resistance and foliage maturity type are affected by such environmental conditions as photoperiod (Umaerus 1959). Further support for this suggestion has resulted from recent studies of the association between

foliar late blight resistance and foliage maturity type using molecular markers. These studies have shown that all loci for foliage maturity type correlate with loci for foliar late blight resistance (Coolins et al. 1999; Ewing et al. 2000). All these observations support a physiological linkage between race-non-specific resistance to foliar late blight and foliage maturity type (Visker et al. 2004). However, genes with pleiotropic effects, or different genes with different functions that are closely linked on the same loci, cannot be excluded yet (Visker et al. 2003).

In general, cultivars that were more resistant to foliar late blight had rougher skin, whereas the skins of the more susceptible cultivars were smoother. The probable reason for this finding is that when breeding programmes seek potato cultivars with market characteristics such as high yield, clear and smoother skin, and flat buds, the genes that provide disease resistance can be lost, and the cultivars thus present lower resistance levels. For example, the *Ágata*, *Asterix*, *Cupido* and *Monalisa* cultivars have been widely cultivated because they present suitable characteristics for the market, and they are classified as susceptible to foliar late blight. The skin type of the potato tuber is of extreme importance for the “in natura” consumption because smoother-skinned tubers are preferred in this market. However, tubers having rougher skins would be suitable for industrial processing and for the organic market.

Thus, the potato grower must evaluate each characteristic of the plantation-available cultivars and choose the best cultivar to plant. With this classification of potato cultivars resistance to foliar late blight, the grower will find it easier to choose the most suitable cultivars for his purpose in Brazilian environmental conditions. To date, the most important foliar late blight control measure is fungicide application, which may contribute up to 20% of the total cost of the cultivars (Mizubuti 2001). Obtaining potato cultivars resistant to foliar late blight will reduce the costs of production and result in less contamination of the environment from fungicide residues on the tubers. As a consequence, the market competitiveness of the growers will increase and higher sustainability of the entire potato production chain should be ensured.

## **5 Acknowledgements**

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq and Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) for providing the financial support and the student grants.

## 6 References

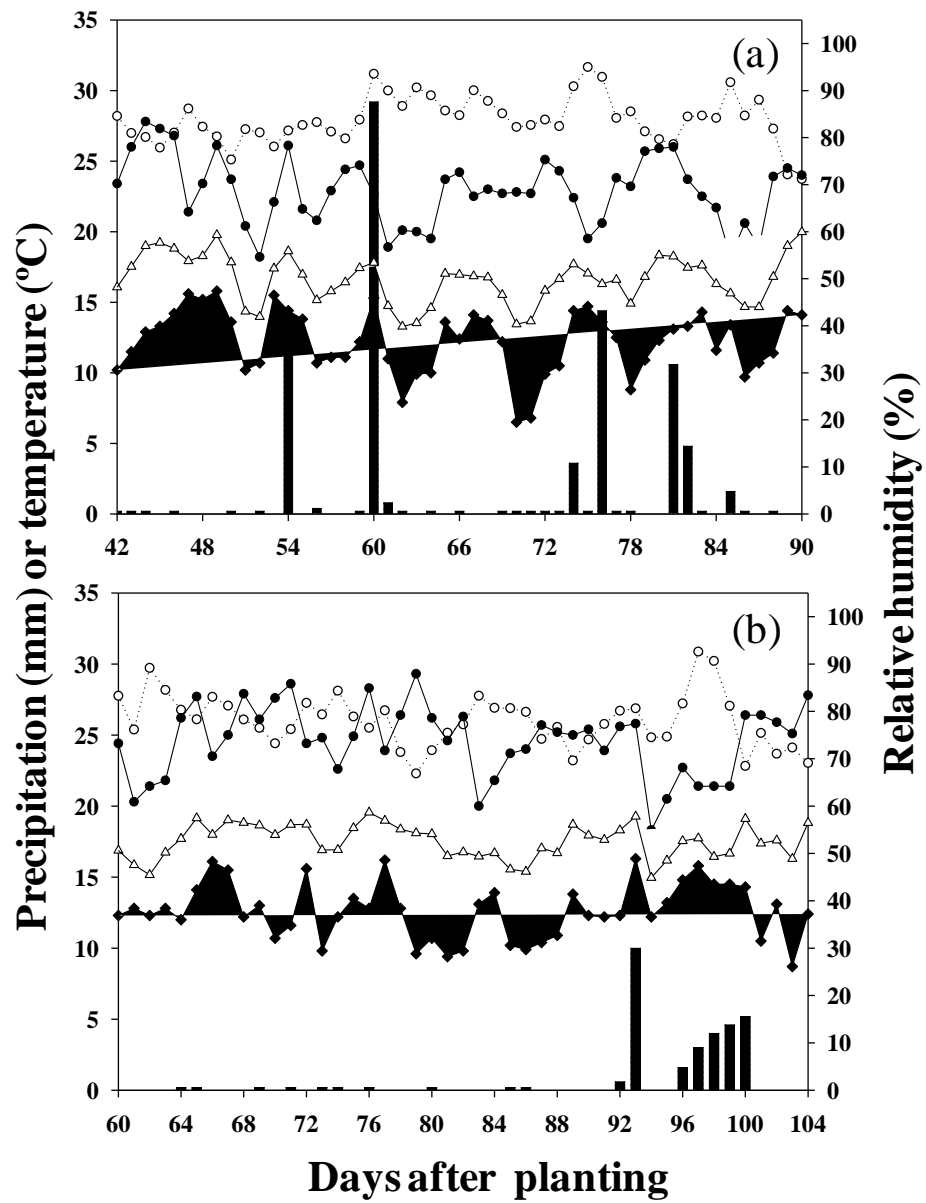
- Aylor DE, Fry WE, Mayton H, Andrade Piedra, JL (2001) Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology* 91:1189-1196
- Brommonschenkel SH (1988) Patogenicidade, compatibilidade, citogenética e padrões isoenzimáticos de isolados de *Phytophthora infestans* (Mont.) de Bary no Brasil. Dissertation, Viçosa Federal University
- Cadena-Hinojosa MA, Díaz-Valasis M, Guzmán-Plazola RA, Fernández-pavía S, Grünwald NJ (2007) Late Blight Resistance of Five Mexican Potato Cultivars in the Eastern Sierra of the State of Mexico. *Am J Pot Res* 84:385-392
- Collins A, Milbourne D, Ramsay L, Meyer R, Chatot- Balandras C, Oberhagemann P, De Jong W, Gebhardt C, Bonnel E, Waugh R (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. *Mol Breed* 5:387–398
- Colon LT, Turkensteen LJ, Prummel, W, Budding DJ, Hoogendoorn J (1995) Durable resistance to late blight (*Phytophthora infestans*) in old potato cultivars. *Eur J Plant Pathol* 101: 387-397
- Costa RV, Zambolim L, Vale FXR, Mizubuti ESG (2002) Previsão da requeima da batateira. *Fitopatol Bras* 27: 349-354
- Duarte HSS, Zambolim L, Mizubuti ESG, Pádua JG, Ribeiro Júnior JI, Carmo EL, Nogueira Júnior AF (2010) Proposal of a classification system for assessing resistance of potato cultivars to late blight. In: III Workshop de Epidemiologia de Doenças de Plantas, 2010, Bento Gonçalves-RS. III Workshop de Epidemiologia de Doenças de Plantas, 2010. v. 1. p. 72-72.
- Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitative resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. *Mol Breed* 6: 25–36
- Flier WG, Turkensteen LJ, Van Den Bosch GBM, Vereijken PFG, Mulder A (2001) Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different of blight resistance. *Plant Pathol* 50: 292-301

- Flier WG, Van Den Bosch GBM, Turkensteen LJ (2003) Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. Plant Pathol 52: 326-337
- Fohner GR, Fry WE, White GB (1984) Computer simulation raises question about timing protecting fungicide application frequency according to potato late blight forecast. Phytopathology 74: 1145-1147
- Forbes GA, Chacón MG, Kirk HG, Huarte MA, Van Damme M, Distel S, Mackay GR, Stewart, HE, Lowe R, Duncan JM, Mayton HS, Fry WE, Andrivon D, Ellissèche D, Pellé R, Platt HW, Mackenzie G, Tarn TR, Colon LT, Budding DJ, Lozoya-Saldaña H, Hernandez-Vilchis A, Capezio S (2005) Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathol 54: 364-372
- Fry WE (1977) Integrated control of potato late blight: Effects of polygenic resistance and techniques of timing fungicide applications. Phytopathology 67:415-420.
- Fry WE (1978) Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68:1650-1655
- Fry WE, Apple AE, Bruhn JA (1983) Evaluation of potato late blight forecast modified to incorporation host resistance and fungicide weathering. Phytopathology 73:1054-1059
- Garrett KA, Nelson RJ, Mundt CC, Chacón G, Jaramillo RE, Forbes GA (2001) The effects of host diversity and other management components on epidemics of potato late blight in the humid highland tropics. Phytopathology 91:993-1000
- Grünwald NJ, Rubio-Covarrubias OA, Fry WE (2000) Potato late-blight management in the Toluca Valley: Forecasts and resistant cultivars. Plant Dis 84:410-416
- Hansen JG, Koppel MB, Valskyte AC, Turka ID, Kapsa J (2005) Evaluation of foliar resistance in potato to *Phytophthora infestans* based on an international field trial network. Plant Pathol 54:169-179
- Haynes KG, Weingartner DP (2004) The use of area under the disease progress curve to assess resistance to late blight in potato germplasm. Am J Pot Res 81:137-141
- James WC 1971. An illustrated series of assessment keys for plant disease, their preparation and usage. Can Plant Dis Surv 51, 39-65.
- Jones AL (1986) Role of wet periods in predicting foliar disease. In: Leonard KJ, Fry WE (Eds) Plant disease epidemiology: population dynamics and management. New York, USA: MacMillan Publishing Company, pp 87-89

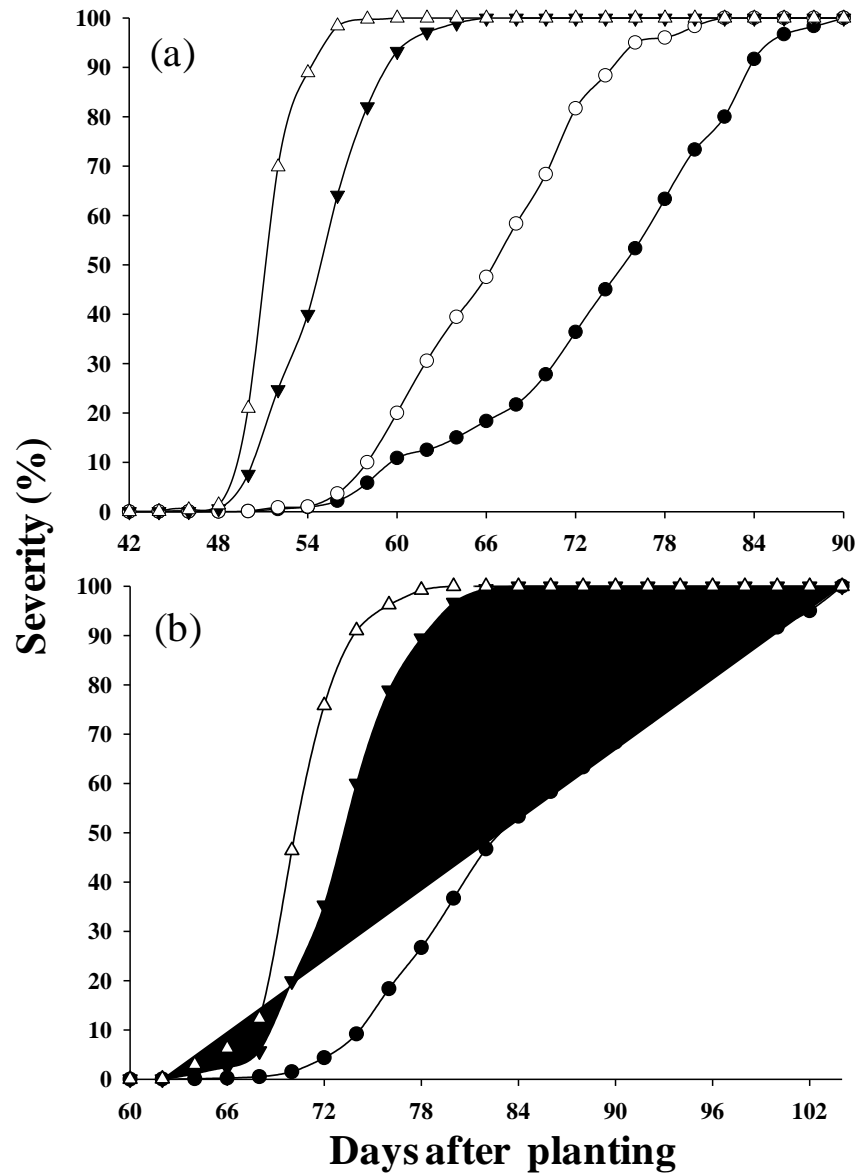
- Kaiser HF (1958) The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 23: 187-200
- Lima MA, Maffia LA, Barreto RW, Mizubuti ESG (2009) *Phytophthora infestans* in a subtropical region: survival on tomato debris, temporal dynamics of airborne sporangia and alternative hosts. *Plant Pathol* 58: 87-99
- Madden LV, Hughes G, Van Den Bosh F (2007) *The Study of Plant Disease Epidemics*. St. Paul, APS Press
- Minitab Statistical Software, version 14.
- Mizubuti ESG (2001) Requeima ou mela da batata e do tomate. In: Luz EDN, Santos AF, Matsuoka, K, Bezerra JL (Eds) *Doenças causadas por Phytophthora no Brasil*. Campinas, BR: Livraria Editora Rural, pp 100-174
- Mizubuti ESG, Fry WE (2006) Potato Late Blight. In: Cooke BM, Jones DG, Kaye B (Eds) *The epidemiology of plant disease*. Dordrecht:Springer, pp 445-471
- Nazareno NRX, Scotti CA, Mafioletti RL, Boschetto N (1999) Controle da requeima da batata através do monitoramento de variáveis climáticas. *Fitopatol Bras* 24: 170-174
- Ojiambo PS, Nyanapah JO, Lung'aho C, Karinga JK, Kidanemariam HM (2000) Comparing different epidemiological models in field evaluations of selected genotypes from *Solanum tuberosum* CIP population A for resistance to *Phytophthora infestans* (Mont.) de Bary in Kenya. *Euphytica* 111:211-218
- Platt HW, Tai G (1998) Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. *Am J Pot Res* 75:173-178
- Reis A, Smart CD, Fry WE, Maffia LA, Mizubuti ESG (2003) Characterization of isolates of *Phytophthora infestans* from southern and southeastern Brazil from 1998 to 2000. *Plant Dis* 87: 896-900
- Reis A, Ribeiro FHS, Mizubuti ESG (2006) Caracterização de isolados de *Phytophthora infestans* do Distrito Federal e de Goiás. *Fitopatol Bras* 31: 270-276
- SAS Statistical Analysis System program, version 9.0.
- Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* 67:1051-1056
- Suassuna ND, Maffia LA, Mizubuti ESG (2004) Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. *Plant Pathol* 53:405-413
- Swiezynski KM (1990) resistance to *Phytophthora infestans* in potato cultivars and its relation to maturity. *Genetic Pol* 31:99-106

- Toxopeus HJ (1958) Some notes on the relations between field resistance to *Phytophthora infestans* in leaves and tubers and ripening time in *Solanum tuberosum* subsp. *tuberosum*. Euphytica 7:123-130
- Umaerus V (1959) The relationship between peroxidase activity in potato leaves and resistance to *Phytophthora infestans*. Am Potato J 36:124–131
- Visker MHPW, Keizer LCP, Van Eck HJ, Jacobsen E, Colon LT, Struik PC (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor Appl Genet 106: 317–325
- Visker MHPW, Van Raaij HMG, Keizer LCP, Struik PC, Colon LT (2004) Correlation between late blight resistance and foliage maturity type in potato. Euphytica 137:311-323

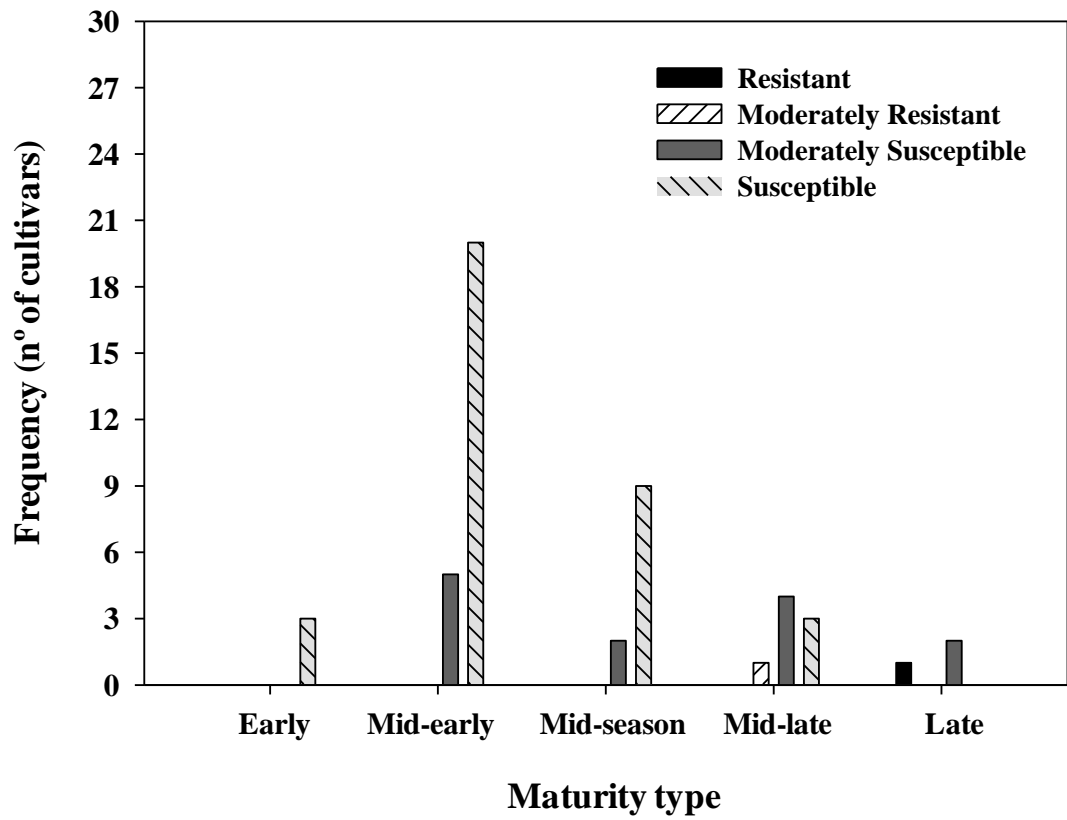
7 Figures



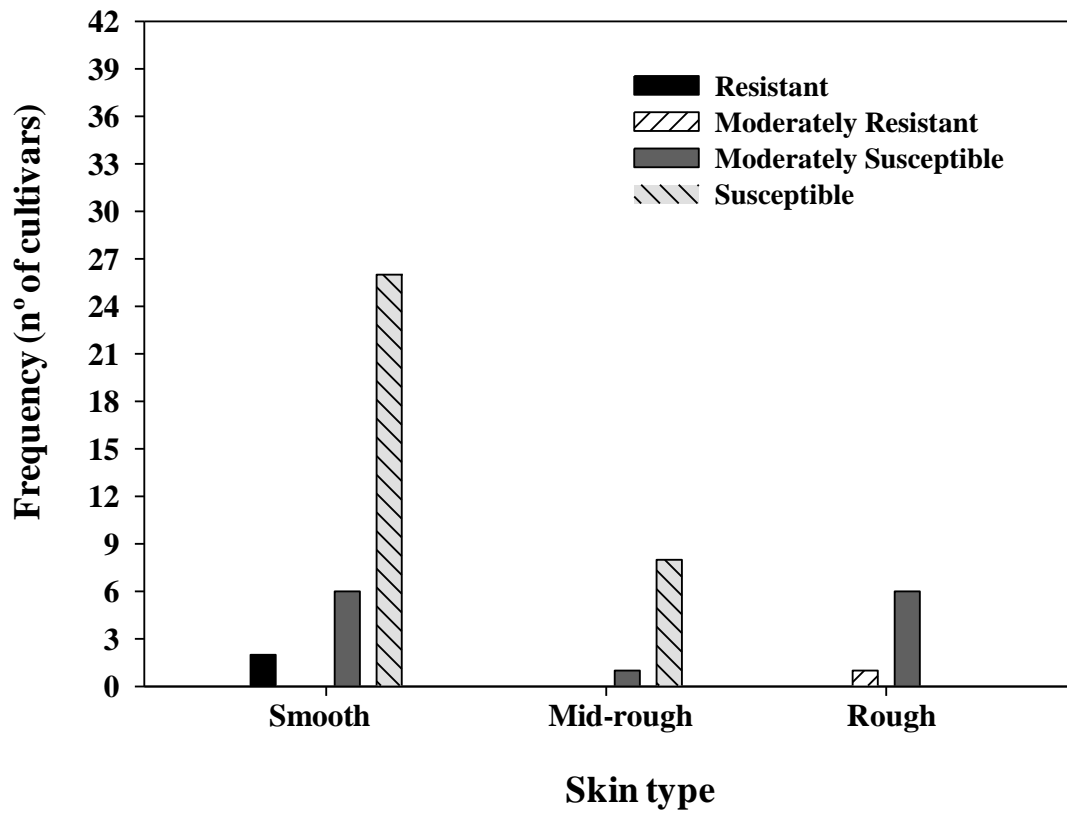
**Fig. 1** Values of meteorological variables recorded during the epidemic in experiments 1 (a) and 2 (b). Average relative humidity is represented by a dotted line with empty circles, pluviometric precipitation by vertical bars and temperature by a solid line. Maximum, medium and minimum temperatures are represented by  $\bullet$ —,  $\triangle$ — and  $\blacklozenge$ —, respectively.



**Fig. 2** Progression curves of each level of cultivar resistance to foliar late blight recorded in experiments 1 (a) and 2 (b). Resistant, moderately resistant, moderately susceptible and susceptible are represented by  $\bullet$ ,  $\circ$ ,  $\blacktriangledown$  and  $\triangle$ , respectively.



**Fig. 3** Relationship between resistance to foliar late blight in potato and foliage maturity type for the cultivars evaluated in experiments 1 and 2.



**Fig. 4** Relationship between resistance to foliar late blight in potato and skin type for the cultivars evaluated in experiments 1 and 2.

## 8 Tables

**Table 1** Origins, foliage maturity type and skin type of potato cultivars evaluated in experiment 1.

<b>Cultivars</b>	<b>Origins</b>	<b>Maturity type<sup>a</sup></b>	<b>Skin type</b>
Ágata	Netherlands	Early	Smooth
Almera	Netherlands	Mid-early	Smooth
Aracy	Brazil	Late	Rough
Aracy Ruiva	Brazil	Late	Rough
Asterix	Netherlands	Mid-late	Mid-rough
Atlantic	USA	Mid-season	Rough
Baraka	Netherlands	Mid-late	Rough
Baronesa	Brazil	Mid-season	Smooth
BRS Ana	Brazil	Mid-late	Mid-rough
BRS Elisa	Brazil	Mid-season	Smooth
Caesar	Netherlands	Mid-late	Smooth
Canelle	France	Mid-early	Smooth
Catucha	Brazil	Mid-season	Smooth
Chipie	France	Mid-season	Mid-rough
Colorado	France	Mid-late	Rough
Cupido	Netherlands	Mid-early	Smooth
Éden	France	Mid-season	Smooth
<b>Elodie (susceptible standard)</b>	France	Mid-early	Smooth
Emeraude	France	Mid-early	Smooth
Eole	France	Mid-early	Smooth
Florice	France	Mid-early	Smooth
Fontane	Netherlands	Mid-season	Smooth
Gourmandine	France	Mid-season	Smooth
Gredine	France	Mid-early	Smooth
IAPAR Cristina	Brazil	Mid-late	Rough
Itararé	Brazil	Mid-late	Mid-rough
<b>Ibituaçu (resistant standard)</b>	Brazil	Late	Smooth
Markies	Netherlands	Mid-late	Smooth
Melody	Netherlands	Mid-early	Smooth
Monalisa	Netherlands	Mid-early	Smooth
Naturella	France	Mid-late	Smooth
Opaline	France	Mid-early	Smooth
Soléia	France	Mid-season	Smooth
Voyager	Netherlands	Early	Smooth

### Footnotes

<sup>a</sup> The foliage maturity type is based on the number of days from planting until maturity. Early = < 90 days; Mid-early = 91-100 days; Mid-season = 101-110 days; Mid-late = 111-120 days; Late = > 121days.

**Table 2** Origins, foliage maturity type and skin type of potato cultivars evaluated in experiment 2.

<b>Cultivars</b>	<b>Origins</b>	<b>Maturity type<sup>a</sup></b>	<b>Skin type</b>
Ambra	Netherlands	Early	Smooth
Amorosa	Netherlands	Mid-early	Smooth
Annabelle	Netherlands	Early	Smooth
Armada	Netherlands	Mid-early	Mid-rough
<b>Elodie (susceptible standard)</b>	France	Mid-early	Smooth
Fabula	Netherlands	Mid-season	Smooth
<b>Ibituaçu (resistant standard)</b>	Brazil	Late	Smooth
Innovator	Netherlands	Mid-early	Rough
Madeleine	Netherlands	Mid-early	Smooth
Maranca	Netherlands	Mid-season	Mid-rough
Marlen	Netherlands	Mid-season	Mid-rough
Matador	Netherlands	Mid-early	Smooth
Mozart	Netherlands	Mid-season	Smooth
Rodeo	Netherlands	Mid-season	Mid-rough
Sinora	Netherlands	Mid-early	Mid-rough
Victoria	Netherlands	Mid-late	Rough
Vivaldi	Netherlands	Mid-early	Smooth

#### **Footnotes**

<sup>a</sup> The maturity type is based on the number of days from planting until maturity. Early = < 90 days; Mid-early = 91-100 days; Mid-season = 101-110 days; Mid-late = 111-120 days; Late = > 121days.

**Table 3** PC1 scores obtained for the four variables, the percentages of cultivar standardized values in relation to the standardized values of Elodie cultivars and levels of resistance to foliar late blight for experiments 1 and 2.

<b>Cultivars</b>	<b>PC1 Exp. 1<sup>b</sup></b>	<b>%<sup>c</sup></b>	<b>Resistance Exp. 1<sup>a</sup></b>	<b>Cultivars</b>	<b>PC1 Exp. 2<sup>b</sup></b>	<b>%<sup>c</sup></b>	<b>Resistance Exp. 2<sup>a</sup></b>
Ágata	-1.38	94.52	S	Ambra	-0.59	90.71	S
Almera	-1.26	93.11	S	Amorosa	-0.80	93.24	S
Aracy	0.77	70.13	MS	Annabelle	-0.36	87.91	S
Aracy Ruiva	1.40	62.92	MS	Armada	-1.51	101.82	S
Asterix	-1.48	95.67	S	Elodie	-1.36	100.00	S
Atlantic	-1.11	91.43	S	Fabula	-0.43	88.72	S
Baraka	0.43	74.02	MS	Ibituaçu	6.93	0.00	R
Baronesa	0.60	72.05	MS	Innovator	0.64	75.81	MS
BRS Ana	0.63	71.71	MS	Madeleine	-0.65	91.42	S
BRS Elisa	0.61	71.91	MS	Maranca	-0.31	87.37	S
Caesar	0.53	72.78	MS	Marlen	-1.12	97.04	S
Canelle	-1.82	99.53	S	Matador	-0.40	88.37	S
Catucha	0.95	68.06	MS	Mozart	-0.09	84.62	S
Chipie	-0.39	83.29	S	Rodeo	-0.45	89.00	S
Colorado	4.96	22.52	MR	Sinora	-0.66	91.51	S
Cupido	-1.29	93.54	S	Victoria	0.74	74.62	MS
Éden	-0.91	89.24	S	Vivaldi	0.63	75.42	MS
Elodie	-1.86	100.00	S	-	-	-	-
Emeraude	-0.45	83.91	S	-	-	-	-

Eole	-1.14	91.78	S	-	-	-	-
Florice	0.40	74.32	MS	-	-	-	-
Fontane	-1.41	94.91	S	-	-	-	-
Gourmandine	-0.72	87.01	S	-	-	-	-
Gredine	-1.61	97.17	S	-	-	-	-
IAPAR Cristina	1.49	61.97	MS	-	-	-	-
Itararé	0.14	77.26	S	-	-	-	-
Ibituaçu	6.94	0.00	R	-	-	-	-
Markies	-0.12	80.22	S	-	-	-	-
Melody	-0.55	85.05	S	-	-	-	-
Monalisa	-1.13	91.73	S	-	-	-	-
Naturella	-0.62	85.93	S	-	-	-	-
Opaline	-0.97	89.87	S	-	-	-	-
Soléia	0.19	76.70	S	-	-	-	-
Voyager	0.19	76.70	S	-	-	-	-

### Footnotes

<sup>a</sup> Cultivar levels of resistance to late blight: R = resistant; MR = moderately resistant; MS = moderately susceptible and S= susceptible

<sup>b</sup> PC1 scores obtained for the four selected response variables.

<sup>c</sup> Percentages of standardized values of cultivars in relation to Elodie cultivar standardized values.

**Table 4** Averages of epidemiological variables recorded for each level of resistance to foliar late blight in experiment 1 and 2.

RESISTANCE LEVEL	Variables <sup>a</sup>			
	Y <sub>50</sub>	RAUDPC	T <sub>0.5</sub>	<i>r</i>
<b>Experiment 1</b>				
<b>R</b>	17.5	0.33	11.0	0.20
<b>MR</b>	45.5	0.48	10.3	0.25
<b>MS</b>	99.8	0.72	7.3	0.56
<b>S</b>	100.0	0.78	6.1	1.00
<b>Experiment 2</b>				
<b>R</b>	44.2	0.42	9.0	0.17
<b>MR</b>	-	-	-	-
<b>MS</b>	98.7	0.68	4.0	0.50
<b>S</b>	100	0.73	3.5	0.70

**Footnotes**

R = resistant; MR = moderately resistant; MS = moderately susceptible and S = susceptible

<sup>a</sup> Y<sub>50</sub> = severity in the middle of the epidemic; RAUDPC = relative area under the disease progress curve; T<sub>0.5</sub> = time in days from the onset of the first symptoms in the experiment until 0.5% severity and *r* = disease progression rate.

## ARTICLE 2

### Development and validation of a set of standard area diagrams and field key to estimate severity of potato early blight

#### Abstract

A standard area diagram (SAD) and a field key (FK) to assess the severity of potato early blight (*Alternaria grandis*) on the leaves of potato (*Solanum tuberosum* L.) and in research plots, respectively, were developed and validated. The proposed SAD contains illustrations of leaves with twelve disease severity levels (0.05, 0.5, 2, 4, 8, 16, 32, 48, 62, 82, 96 and 100%). The proposed FK was an adaptation of a FK developed to assess the severity of potato late blight. The SAD and FK were validated by 14 and 10 raters, respectively, with no previous experience in evaluating plant disease. Lin's concordance correlation ( $\rho_c$ ) analysis of estimated vs. actual disease severity (based on image analysis software program) showed that precision and accuracy improved when using the SAD and FK, for most raters, compared to assessments made without the SAD and FK. The  $r$  value (the precision component of  $\rho_c$ ),  $u$ ,  $v$  (the accuracy component of  $\rho_c$ ) and  $\rho_c$  (a combined measure of accuracy and precision) improved in 85.7, 85.7, 64.3 and 100% of the raters, respectively, when the SAD was used. When the FK was used, the  $r$ ,  $u$ ,  $v$  and  $\rho_c$  values improved in 80, 90, 70 and 80% of the raters, respectively. Severity estimates were more reliable using the SAD ( $R^2=0.70-1.00$  and intra-class correlation ( $\rho$ ) of 0.853 without SAD, and  $R^2=0.80-1.00$  and  $\rho$  of 0.963 using the SAD). Severity estimates were also more reliable using the FK ( $R^2=0.70-1.00$  and  $\rho$  of 0.934 without FK, and  $R^2=0.90-1.00$  and  $\rho$  of 0.975 using the FK). Both the SAD and FK improved raters' ability to accurately, precisely and reliably estimate potato early blight severity, and as such these scale can be used for assessing severity for many purposes, including plant breeding for resistance, fungicide screening, and pathotype characterization.

**Keywords:** *Alternaria grandis*, *Alternaria solani*, disease severity, phytopathometry, *Solanum tuberosum* L..

## 1 Introduction

Potato (*Solanum tuberosum* L.) is the fourth most cultivated food crop worldwide (Fao, 2010), being grown in approximately 125 countries. Every day, more than a billion people eat potatoes around the world (Mullins et al. 2006). The productivity of this crop is often affected by numerous pests and disease, of which early blight is one of the most important yield-limiting factors (Shuman and Christ 2005; Guenther et al. 1999). For decades the mitosporic fungus, *Alternaria solani* Sorauer (Rotem 1994), had been considered the causal agent of this disease on both potato and tomato. However, in 2000, based on a review and morphological studies of the genus *Alternaria*, Simmons (2000) reported that *A. tomatophila* Simmons was the causal agent of early blight on tomato, and *A. solani* was the causal agent of early blight on potato. A recent study in Brazil also revealed a similar association between *A. grandis* and *A. tomatophila* and early blight on potato and tomato, respectively, but showed that cross infections were possible (Rodrigues et al. 2010). Interestingly, in that same study, isolates of *A. solani* were not detected causing early blight on potato. Nevertheless, the foliar symptoms of potato early blight caused by *A. grandis* are the same to those caused by *A. solani*.

Early blight causes necrotic lesions on leaves, stems, fruits and tubers (Rotem 1994), which can be observed at any stage of the plant's development, but the greatest intensity is usually observed on mature, senescent tissues (Dita Rodriguez et al. 2006; Johnson and Teng 1990). Typical lesions are dark, elongated or circular, with concentric rings (Jones 1991). In Brazil, the damage may occur at any time of the year (Batista et al. 2006). The pathogen has a short incubation period, and as such, several cycles may occur during the development of the crop (Rotem 1994). Under hot, humid conditions, when the disease is most severe, destruction of the leaves and defoliation of the plant may occur, decreasing the yield and quality of tubers, if control measures are not implemented (Castro et al. 2000). Because of these characteristics, the application of fungicide to control early blight has become intensive (Christ and Shuman 2005; Guenther et al. 1999).

Disease quantification is an integral part of the epidemiology and management of early blight of potatoes, as well as other plant diseases. For instance, estimates of disease intensity are commonly used to evaluate control measures, determine varietal resistance, assess the efficacy of fungicides, and develop crop loss models. In the case of early blight of potatoes, severity is often the most adequate method of assessment,

where the percentage of diseased leaf tissue in relation to the total leaf area is estimated (Amorim 1995). It is important to ensure that estimates of disease intensity are both accurate and precise. Methods exist for determining the quality and for improving the precision and accuracy of disease assessments (Bock et al. 2010; Madden et al. 2007; Nutter et al. 1993; Vale et al. 2004). These methods usually emphasize the use of standard area diagrams (SAD) and field keys (FK) (Bergamin Filho and Amorim 1996). SADs are illustrations of diseased plants, leaves, or other plant parts, with different levels of severity that are used as references to compare sample to when quantifying disease. Field Keys also referred to as descriptive scales, are rating scales in which numbers are used to represent different levels of disease intensity, with each number or level accompanied by a verbal description of the level of damage caused by the disease (Bergamin Filho and Amorim 1996).

A commonly used SAD for early blight of potatoes is the scale proposed by Reifschneider et al., (1984) that is comprised of diagrams of potato leaves with severity levels ranging from 0 to 50%. However, this scale has two major limitations: it lacks severity levels greater than 50%, and to our knowledge, it has not been formally validated. It is unclear how precise and accurate raters would be when using this scale. A good SAD should be easy to use, provide reliable results, and applicable under a wide variety of conditions. Moreover, the severity levels on the scale should represent different stages of disease development and encompass the full range of severity commonly encountered in the field (Berger 1980). Representative symptoms of the disease must be identified, and the maximum and minimum disease severity in the field must be ascertained.

The use of SAD is desirable in many situations, such as when estimating disease severity on individual leaves or entire plants (Campbell and Madden 1990). Severity assessments in research plots using SAD could be done in multiple ways. For instance, when leaves are the sampling units, all leaves of a plant should be evaluated, and then the average severity of each rated plant is obtained. On the other hand, when the plants are the sampling units, the severity of each rated plant is estimated directly. In both cases, the average severity of each plot is estimated based on the average severity of the rated plants. Both approaches may be very laborious and time-consuming, therefore, the use of FK (instead of SAD) may be preferred to quantify the severity of early blight in potato plants in research plots (Campbell and Madden 1990). Additionally, using SAD

alone to rate early blight at the plot level is not a trivial task, therefore the accuracy and precision of disease intensity can be improved with a combination of SAD and FK.

The SAD and FK must be validated before being recommended as tools for improving the accuracy and precision of disease severity estimation, and in the event that they produce unsatisfactory results, they must be corrected before being used (Martin et al. 2004).

The goals of this study were to 1) develop and validate a set of SAD with a full range of disease levels to quantify early blight severity on potato leaves and provide assistance in the use of the FK, 2) develop and validate a FK to quantify the severity of early blight on potato plants in research plots.

## **2 Material and Methods**

### **2.1 Development of the standard area diagram (SAD) set**

To develop the SAD set, 34 plots, each planted with a different cultivar, were established in March of 2009 in an experimental field located in Viçosa, Minas Gerais, Brazil (20°44'44 "S, 42°50'59" W, 661 m above sea level). Each plot consisted of a single 3.0 m-long row. The 34 cultivars (Ágata, Almera, Aracy, Aracy Ruiva, Asterix, Atlantic, Baraka, Baronesa, BRS Ana, BRS Elisa, Caesar, Canelle, Catucha, Chipie, Colorado, Cupido, Éden, Elodie, Emeraude, Eole, Florice, Fontane, Gourmandine, Gredine, IAPAR Cristina, Itararé, Ibituaçu, Markies, Melody, Monalisa, Naturella, Opaline, Soléia and Voyager) varied in their levels of resistance to early blight, ranging from susceptible to resistant. This was very useful for obtaining plants with different levels of disease severity. To ensure the development of disease in the field, all plots were spray-inoculated at 30 days after planting with a spore suspension of *A. grandis* (a mixture of nine isolates) containing  $1 \times 10^3$  conidia/mL.

Between 10 and 38 days after inoculation, 150 leaves with different levels of early blight severity were arbitrarily collected from all plots. Each leaf was individually scanned using a HP Photosmart scanner (Model C3180) and the images, with a resolution of 300 dpi, were transferred to a computer. The proportion of diseased leaf area was then estimated using QUANT (Vale et al. 2003), based in the procedure called discriminant function. These scores were considered the actual (true) disease severity (%) and as the references for evaluating the accuracy and precision of assessments done with and without the SAD. QUANT is a software that uses image analysis, based on

different procedures, to quantify disease severity on leaves and plants. The images from QUANT along with their respective actual severity were then used to develop a set of SADs with different level of severity (0.05, 0.5, 2, 4, 8, 16, 32, 48, 62, 82, 96 and 100%, Fig. 1). Potato leaf pattern were used as the template and then diagrams with the twelve severity levels were created using the PAINT.NET image editing software (<http://www.getpaint.net/>) following the same patterns of lesion distribution found on the scanned leaves.

## 2.2 Validation of the SADs to determine potato early blight severity on leaves

Validation of the SAD was performed in two steps. First, 14 raters with no experience quantifying early blight of potato analysed 50 images of potato leaves with different disease severities. Each image was projected onto a separate MS PowerPoint slides for visualisation and quantification by the raters, without using the SAD. In the second step, the raters received another set of 50 images of diseased potato leaves to perform estimates of severity using the SAD. Using on the data obtained, accuracy and precision of each rater, with and without the use of the SAD, was determined based on Lin's concordance correlation coefficient ( $\rho_c$ ) (Lin, 1989), as described by Nita et al (2003). This coefficient, which combines measures of accuracy and precision to assess the relational fit of the data to the line of concordance ( $45^\circ$ ), is defined as:  $\rho_c = r \times C_b$ , where  $C_b$  is a bias correction factor that measures how far the best-fitting line deviates from  $45^\circ$  and is thus a measure of bias or accuracy, and  $r$  is the correlation coefficient between estimated severity ( $Y$ ) and actual severity ( $X$ ), which measures precision (variation) or the scattering of points around the best-fitting line.  $C_b$  is estimated as:  $C_b = 2 / [(v + 1/v + u^2)]$ , where  $v = \sigma_y / \sigma_x$  is a measure of the difference between actual and estimated severity (defined by the difference in slope of the two lines), with  $\sigma_y$  and  $\sigma_x$  being the standard deviation of  $Y$  and  $X$ , respectively; and  $u = (\mu_y - \mu_x) / \sqrt{(\sigma_y \cdot \sigma_x)}$  provides a measure of the difference in heights of the lines, where  $\mu$  is the mean value for  $Y$  and  $X$ , and  $\sigma$  is defined as above. A  $v$  value of 1 occurs when the slopes are equal. When the heights of the lines are the same,  $u = 0$ . When there is perfect agreement between measured and actual severity (i.e. the measurements are accurate), the points fall on the concordance line and  $\mu_y = \mu_x$ ,  $\sigma_y = \sigma_x$ ,  $r = 1$ , and by definition,  $v = 1$ ,  $u = 0$ ,

$C_b = 1$ , and  $\rho_c = 1$  (Bock et al. 2010; Nita et al. 2003). The absolute error (estimated severity minus actual severity) was also obtained.

Inter-rater reliability of the estimates was determined in two ways. First, using the coefficient of determination ( $R^2$ ) from linear regression analyses of relationships between severity estimates for all pairs of raters (Nutter Jr. and Schultz 1995). Second, using the the intracluster (or intra-class) correlation ( $\rho$ ) for all raters combined as described by Shoukri and Pause (1999) and Nita et al. (2003).

### **2.3 Development of the field keys (FK)**

Plot establishment, inoculation, sampling, and image analysis for the FK development were very similar to that described above for the SAD development, with a few modifications. The plots were established in April of 2009 and each consisted to two 3-m-long rows, spaced 0.8 m apart. Plots were again planted with cultivars with different levels of resistance to early blight and plants were collected at multiple times during the development of the crop (between 40 and 70 days after planting) in order to obtain a range of severity levels. Ten plants from each of ten plots with different levels of severity were chosen for the development of the FK. In this study, a plant was defined as a single stem with all of its leaves. After the plants were collected, the leaves were detached and individually digitized, and then all images (with a resolution of 300 dpi) were transferred to a computer and evaluated using QUANT (Vale et al. 2003) as described above. Total (healthy + diseased) area was determined for each plant and subsequently used to estimate the average area of plants. The average size of the lesions on all leaf was also determined. Therefore, it was possible to develop a FK (Table 2) for assessing disease severity in research plots by modifying the FK developed by James (1971) to assess the severity of potato late blight in research plots.

### **2.4 Validation of the FK and SAD for the assessment of potato early blight severity in experimental plots**

To validation of the FK and SAD, the same thirty-four cultivars used to development of the SAD were planted in September of 2010 in Viçosa, Minas Gerais, Brazil. There were two plots of each cultivar, each consisting of two 3.0-m-long row, spaced 0.80 m apart. The plants in one of the plots of each cultivar were inoculated at

25 days after planting with an *A. grandis* (nine isolates) spores suspension containing  $1 \times 10^3$  conidia/ml, while plants in the other plot were not inoculated. Again, cultivar resistance and inoculation were used to generate plots with different levels of early blight, and ten plots with different severity levels were used to validate the FK and SAD.

FK validation was performed at 55 days after planting in two steps in the field using five plants per plot. At first, 10 raters, with no experience quantifying early blight of potato estimated early blight severity in 10 plots without the use of the FK. In the second stage of validation, the raters estimated severity of the same 10 plots using the FK, with the aid of the SAD.

SAD validation was performed at the field level on the same day as the FK validation. This validation aimed at determining whether the use of the FK allowed the same level of accuracy and precision in disease severity assessment in research plots as that obtained by using the SAD, considered here as the standard method. Validation of the SAD was also done in two stages, on five plants in each of 10 plots with different levels of severity. Raters first assessed disease severity without the aid of the SAD, and then repeated the assessment using the SAD. A total of 463 leaves were assessed by each rater. The estimated plant severity was obtained by averaging the severity of each leaf assessed with the SAD, and then plot severity was determined as the average severity of the five plants assessed.

The 50 plants used for in-field validation of the SAD and FK were collected and a total of 463 leaves were detached, individually scanned, and the images (300 dpi resolution) were transferred to a computer on the day of validation. Then, each leaf was evaluated for the proportion of the diseased area using the QUANT program (Vale et al. 2003). Percentage actual disease severity (%) was then determined for each plot by dividing diseased area of the five plants by the total area (healthy + diseased) of the same five plants and multiplying by 100.

Accuracy, precision, and reliability of the estimates were determined in a manner similar to that described above when validating SAD for assessing early blight severity on leaves.

### **3 Results**

#### **3.1 The use of the SAD to determine potato early blight severity on leaves**

The scale has twelve severity levels: 0.05, 0.5, 2, 4, 8, 16, 32, 48, 64, 82, 96 and 100%. Coalescent lesions were observed at levels of severity higher than 4% and were considered in the development of the scale (Fig. 1).

Based on Lin's concordance correlation analysis and estimated and actual severity, e assessments made by the 14 raters were closer to the actual values when the SAD were employed, compared to when the raters assessed the leaves without the aid of SAD. The parameters and statistics of Lin's concordance correlation analysis showed that accuracy and precision improved with the use of the SAD for most raters (Fig. 2). For individual raters, there was a linear relationship between estimated and actual severity when the SAD were employed. For 100% of the raters, the Lin's concordance correlation coefficient ( $\rho_c$ ) was higher with the use of the SAD than without the use of the SAD. When SAD was not employed, the  $\rho_c$  ranged from 0.757 to 0.980, with a mean of 0.917, however, when the SAD were used, the  $\rho_c$  ranged from 0.964 to 0.990, with a mean of 0.979 (Fig. 2). The  $u$  and  $v$  values, which are used to compute the correction factor  $C_b$ , improved for 85.7 and 64.3% of the raters, respectively, and the  $r$  values improved for 85.7% of the raters, when the SAD was used.

Furthermore, improvements when using the SAD was also gauged by determining the absolute error associated with each rater's estimate (Fig. 3). Without the use of the SAD, raters had higher deviations in the errors, and for 78.57% of the raters (1, 2, 5, 6, 7, 8, 9, 10, 12, 13 and 14), the errors exceeded 25% (Fig. 3). However, when the SAD was used, 50% of the raters (1, 2, 3, 5, 6, 7 and 14) had no errors above 15%, and the 50% had few cases in which the errors surpassed 15%. For all the raters, the errors were lower than 19% and were concentrated at severity levels close to 10% when the SAD was used (Fig. 3).

With the use of the SAD, there was a slight tendency to underestimate disease severity. This was made evident by the fact that 85.71% of the raters showed negative  $u$  values (Fig. 2). Moreover, this trend was further confirmed for the majority of the raters by the fact that negative absolute errors were detected in most estimates (Fig. 3).

In addition to accuracy and precision, the inter-rater reliability of the estimates (Berger 1980) was another indicator of the value of using the SAD assess early blight severity. Different raters, when using the same scale to assess of the same plant material must estimate the same severity values (Nutter Jr. and Schultz 1995). Without the use of the SAD, 85.71% of the pairwise comparisons had an  $R^2 < 0.89$ , but when the SAD was employed, only 3.30% of pairwise comparisons had an  $R^2 < 0.89$ ; i.e in 96.70% of the

comparisons, the use of the SAD resulted in an  $R^2 > 90\%$  (Table 1). The  $\rho$  value was greater when the SAD was used ( $\rho = 0.853$  unaided and  $\rho = 0.963$  using SAD), indicating that the SAD provided more reliable results.

### **3.2 The FK and SAD for the assessment of potato early blight severity in experimental plots**

The mean area of early blight lesions on potatoes was  $0.51 \text{ cm}^2$ , and the mean area of each plant was  $1503 \text{ cm}^2$ . With these data, it was possible to modify the descriptive scale developed by James (1971) for the assessment of early blight severity in potato research plots. The modified scale is presented in Table 2. Based on the Lin's concordance correlation analysis and estimated and actual severity, the assessments made by the 10 raters were closer to the actual values when the FK and SAD were employed, compared to when the raters assessed the plots without the aid of FK and SAD. The parameters and statistics from Lin's concordance correlation analysis showed that accuracy and precision improved with the use of the FK and SAD for most raters (Fig. 4). There was a linear relationship between estimated and actual severity when the FK and SAD were employed. For 80 and 90% of the raters, the Lin's concordance correlation coefficient ( $\rho_c$ ) was higher with the use of the FK and SAD, respectively, than without the use of the FK and SAD. Without use of the FK and SAD,  $\rho_c$  ranged from 0.843 to 0.981, with a mean of 0.934. However, when the FK were employed, the  $\rho_c$  values ranged from 0.950 to 0.998, with a mean of 0.983 and when the SAD were employed,  $\rho_c$  ranged from 0.957 to 0.995, with a mean of 0.983. The  $u$  and  $v$  values improved for 90 and 70% of the raters, respectively, and the  $r$  values improved for 80% of the raters, when the FK was used. When the SAD was used, the  $u$  and  $v$  values improved for 90 and 50% of the raters, respectively, and the  $r$  values improved for 90% of raters (Fig. 2).

Without the use of the scales, there was again a major deviation in the absolute errors for the raters, with only 20% of them (3 and 4) having errors lower than 15%. However, when the FK was used 70% of the raters (1, 2, 5, 6, 7, 9 and 10) had errors below 15%, and the others very had few cases in which the errors surpassed 15%. For all of the raters, errors were lower than 20.5% and were concentrated at about 10% severity, when the FK was used. When using the SAD, 80% of the raters (1, 2, 4, 5, 6, 7, 9 and 10) had errors below 15%, and, for the others, the errors were higher than 15%

in only a few cases. For all of the raters, when the SAD was used, all of the errors were below 18.27%, with most being concentrated at about 10% severity (Fig. 5).

Again, when using the FK and SAD, there was a slight tendency to underestimate disease, as indicated by the fact that 80 and 60% of the raters showed negative  $u$  values for FK and SAD, respectively (Fig. 4). This was confirmed by the negative absolute error detected in most estimates for the majority of the raters (Fig. 5).

Without the use of the scales, 26.67% of the pairwise comparisons had an  $R^2 < 0.89$ , but the FK and SAD were used, 0% of the pairwise comparisons had an  $R^2 < 0.89$ . Furthermore, in 100% of the comparisons, using the FK and SAD resulted in an  $R^2 > 90\%$ . The  $\rho$  value was greater when the FK and SAD was used than when these scales were not employed ( $\rho = 0.934$  unaided,  $\rho = 0.975$  using FK and  $\rho = 0.987$  using SAD), indicating that the FK and SAD provided reliable results (Table 3).

#### **4 Discussion**

Disease quantification is an important part of the epidemiology and management of early blight of potatoes and many methods are used for this purpose. These methods usually emphasize the use of standard area diagrams (SAD) and field keys (FK) to aid disease assessment. A commonly used SAD for early blight of potatoes on leaves is the scale proposed by Reifschneider et al., (1984). While very useful, this scale has two major limitations: it lacks severity levels greater than 50%, and there is no published information on its validation. In this study we developed and validated a set of SAD with a full range of disease levels to quantify early blight severity on potato leaves. In addition, since there are no FKs to quantify the severity of early blight on potato plants in research plot, we also developed and validated a FK for early blight as part of this research. The use of FK to quantify disease severity in research plots is very common and often more appealing than the use of SAD, mainly because the assessments made using FK tend to be less time-consuming. Both the SAD and FK developed and validated in this study are good tools for quantifying early blight severity because they improved the accuracy, precision and reliability of disease severity estimates.

In most studies involving the validation of SAD to determine disease severity on leaves, raters have shown a tendency to overestimate the levels of disease severity (Capucho et al. 2010; Diaz et al. 2001; Leite et al. 2002; Newton and Hackett 1994; Parker et al. 1995; Sherwood et al. 1983). However, in some cases, such as early blight

in potato, the levels of foliar disease severity were underestimated (Gomes et al. 2004; Michereff et al. 1998; Michereff et al. 2000). According to Nutter Jr. and Schultz (1995), solutions to correct or minimize inaccurate disease severity assessments vary according to the magnitude of the error and the amount of trainings received by the rater. In the current investigation, using the SAD to estimate potato early blight severity on leaves led to an overall reduction of the absolute error (the difference between estimated and actual severity). When SAD was not utilized, close to 80% of the raters had absolute errors above 25%, however, when SAD was used all raters had absolute errors below 19%. In spite of the overall improvement in the accuracy of disease assessment with the use of the SAD, there was still a tendency to underestimate severity, with close to 86% of the raters having negative  $u$  values. Computer-based training of raters is one approach that could be used to further reduce errors and increase the accuracy of potato early blight severity estimates (Nutter Jr. and Schultz 1995; Nutter Jr. and Worawitlikit 1989; Nutter et al. 1998).

In addition to improving the accuracy of disease assessments, the use of a SAD can also reduce the time taken to quantify disease as well as standardize results among raters and experiments (Stonehouse 1994). Based on the coefficient of determination ( $R^2$ ) for relationships between severity estimates made by different raters and the intracluster correlation coefficient, the reliability of estimates among raters (inter-rater reliability) improved when the SAD was employed. Without SAD, 86% of the pairwise relationships between severity estimates had  $R^2$  values below 0.89 compared to only 3% when SAD was used. Similarly,  $\rho$  was 0.85 without SAD and 0.96 with SAD. These results are very consistent with those observed in other studies (Belasque et al. 2005; Capucho et al. 2010; Leite and Amorin, 2002).

Similar trends to those observed when using the SAD to rate early blight on leaves were observed when using the FK in combination with the SAD to quantify severity in research plots. Using the two assessment tools also reduced the absolute error and increased inter-rater reliability for estimates made at the plot level. Both the FK and SAD resulted in more accurate, precise and reliable estimates of disease severity in research plots, however, the use of the FK may be preferred since it usually allows for faster severity assessment when compared to the SAD.

When using the FK each lesion should be considered to be approximately 0.51 cm<sup>2</sup> (~0.8 cm diameter), therefore the number of lesions used on the scale will depend on the size of the lesions. For example, if you see 30 lesions on 30 plants, and each

lesion has a diameter of approximately 0.4 cm, estimated severity in the research plot will be 0.05% and not 0.1%. The SAD should be used in conjunction with the FK.

One of the most commonly used approach for validating disease assessment scales and estimating the accuracy and precision of assessments is linear regression. However, in this investigation, we opted not to rely on linear regression to validation the SAD and FK. This is because erroneous conclusions may be drawn from linear regression analysis under certain circumstances (Lin 1989; Madden et al. 2007). Linear regression analysis may not detect departure from intercept 0 and slope 1 if the data are very scattered. The less precise the data, the less likely the null hypothesis ( $\beta_0 = 0$  and  $\beta_1 = 1$ ) will be rejected, and conversely, if the system is highly reproducible, the hypothesis could be easily rejected due to very small error (Bock et al., 2010). Thus, we used here Lin's concordance correlation coefficient ( $\rho_c$ ) because it combines measures of accuracy and precision and avoids the problems associated with linear regression analysis discussed above.

The SAD developed and validated in this study to quantify the severity of early blight on leaves of potato and to provide assistance in the use of the FK, also developed and validated to quantify the severity of early blight in potato plants in experimental plots, is a useful tool for improving the quality of assessments (accuracy, precision and reliability) of potato early blight severity and consequently minimize incorrect results. The SAD and FK developed here will therefore be useful for epidemiological research, comparative studies on disease control methods, pathotype characterization, and other studies where quality assessment of potato early blight is required.

## **5 Acknowledgements**

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for providing the financial support and the student grants.

## **6 References**

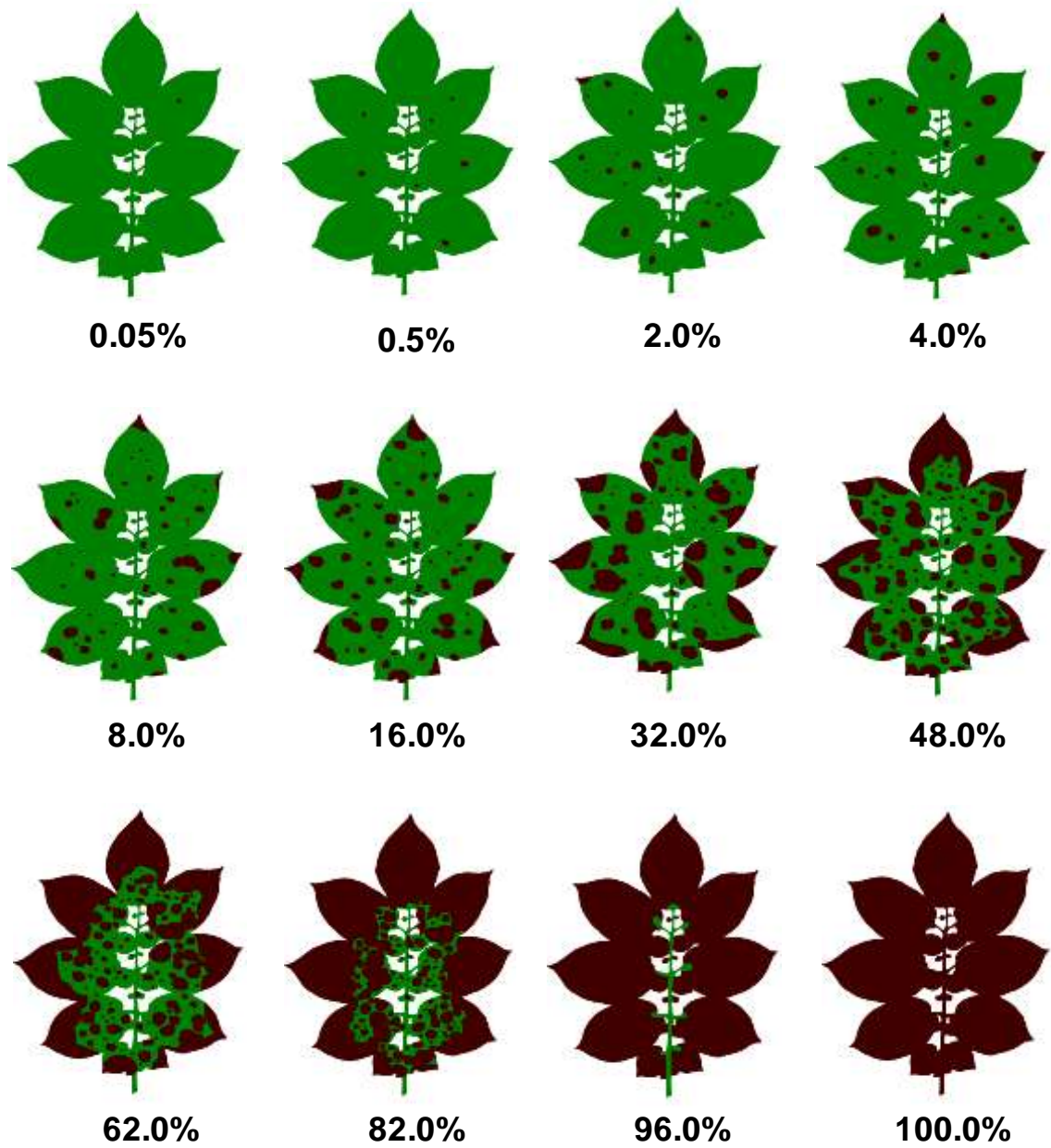
Amorim, L. (1995) Avaliação de doenças. In A. Bergamim Filho, H Kimati, Amorim L (eds) *Manual de fitopatologia* (pp 647-671) São Paulo: Ceres.

- Batista, D. C., Lima, M. A., Haddad, F., Maffia, L. A., Mizubuti, E. S. G. (2006) Validation of decision support systems for tomato early blight and potato late blight, under Brazilian conditions. *Crop Protection* 25, 664-670.
- Belasque, J., Bassanezi, R.B., Spósito, M.B., Ribeiro, L.M., Jesus Júnior, W.C., Amorim, L. (2005) Escalas diagramáticas para a avaliação da severidade do cancro cítrico. *Fitopatologia Brasileira* 30, 387-393.
- Bergamim Filho, A., & Amorim, L. (1996) *Doenças de plantas tropicais: Epidemiologia e controle econômico*. São Paulo: Ceres.
- Berger, R. D. (1980) Measuring Disease Intensity. In P. S. Teng, & S.V. Krupa (eds) *Crop loss assessment wich constrain production and crop improvement in agriculture and forestry* (pp 28-31) St. Paul: University of Minnesota.
- Bock, C. H., Poole, G., Parker, P. E., Gottwald, T.R. (2010) Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *Critical Review Plant Science* 29, 59-107.
- Campbell C.L., Madden, L.V. (1990) *Introduction to Plant Disease Epidemiology*. New York: John Willey & Sons.
- Capucho, A. S., Zambolim, L., Duarte, H. S. S., Parreira, D. F., Ferreira, P. A., Lanza, F. E., Costa, R. V., Casela, C.R., Cota, L. V. (2010) Influence of leaf position that correspond to whole plant severity and diagrammatic scale for white spot of corn. *Crop Protection*, 29, 1015-1020.
- Castro, M. E. A., Zambolim, L., Chaves, G.M., Cruz, C.D., Matsuoka, K. (2000) Variabilidade patogênica de *Alternaria solani*, agente causal da pinta-preta do tomateiro. *Summa Phytopathologica*, 8, 24-28.
- Diaz, C. G., Bassanezi, R. B., Bergamin Filho, A. (2001) Desenvolvimento e validação de uma escala diagramática para *Xanthomonas axonopodis* pv. *phaseoli* em feijoeiro. *Summa Phytopathologica*, 27, 35-39.
- Dita-Rodríguez, M. A., Brommonschenkel, S. H., Matsuoka, K., Mizubuti, E. S. G. (2006) Components of resistance to early blight in four potato cultivars: effect of leaf position. *Journal of Phytopathology*, 154, 230-235.
- Fao (2010) Food and Agriculture Organization. <http://faostat.fao.org>. Accessed 28 august 2010
- Gomes, A. M. A., Michereff, S. J., Mariano, R. L. R. (2004) Elaboração e validação de escala diagramática para cercosporiose da alface. *Summa Phytopathologica*, 30, 38-42.

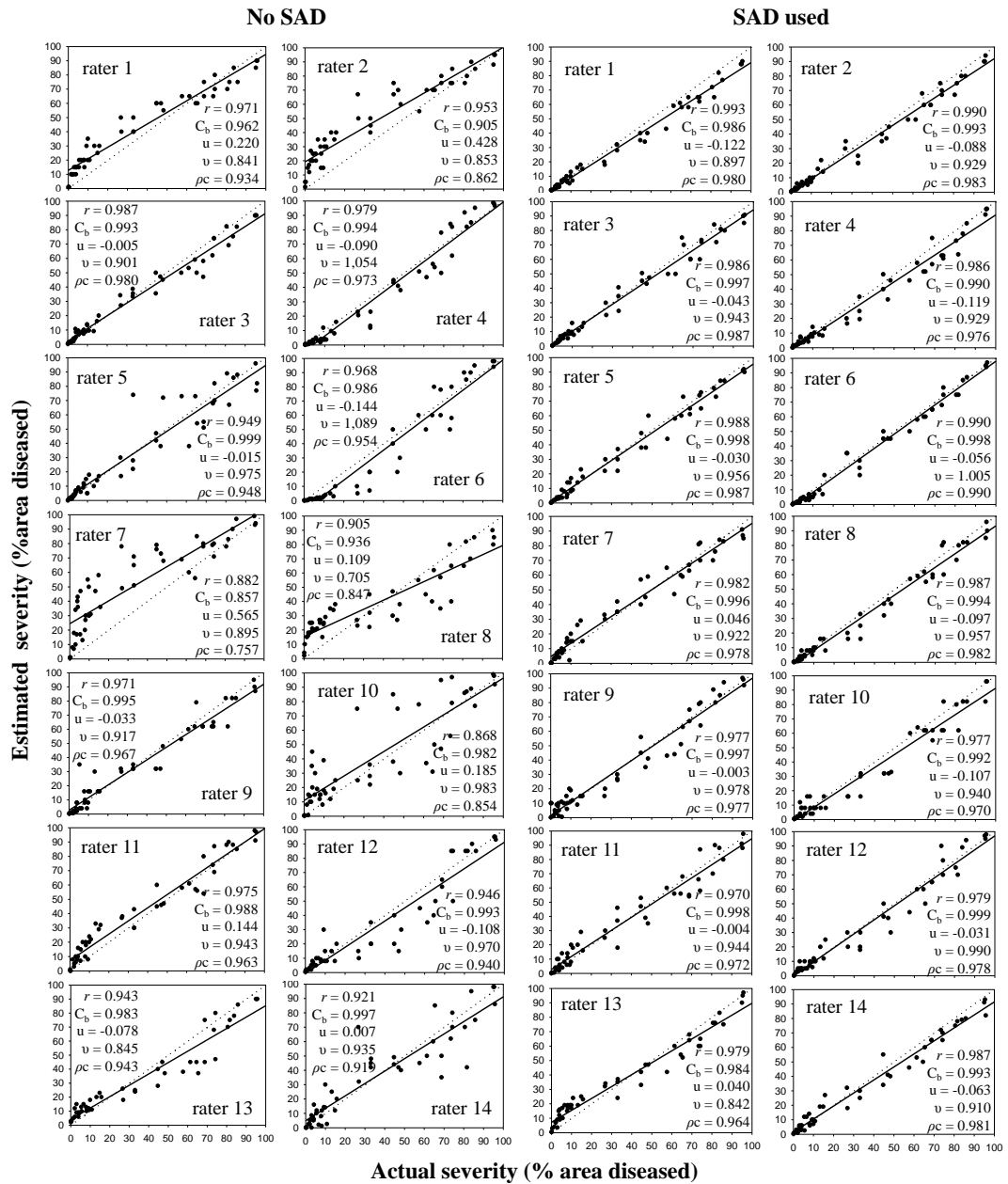
- Guenther, J. F., Wiese, M. V., Pavlista, A. D., Sieczka, J. B., Wyman, J. (1999) Assessment of pesticide use in the US potato industry. *American Journal Potato Research*, 76, 25-29.
- James WC (1971) An illustrated series of assessment keys for plant disease, their preparation and usage. *Canadian Plant Disease Survey*, 51, 39-65.
- Johnson, K.B, & Teng, P. S. (1990) Coupling a disease progress model for early blight to a model of potato growth. *Phytopathology*, 80,416-425.
- Jones, J. B., Stall, R.E., Zitter, T.A. (1991) Compendium of tomato disease. St. Paul: APS Press.
- Leite, R. M. V. B. C., Amorim, L. (2002) Elaboração e validação de escala diagramática para Mancha de Alternária em girassol. *Summa Phytopathologica* 28:14-19
- Lin, L. I. (1989) A concordance correlation coefficient to evaluate reproducibility. *Biometrics*. 45, 255-268.
- Madden, L.V., Hughes, G., van den Bosch, F. (2007) *The Study of Plant Disease Epidemics*. Press, St. Paul: APS.
- Martins, M.C., Guerzoni, R. A., Câmara, G. M. S., Mattiazzi, P., Lourenço, S. A., Amorim, L. (2004) Escala diagramática para a quantificação do complexo de doenças foliares de final de ciclo em soja. *Fitopatologia Brasileira*, 29,179-184.
- Michereff, S. J., Maffia, L. A., Noronha, M. A. (2000) Escala diagramática para avaliação da severidade da queima das folhas do inhame. *Fitopatologia Brasileira*, 25,612-629.
- Michereff, S. J., Pedrosa, R. A., Noronha, M.A., Martins, R. B, Silva, F. V. (1998) Escala diagramática e tamanho de amostras para avaliação da severidade da mancha parda da mandioca (*Cercosporidium henningsii*). *Agrotrópica*, 10, 143-148.
- Mullins, E., Milbourne, D., Petti, C., Doyle-Prestwich, B.M., Meade, C. (2006) Potato in the age of biotechnology. *Trends Plant Science*, 11:254-260.
- Newton, A. C., Hackett, C. A. (1994) Subjective components of mildew assessment on spring barley. *European Journal Plant Pathology*, 100,395-412.
- Nita, M., Ellis, M. A., Madden, L. V. (2003) Reliability and accuracy of visual estimation of Phomopsis leaf blight of strawberry. *Phytopathology* 93, 995-1005.
- Nutter, F. W., Worawitlikit, O. (1989) Disease.Pro: a computer program for evaluating and improving a person ability to assess disease proportion. *Phytopathology*, 79,111-135.

- Nutter, F. W., Schultz, P. M. (1995) Improving the accuracy and precision of disease assessments: selection of methods and use of computer-aided training programs. *Canadian Journal Plant Pathology*, 17,174-184.
- Nutter, F. W., Gleason, M. L., Jenco, J. H., Christinas, N. C. (1993) Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. *Phytopathology*, 83,806-812.
- Parker, S. R., Shaw, M. W., Royle, D. J. (1995) The reliability of visual estimates of disease severity on cereal leaves. *Plant Pathology*, 43, 856-865.
- Reifschneider, F. J. B., Furumoto, O., Filgueira, F. A. R. (1984) Illustrated key for the evaluation of early blight of potatoes. *FAO Plant Protect Bulletin*, 32,91-94.
- Rodrigues, T. T. M. S., Berbee, M. L., Simmons, E. G., Cardoso, C. R., Reis, A., Maffia, L. A., Mizubuti, E. S. G. (2010) First report of *Alternaria tomatophila* and *A. grandis* causing early blight on tomato and potato in Brazil. *New Disease Reports*, 22, 28-28.
- Rotem, J. (1994) *The genus Alternaria: Biology, epidemiology and pathogenicity*. St Paul: APS Press.
- Sherwood, R. T., Berg, C. C., Hoover, M. R., Zeiders, K. E. (1983) Illusions in visual assessment of *Stagonospora* leaf spot of orchardgrass. *Phytopathology*, 73, 173-177.
- Shoukri, M. M., & Pause, C. A. (1999) *Statistical Methods for Health Science*. 2nd ed. CRC Press, Boca Raton, FL.
- Shuman, J. L., Christ, B. J. (2005) Integrating a host-resistance factor into the fast system to forecast early blight of potato. *American Journal Potato Research*, 82, 9-19.
- Stonehouse, J. (1994) Assessment of Andean bean diseases using visual keys. *Plant Pathology*, 43, 519-527.
- Vale, F. X. R., Fernandes Filho, E.I., Liberato, J. R. (2003) QUANT: A software plant disease severity assessment. In R. Close, M. Braithwaite, I. Havery I (eds) *Proceedings of the 8th International Congress of Plant Pathology*, New Zealand, pp105
- Vale FXR, Jesus Junior WC, Zambolim (2004) *Epidemiologia aplicada ao manejo de doenças de plantas*. Belo Horizonte: Perffil.

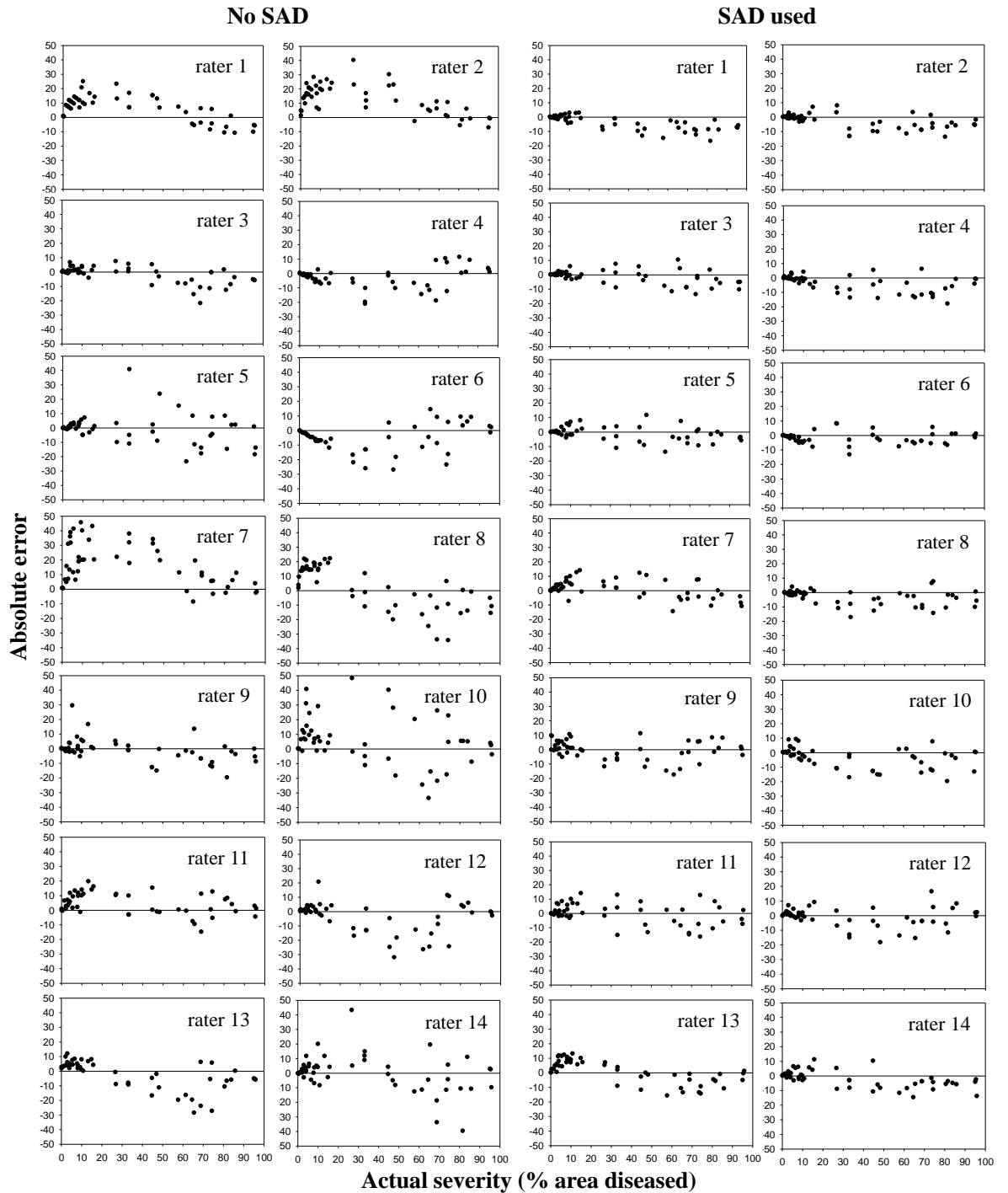
**7 Figures**



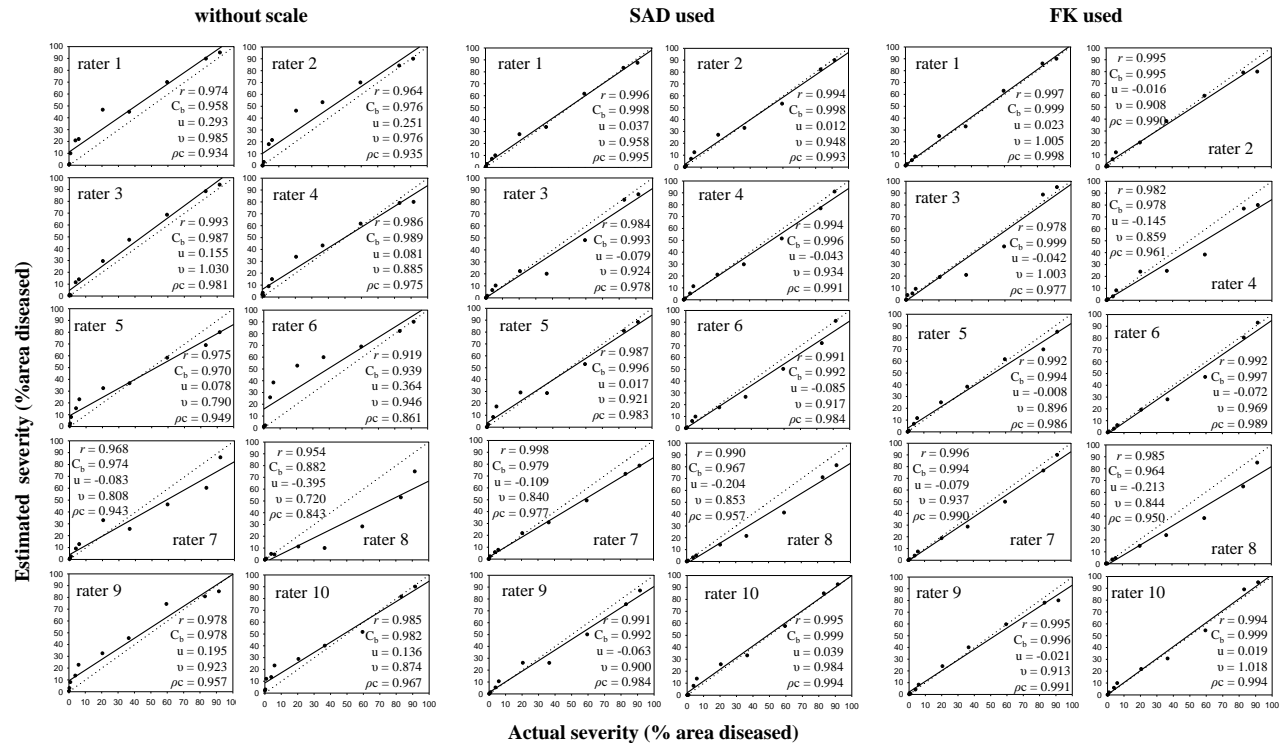
**Fig. 1** Standard area diagrams for early blight (*Alternaria grandis*) severity on potato (*Solanum tuberosum*) leaves. The numbers represent percent (%) leaf area with visual symptoms of the disease.



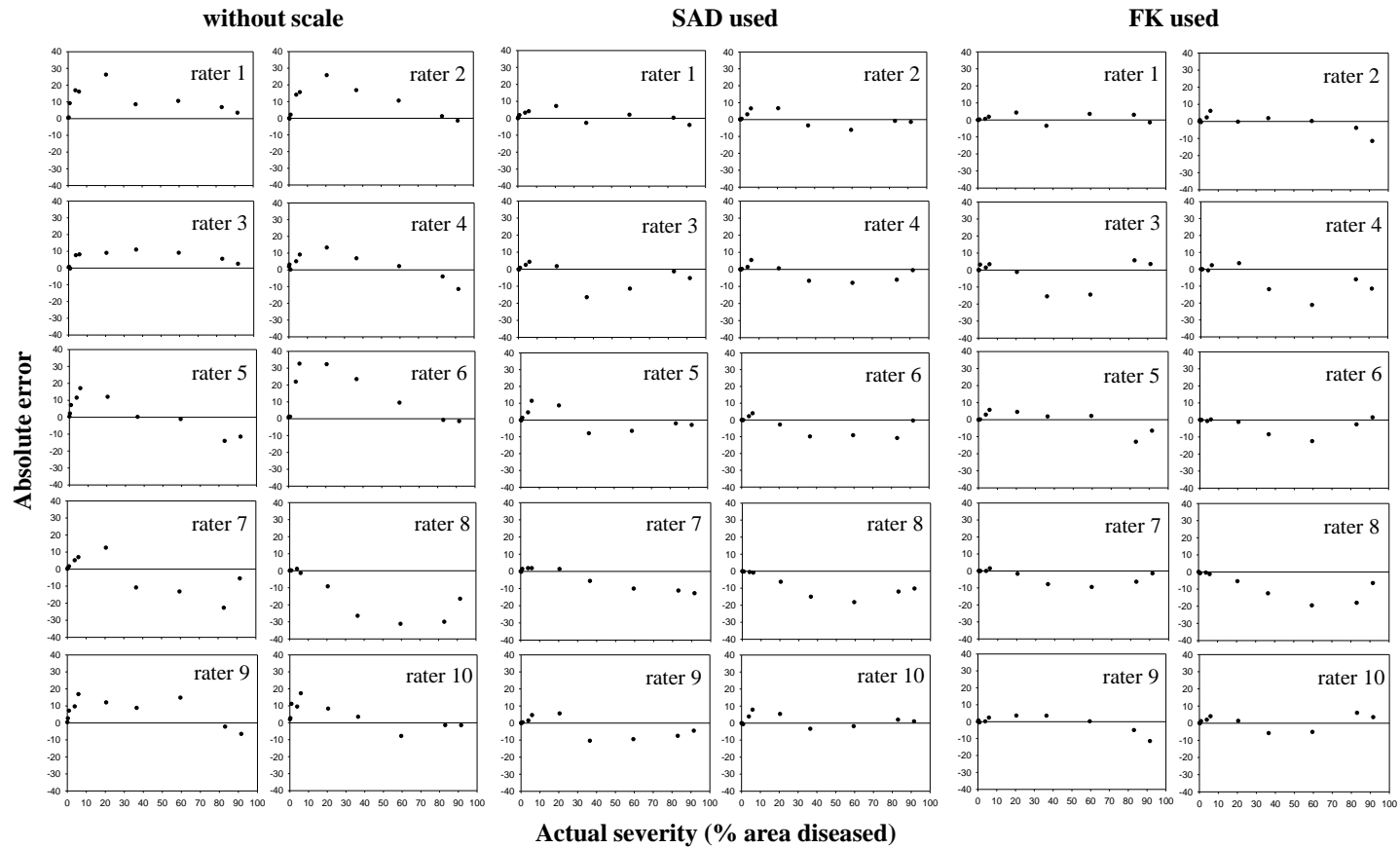
**Fig. 2** Relationship between actual and estimated severity of early blight (*Alternaria grandis*) of potato (*Solanum tuberosum*) assessed on leaves by fourteen different raters without and with the use of standard area diagrams. In each graph, the solid line represents the best-fit linear regression line, whereas the dotted line is the concordance line, which represents perfect agreement between actual and estimated severity (slope of 1, intercept of 0). A total of 50 leaves were assessed by each rater. Accuracy was determined with Lin's concordance correlation coefficient ( $\rho_c$ ), calculated as the product of the correlation coefficient ( $r$ ) and the bias correction factor ( $C_b$ ).  $C_b$  is a function of location shift ( $u$ ) and scale shift ( $v$ ), indicating changes in line height and slope, respectively.



**Fig. 3** Absolute error (estimated severity minus actual severity) of estimates of severity of early blight (*Alternaria grandis*) on leaves of potato (*Solanum tuberosum*) for each of 14 raters, without and with the use of a set of standard area diagrams (SAD). Low absolute errors indicate that the estimated severity was similar to the actual severity.



**Fig. 4** Relationship between actual and estimated severity of early blight (*Alternaria grandis*) of potato (*Solanum tuberosum*) assessed in research plots by ten different raters without the use of a scale, using standard area diagrams, and using a descriptive field key. In each graph, the solid line represents the best-fit linear regression line, whereas the dotted line is the concordance line, which represents perfect agreement between actual and estimated severity (slope of 1, intercept of 0). A total of 10 plots were assessed by each rater. Accuracy was determined with Lin's concordance correlation coefficient ( $\rho_c$ ), calculated as the product of the correlation coefficient ( $r$ ) and the bias correction factor ( $C_b$ ).  $C_b$  is a function of location shift ( $u$ ) and scale shift ( $v$ ), indicating changes in line height and slope, respectively.



**Fig. 5** Absolute error (estimated severity minus actual severity) of estimates of severity of early blight (*Alternaria grandis*) in potato (*Solanum tuberosum*) for each of 10 raters, without the use of a scale, using standard area diagram (SAD), and with the use of a field key (FK). Low absolute errors indicate that the estimated severity was similar to the actual severity.

## 8 Tables

**Table 1.** Inter-raters reliability of estimates of early blight (*Alternaria grandis*) severity on leaves of potato (*Solanum tuberosum*) based on the coefficient of determination ( $R^2$ ) from linear regression analyses of severity estimates for all pairwise comparisons of 14 raters assessing severity with and without the use of a set of standard area diagrams (SAD).

$R^2$ values	Frequencies (%)*	
	No SAD	SAD used
0.70 - 0.79	46.15	0
0.80 - 0.89	39.56	3.30
0.90 - 1.00	14.29	96.70

### Footnotes

\* Calculated based on the number of occurrences of values within each interval, relative to the total of 91 possible combinations of the fourteen raters.

**Table 2.** The field key (FK) for assessing the severity of early blight (*Alternaria grandis*) of potato (*Solanum tuberosum*) in research plots.

Severity (%)	Description
0.0	No disease observed
0.001	Approximately 1 lesion/30 plants
0.01	Approximately 3 lesions/10 plants
0.05	Approximately 15 lesions/10 plants
0.1	Approximately 30 lesions /10 plants
1.0	Approximately 30 lesions /plant
5.0	Approximately 150 lesions /plant
25	Approximately 25% of leaf area destroyed. Nearly every leaflet infected, but plants retain normal form; plants may smell of blight; field looks green although every plant is affected.
50	Approximately 50% of leaf area destroyed. Every plant affected. Field appears green, flecked with brown
75	Approximately 75% of leaf area destroyed; field appears neither predominantly brown nor green
95	Only a few leaves on plants, but stems green
100	All leaves dead, stems dead or dying

**Observations:**

- When using the FK, each lesion should be considered to be approximately 0.51 cm<sup>2</sup> (~0.8 cm in diameter).
- A plant is defined as a single stem with its leaves.
- The standard area diagram (SAD) should be used to aid severity assessments using the FK.

**Table 3.** Inter-raters reliability of estimates of early blight (*Alternaria grandis*) in potato (*Solanum tuberosum*) based on the coefficient of determination ( $R^2$ ) from linear regression analyses of severity estimates for all pairwise comparisons of 10 raters assessing severity without the use of a scale, using a set standard area diagrams (SAD) and with the use of the field key (FK).

$R^2$ values	Frequencies (%)*		
	Without scale	SAD used	FK used
0.70 - 0.79	0.04	0	0
0.80 - 0.89	26.63	0	0
0.90 - 1.00	73.33	100	100

**Footnotes**

\* Calculated by considering the number of occurrences in the interval compared to the total of 45 possible combinations among the ten raters.

## ARTICLE 3

### Field resistance of potato cultivars to early blight and its relationship with foliage maturity type and skin type in Brazil

#### Abstract

The use of resistant cultivars is the preferred control method for plant diseases. When available, this approach is attractive owing to its high efficacy, low cost, and ease of implementation. The objectives of this study were to assess the resistance of potato cultivars to early blight (*Alternaria grandis*) under different environmental conditions and to assess relationships among resistance to early blight, foliage maturity type, and tuber skin type. Three experiments were performed in Viçosa, Minas Gerais, Brazil. The first and second experiments used 26 cultivars (treatments), whereas the third used 24 cultivars. The severity of early blight in each plot was quantified every seven days using a diagrammatic scale. The values of the epidemiological variables severity in the middle of the epidemic, relative area under the disease progress curve, time in days from the onset of the first symptoms until 0.5% severity and disease progression rate were then obtained. The proposed levels of resistance to early blight were defined as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Most of the cultivars were classified as either susceptible and moderately susceptible to early blight. However, the resistance classification of some cultivars changed according to the environmental conditions. Resistant cultivars had mid-season, mid-late or late maturity. In most cases, the cultivars found to be susceptible to foliar early blight had earlier maturity (early or mid-early maturity). None of the susceptible cultivars had later maturity (mid-late or late maturity). The cultivars found to be resistant had a rough, mid-rough or smooth skin. Cultivars that were susceptible to early blight and had rough skin were not observed. In most cases, the cultivars that were found to be susceptible or moderately susceptible to foliar early blight had smooth skin.

**Keywords:** *Alternaria grandis*; potato disease; *Solanum tuberosum* L..

#### 1 Introduction

Potato (*Solanum tuberosum* L.) ranks fourth among the food crops produced worldwide and is cultivated in more than one hundred countries. In 2009, the world and

Brazilian production of potatoes was approximately 329 and 3.5 million tons, respectively (FAO, 2011).

Potato production is limited by several factors, including diseases. From planting to harvest, the potato crop is subject to a large number of diseases. For this reason, the production of potatoes is dependent on fungicide application. Among the diseases that affect potatoes, early blight is considered one of the most important foliar diseases (Shuman & Christ, 2005; Guenther *et al.*, 1999). In recent years, disease severity has increased under field conditions. In Brazil, during the rainy season, early blight is the most limiting disease for potato production (Reifschneider *et al.*, 1984).

The causal agent of the disease has been attributed to the mitosporic fungus, *Alternaria solani* Sorauer (Rotem, 1994). However, in a review of the genus *Alternaria* supported by morphological studies, Simmons (2000) reported that *A. tomatophila* Simmons was the causal agent early blight in tomato and that *A. solani* was the causal agent of early blight in potato. Subsequent studies in Brazil (Rodrigues *et al.*, 2010) revealed the widespread occurrence of *A. grandis* and *A. tomatophila* causing early blight in potato and tomato, respectively. Interestingly, in the same study, isolates of *A. solani* were not detected causing early blight in potato. Nevertheless, the symptoms of early blight caused by *A. grandis* are similar to those caused by *A. solani*.

The disease causes necrotic lesions on leaves, stems and tubers (Rotem, 1994). These lesions can be observed at any plant growth development, but greater intensity is observed in mature and senescent tissues (Dita Rodriguez *et al.*, 2006; Johnson & Teng, 1990). The lesions are dark, elongated or circular, with concentric rings (Jones, 1991). At high severity, the disease leads to the destruction of the leaves and defoliation, thereby decreasing the yield and quality of tubers (Castro *et al.*, 2000).

An epidemic of potato early blight may occur at any time of the year (Batista *et al.*, 2006). The pathogen has a short life cycle, and several cycles may occur during the life cycle of the crop (Rotem, 1994). However, during hot and humid periods in which the disease is most severe, it usually destroys the crop completely if control measures are not implemented (Adams & Stevenson, 1990). The disease represents a particular threat because potatoes are cultivated in regions and seasons in which the conditions are favourable to development of early blight and because the cultivars commonly used are susceptible to this disease (Pelletier & Fry, 1990). Because of these characteristics, the requirement to apply fungicide to control foliar early blight has become intense. This regular use of expensive fungicides throughout the entire crop cycle significantly

increases production costs. It is also responsible for greater risks to human health; environmental contamination caused by runoff; leaching of excessively sprayed products; fungicide residues in tubers; and the risk of selection for fungicide-resistant isolates, especially those with more specific mechanisms of action. All of these factors are matters of concern and have led to an increase in research related to practices and management strategies that are rational, efficient and economically viable. Among these strategies is the use of cultivars with higher levels of resistance to early blight. The use of these resistant cultivars can at least reduce the need for chemical control, although fungicides cannot be completely removed from the disease management program.

The use of resistant cultivars is the preferred control method owing to its high efficacy, low cost, and simplicity to implement. In disease management programmes, host resistance may be combined and/or complemented with the use of fungicides to improve foliar early blight control under highly favourable disease conditions. Potato cultivars with higher levels of resistance demand less fungicide than cultivars with lower levels of resistance. Furthermore, the use of cultivars with higher resistance levels may reduce yield losses and dependence on chemical control. For this purpose, it is vital to evaluate the resistance of cultivars. However, the main cultivars planted in Brazil are still unclassified in terms of their resistance to early blight.

In addition to disease resistance, the foliage maturity type and the skin type of the potato tuber are factors that growers may consider when they select a cultivar to plant. Their selection will also depend on the culinary attractiveness of the cultivar in the consumer market. The current ideal trend is to use earlier maturing cultivars that also have smooth skin and with disease resistance. For this reason, it is important to know whether it is possible to obtain all three characteristics in the same cultivar.

The works of cultivars' screening for disease resistance are also very important to support the work of molecular biology studies. If we know the resistance' level of cultivars to early blight and type of resistance is possible to do the identifying and mapping early blight resistance genes (QTLs-Quantitative trait loci) using DNA marker and thus can improve our understanding of the genetics of disease resistance and facilitate a breeding program through marker-assisted selection. Due the resistance to early blight be negatively correlated with earliness, thus early blight resistance increases with late-maturing cultivars (Douglas & Pavek 1972, Mendoza et al., 1986, Pelletier & Fry 1989, Herriott et al., 1990, Zhang 2004) is possible also to identify QTLs for early

blight resistance with a pleiotropic effect on foliage maturity or closely linked to genes governing foliage maturity, as reported by Zhang (2004).

This work aimed to assess the resistance of potato cultivars to foliar early blight. Likewise, it aimed to assess the relationships between resistance to foliar early blight and foliage maturity type and also between resistance to foliar early blight and skin type in potato cultivars.

## **2 Material and Methods**

### **2.1 General Aspects**

Three field experiments were carried out under different environmental conditions to study the resistance of potato cultivars to foliar early blight. Each plot consisted of two rows measuring 3.0 m x 0.80 m. Each cultivar had 15 stems/m<sup>2</sup>, and the plots were 1.60 m apart.

The climate variables measured were: precipitation; maximum, average, and minimum relative humidities; and maximum, average and minimum temperatures.

Plants were fertilized according to the soil analyses and local recommendations. Sprinkle irrigation was used over the entire experimental area to favor foliar early blight epidemic.

After inoculation, specific fungicides were applied to control late blight allowing only the establishment of foliar early blight in the field. Fungicide were applied with a backpack sprayer with a pressure-regulating valve to obtain a 30 lbs/pol<sup>2</sup> working pressure. A conic tip was used and fungicide mix was applied at a rate of 600 L/ha.

The experiments were performed in a randomized complete block design with three replications, and each treatment was represented by one cultivar.

### **2.2 Experiment 1**

This trial was carried out in an experimental field at the Universidade Federal de Viçosa in Viçosa, which is in the southeast area of the state of Minas Gerais, at 20°44'44"S, 42°50'59"W and 661 m above sea level. The experiment began on June 13rd and was finished in September 8th of 2009 using 26 potato cultivars. The cultivars, origins, foliage maturity type and skin type are in table 1.

Climate data were collected at a meteorological station located inside the experimental potato field. The leaves of potato plants were artificially inoculated with a backpack sprayer 31 days after planting with one isolate of *A. grandis* collected from each potato region of Brazil (Camanducaia-MG, Araxá-MG, Cristalina-GO, Bom Repouso-MG, Bueno Brandão-MG, Maria da Fé-MG, Cachoeira de Minas-MG and Cristina-MG). The eight isolates were mixed to obtain 1000 conidia/mL. Each plot received about 200 ml of the inoculum suspension.

### **2.3 Experiment 2**

This experiment was a repetition of experiment 1 and was used to assess if have different in the resistance level to early blight under different environmental conditions. This trial was carried out in an experimental field at the Universidade Federal de Viçosa from April 14th to July 17th of 2010 using the same 26 potato cultivars of experiment 1.

Climate data were collected at a meteorological station located inside the experimental potato field. Potato plants were artificially inoculated 31 days after planting with the same methodology of experiment 1.

### **2.4 Experiment 3**

This trial was carried out in an experimental field at the Universidade Federal de Viçosa from April 14th to July 17th of 2010 to assess the resistance to foliar early blight of 24 potato cultivars, which was not used in the experiments 1 and 2. The cultivars, origins, foliage maturity type and skin type are in table 2.

Climate data were collected at a meteorological station located inside the experimental potato field. Potato plants were artificially inoculated 31 days after planting with the same methodology of experiment 1.

### **2.5 Disease assessment**

Early blight severity in each plot was quantified every seven days from the inoculation with a standard area diagram (Duarte et al. 2010). The value of severity for each plot was measured by examining five plants and averaging their severity values. The severity value of each plant was defined as the average severity of all leaves evaluated on that plant.

## 2.6 Data analysis

### 2.6.1 Experiment 1

The epidemiological variables used were the same variables selected by Duarte et al. (2010). These variables discriminate effectively among the different levels of resistance to potato late blight. From the severity data of each cultivar, the following variables were obtained:

- 1- Severity in the middle of the epidemic ( $Y_{50}$ ). An epidemic in the experiment was considered from the onset of the first symptoms in any cultivar until all of them reached 100% severity.
- 2- Area under disease progress curve (AUDPC) (as proposed by Shaner and Finney 1977). An epidemic in the experiment was considered from the onset of the first symptoms in any cultivar until all of them reached 100% severity. Then, the AUDPC was divided by the total area of the graph (= duration of the epidemic in days x 100) to obtain the relative area under disease progress curve (RAUDPC) as proposed by Fry (1978).
- 3- Time in days from the onset of the first symptoms in the experiment until 0.5% severity ( $T_{0.5}$ ). The time for each cultivar was considered from the onset of the first symptoms among all cultivars until a given cultivar reached 0.5% of severity.
- 4- Disease progression rate ( $r$ ). From the disease severity progression data, the exponential, monomolecular, gompertz and logistic models were adjusted with linear and non-linear regressions (Madden et al. 2007). The best model was selected using criteria such as the lower value of the residual mean square, independence and homogeneity, and highest determination coefficient ( $R^2$ ). Analysis were carried out with the SAS (Statistical Analysis System) program, version 9.0.

The average values of the epidemiologic variable was obtained for use in cluster analysis, discriminant analysis and principal component analysis.

To determine levels of resistance of the potato cultivars to foliar early blight, cluster analysis was carried out using the four variable responses simultaneously. This analysis was used as a dissimilarity measure for the standardized Euclidean distance and the following agglomerative hierarchic techniques: single linkage, complete linkage, average linkage, centroid and ward. The agglomerative hierarchic technique that best fit

the data was chosen through visual analysis of the grouping pattern. For the formation of the dendrogram, four groups were defined. Each one pinpointed different resistance levels of potato plants to foliar early blight. Potato plant levels of resistance to early blight were defined as: resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S). Analyses were run using the MINITAB program, version 14.

Discriminant analysis was used to confirm the quality of the grouping formed using the agglomerative hierarchic technique that was chosen to increase the reliability of the formed grouping. Analyses were run with the SAS (Statistical Analysis System) program, version 9.0.

Principal component analysis (PCA) was carried out using a data correlation matrix and the estimated eigenvectors and eigenvalues were obtained for each principal component (PC). The scores of all 26 potato cultivars were estimated through eigenvectors of each response variable for PCs presenting eigenvalues greater than the overall mean (Kaiser 1958). The PCA was used to classify the level of resistance to foliar early blight in experiments 2 and 3. The analysis was carried out with the MINITAB program, version 14.

After the classification of the cultivars based on their early blight resistance through cluster analysis, the average values were obtained for cultivars' epidemiological variables within each resistance level.

The results of experiment 1 suggested a method for classifying potato cultivars according to their level of resistance to early blight in experiments 2 and 3. Four response variables were analysed for this purpose. PC1 scores were obtained from PCA. To allow the scores to have the same sign (standardized value), the PC1 scores of each cultivar were added to the value with the opposite sign from the PC1 score of the most resistant cultivar. The cultivars with, respectively, the lowest (zero) and highest standardized values in module were considered the resistant and susceptible standards. After standardization, the percentage corresponding to the standardized value of each cultivar was obtained by dividing the cultivar standardized value by standardized value of the cultivar considered to be susceptible standard, and multiplying by 100. After obtaining the percentages, a value range for each resistance level was determined and too was possible to obtain a ranking of the cultivars resistance.

### **2.6.2 Experiment 2 and 3**

After obtaining the data for the response variable used to classify the cultivars' level of resistance to foliar early blight, we obtained PC1 scores for experiments 2 and 3. The standardized value of each cultivar was obtained using the Catucha cultivar as a resistant standard, and the corresponding percentage of the standardized value of each cultivar was compared to the susceptible standard (Elodie cultivar) defined in experiment 1. According to the values obtained from that percentage, each cultivar was classified according to its level of resistance to early blight based on the value range defined in experiment 1. After the cultivar classification was obtained according to level of resistance to foliar early blight, the averages of the variables of the cultivars were obtained within each resistance level. With percentages values it was possible to obtain a ranking of the cultivars resistance.

## **2.7 Disease progress curves**

The disease progress curves of the different resistance levels for all three experiments were obtained after classification of the cultivars according to their level of resistance to early blight based on the average of the severity data over time within each resistance level.

## **2.8 Relationships between resistance to early blight and foliage maturity type and between resistance to early blight and the skin type of the potato**

The levels of resistance to early blight for all potato cultivars were determined under different environmental conditions. Graphical analysis and Chi-square test of independence at 5% level were then used to investigate the relationships between resistance to early blight and foliage maturity type and also between resistance to early blight and the skin type of the potato.

## **3 Results**

### **3.1 Meteorological data**

In experiment 1, the temperature ranged from 8.7 to 31.5°C, and the average temperature during the epidemic was 18.2°C. The relative humidity ranged from 26 to

100% (data not shown), and the average humidity was 77.4%. The pluviometric precipitation during the experiment was 33.4 mm (Figure 1a).

In experiment 2 and 3, the temperature ranged from 4.9 to 29.2°C, and the average temperature during the epidemic was 16.2°C. The relative humidity ranged from 36 to 100% (data not shown), and the average relative humidity was 87.2%. The pluviometric precipitation during the experiment was 23.7 mm (Figure 1b).

### **3.2 Disease progress**

In experiment 1, at 38 days after planting DAP (first assessing), the early blight was observed. Only at 87 DAP the Catucha cultivar showed 100% disease severity. Therefore, the epidemic for experiment 1 lasted 50 days.

In experiment 2 and 3, at 38 DAP (first assessing), the early blight was observed. Only at 94 DAP the catucha cultivar showed 100% disease severity. Therefore, the epidemic for experiment 2 and 3 lasted 57 days.

The model that fit the severity data better along the time for all cultivars in all the three experiments was the logistic model with the non-linear regression. Therefore, data on the disease progress values ( $r$ ) were compared among all cultivars.

### **3.3 The level of resistance to early blight in the cultivars of potato**

#### **3.3.1 Experiment 1**

The cultivars were grouped into four resistance levels to foliar early blight, with 100% of hit based on the standardized Euclidean distance and on the method of the complete linkage because it fitted the experiment data best.

The percentages of resistant, moderately resistant, moderately susceptible and susceptible cultivars to early blight were 15.4, 7.7, 34.6 and 42.3%, respectively. The Ibituaçu, Catucha, BRS Ana and Aracy cultivars were classified as resistant; Colorado and Baraka cultivars were classified as moderately resistant; Asterix, Atlantic, Caesar, Chipie, Florice, Markies, Monalisa, Naturella and Soléia cultivars were classified as moderately susceptible and Ágata, Baronesa, Cupido, Éden, Elodie, Emeraude, Eole, Fontane, Gourmandine, Melody and Opaline cultivars were classified as susceptible (Fig. 2).

The average of the eigenvalues obtained from all PCs was 1.0. Therefore, only PC1 was interpreted because it was the only one that presented an eigenvalue higher than the average. The PC1 explained 83.0% of the total data variation (data not shown).

According to Table 3, values tended to increase when the level of resistance to early blight increased for the following variable: time in days from the onset of the first symptoms in the experiment until 0.5% of severity. The severity in the middle of the epidemic, the relative area under disease progress curve and the disease progress rate values tended to decrease when the level of resistance to early blight increased.

With PC1 score estimations of all of the 4 response variables and obtaining the percentage corresponding to the standardized value of each cultivar, a ranking of the cultivars was obtained. It was then possible to identify, which cultivars were more resistant or more susceptible to foliar early blight. The resistance of potato cultivar decreased as the percentage value increased. Conversely, the resistance of potato cultivar increased as the percentage values decreased (Table 4).

The cultivars' resistance level defined by cluster analysis, the PC1 score obtained from the four evaluated variables, the standardized value obtained by the sum of the scores of each cultivar by -4.64 and the percentages of the standardized values for cultivars in relation to the Elodie cultivar standardized value for each level of resistance to early blight are shown in Table 4. The Catucha cultivar was considered the resistant standard, and the Elodie cultivar was considered the susceptible standard.

By obtaining the data of the four epidemiological variables and the percentage of the standardized values of the determined cultivars in relation to the standardized value of Elodie cultivars, it will only be necessary to check if the given range fits to obtain an early blight resistance level for these cultivars. The range of these values will be assigned to classes of resistance levels: cultivars with percentage values between 0.00 and 24.99 will be considered as resistant; between 25.00 and 54.99 as moderately resistant; between 55.00 and 79.99 as moderately susceptible and >80.00 as susceptible (Table 4).

### **3.3.2 Experiment 2**

With the data from the four variables for each cultivar, it was possible to obtain the PC scores. The average of the eigenvalues obtained from all PCs was 1.0. Therefore, only PC1 was interpreted because it was the only one that presented an eigenvalue

higher than the average. PC1 explained 84.4% of the total variation of the data (data not shown).

According to the percentage values of the cultivar standardized values in relation to the Elodie cultivar standardized value and to checking the range of values defined in experiment 1, the Ibituaçu, Catucha, BRS Ana and Aracy cultivars were classified as resistant; Asterix, Atlantic, Baraka, Colorado, Florice, Markies, Monalisa and Naturella cultivars were classified as moderately resistant; Caesar, Chipie, Éden, Emeraude, Eole, Fontane, Gourmandine and Soléia cultivars were classified as moderately susceptible and Ágata, Baronesa, Cupido, Elodie, Melody and Opaline cultivars were classified as susceptible (Table 5). The percentages of resistant, moderately resistant, moderately susceptible and susceptible cultivars to early blight were 15.4, 30.8, 30.8 and 23.0%, respectively.

With PC1 score estimations of all of the 4 response variables and obtaining the percentage corresponding to the standardized value of each cultivar, a ranking of the cultivars was obtained. It was then possible to identify, which cultivars were more resistant or more susceptible to foliar early blight. The resistance of potato cultivar decreased as the percentage value increased. Conversely, the resistance of potato cultivar increased as the percentage value decreased (Table 5).

### **3.3.3 Experiment 3**

Using the data of the four epidemiological variables for each cultivar, PC scores were obtained. The average eigenvalue obtained from all PCs was 1.0. Therefore, only PC1 was interpreted because it was the only one that presented an eigenvalue higher than the average. PC1 explained 82.8% of the total variation of the data (data not shown).

According to the percentage values of the cultivar standardized values in relation to the Elodie cultivar standardized value and in line with the range of values defined in experiment 1, the Aracy Ruiva, Catucha and Itararé cultivars were classified as resistant; the Amorosa, Annabelle, Armada, BRS Elisa, Fabula, IAPAR Cristina, Maranca and Mozart cultivars were classified as moderately resistant; Innovator, Madeleine, Matador, Rodeo, Sinora and Victoria cultivars were classified as moderately susceptible; and the Ambra, Canelle, Elodie, Gredine, Marlen, Vivaldi and Voyager cultivars were classified as susceptible (Table 5). The percentages of resistant,

moderately resistant, moderately susceptible and susceptible cultivars to early blight were 12.5, 33.3, 25.0 and 29.2%, respectively.

With PC1 score estimations of all of the 4 response variables and obtaining the percentage corresponding to the standardized value of each cultivar, a ranking of the cultivars was obtained. It was then possible to identify, which cultivars were more resistant or more susceptible to foliar early blight. The resistance of potato cultivar decreased as the percentage value increased. Conversely, the resistance of potato cultivar increased as the percentage value decreased (Table 5).

### **3.4 The relationship between the resistance to early blight and the foliage maturity type of potato**

A graphical representation of the relationship between the level of resistance to early blight and the foliage maturity type of each cultivar, based on the three experiments, is shown in Figure 4. The resistant cultivars to early blight had mid-season, mid-late or late maturity. No cultivars susceptible to early blight were observed to have later maturity (mid-late or late maturity). In most cases, the cultivars found to be susceptible to early blight had earlier maturity (early or mid-early maturity). This relationship observed between the level of resistance to early blight and the foliage maturity type by graphical representation was confirmed by Chi-square test of independence because there was a relationship significant between the level of resistance to early blight and the foliage maturity type at 5% level ( $P=0.000$ ).

### **3.5 The relationship between resistance to early blight and the skin type of the potato**

A graphical representation of the relationship between the level of resistance of the cultivars to early blight and the skin type of each cultivar, based on the three experiments, is shown in Figure 5. The cultivars found to be resistant had a rough, mid-rough or smooth skin. Cultivars that were susceptible to early blight and had rough skin were not observed. In most cases, the cultivars that were found to be susceptible or moderately susceptible to early blight had smooth skin. This relationship observed between the level of resistance to early blight and the skin type by graphical representation was confirmed by Chi-square test of independence because there was a

relationship significant between the level of resistance to early blight and the skin type at 5% level ( $P=0.012$ ).

#### 4 Discussion

In the present research, we evaluated the resistance to early blight for potato cultivars in Brazil in different climatic conditions. We did not assess the resistance to early blight in tuber because we did not find symptoms of early blight on tubers in the three experiments.

In potato, the trait of resistance to early blight exhibits quantitative inheritance (Frank et al. 1979; Herriott et al. 1990; Christ and Haynes 2001). For this reason, immune genotypes of potato to early blight are difficult to obtain (Zachmann 1982). Moreover, the level of resistance of potato cultivars under field conditions is variable (Douglas and Pavek 1972). Therefore, it is meaningful to define intermediate levels of resistance. Categories such as moderately resistant and moderately susceptible therefore prove useful in practice if the simple dichotomy of resistant and susceptible is not sufficient.

Quantitative resistance implies that the disease progress is reduced. This work has verified that quantitative resistance can be found in potato cultivars. Higher levels of resistance to early blight were associated with lower rates of disease progression.

No potato cultivar was immune to foliar early blight. For this reason, the disease will occur in the field whenever the climatic conditions are favourable and the *A. grandis* inoculum is present. However, the intensity of the disease will depend on each cultivar's resistance level.

The relationship between disease development and plant maturity is well documented. In general, the earlier cultivars are more susceptible and the later cultivars are more resistant to early blight (Harrison et al. 1965; Douglas and Pavek 1972; Rich 1983; Brune et al. 1990; Herriott et al. 1990; Christ 1991). This study also observed such a relationship. Of the five cultivars classified as resistant to foliar early blight, only the Catucha cultivar did not exhibit mid-late or late maturity. The Catucha cultivar was used as resistant standard because it was the most resistant to foliar early blight. This cultivar has mid-season maturity. These results show that the later-maturity cultivars are not necessarily more resistant than the cultivars exhibiting mid-season maturity. Similar results have been found by Christ (1991). The Elodie cultivar, considered a susceptible

standard has a mid-early maturity. Earlier maturity genotypes with field resistance have been identified (Reifschneider and Brune 1992; Herriott et al. 1990, Boiteux et al. 1995; Simon et al. 2009). The identification of such cultivars is important because current breeding programmes seek to obtain cultivars that exhibit earlier maturity and higher levels of resistance to early blight (Brune et al. 1990; Brune et al. 1994; Brune and Reifschneider 1989; Reifschneider and Brune 1992).

Another feature associated with resistance to potato early blight is the plant age. Younger plants are usually more resistant than older plants. Following the initiation of tuberisation, the susceptibility to potato early blight gradually increases (Bussey and Stevenson 1991; Pscheidt and Stevenson 1988).

The resistance level of potato cultivars to early blight changes with the environment. This property results from the fact that resistance to potato early blight is determined by quantitative inheritance. The traits exhibiting quantitative inheritance are characteristically influenced by the environment (Agrios 2005). In experiments 1 and 2, the same cultivars and the same isolates of the fungus were used to inoculate the plots. Some cultivars exhibited a higher resistance level in experiment 2 than in experiment 1. The reason for this difference is that the environment was more favourable for the development of potato early blight in experiment 1 than in experiment 2. This explanation is consistent with the observation that the epidemic was 7 days shorter and exhibited higher values of  $r$  in experiment 1 than in experiment 2.

In general, the skins of the more susceptible (susceptible and moderately susceptible) cultivars are smoother. For example, the *Ágata* and *Cupido* cultivars are widely cultivated because they present suitable characteristics for the market. They are also classified as susceptible to foliar early blight. The probable reason for this association between smooth skin and susceptibility is that when breeding programmes seek potato cultivars with market characteristics such as high yield, clear and smoother skin, and flat buds, the genes that provide disease resistance can be lost, and the cultivars thus present lower resistance levels. The skin type of the potato tuber is of extreme importance for “in natura” consumption because smoother-skinned tubers are preferred in this market. However, tubers having rougher skins would be suitable for industrial processing and for the organic market.

The method used in this study to classify the resistance of potato cultivars to early blight is a standardised method and offers the following advantages: (a) the classification of the cultivars or genotypes according to their resistance to early blight

through comparisons with the reactions of known cultivars (a resistant standard and a susceptible standard); (b) the ability of multivariate analysis techniques to avoid the overlapping classifications that often result from standard multiple comparisons tests and make it difficult to separate cultivars or genotypes according to their levels of resistance to foliar early blight; (c) the ability of principal component analysis techniques to determine the resistance levels and to rank potato cultivars by knowing which cultivars are more resistant or more susceptible and (d) the more comprehensive and simplified interpretation of the resistance of potato to early blight that can be achieved through the simultaneous analysis of four important epidemiological variables with multivariate techniques. Previous field evaluations of the resistance of potato to early blight have used only one or a few epidemiological variables, such as the disease severity at any given time during of the epidemic (Brune et al. 1990; Brune et al. 1994; Brune and Reifschneider 1989; Reifschneider and Brune 1992, Christ 1991), AUDPC (Simon et al. 2009, Dita-Rodríguez et al. 2006, Christ 1991) and  $r$  (Holley et al. 1983). Nazareno et al. (1999) have suggested that caution must be exercised if only one epidemiological variable is used for evaluation. For example, the exclusive use of the AUDPC or of  $r$  may cause a problem of the following sort. Progress curves having the same value of AUDPC may be accompanied by differences in the initial time of occurrence of the epidemic, the final disease severity and the value of  $r$ . Moreover, similar  $r$ -values may be accompanied by differences in AUDPC. Christ (1999) has ranked potato cultivars for resistance to early blight with separate analyses of single epidemiological variables: the disease severity at a given time during the epidemic and AUDPC. Different rankings of the resistance of potato cultivars to early blight resulted from the separate analyses of the two epidemiological variables considered.

The results obtained in the present study are in agreement with the literature regarding the climatic conditions for the development of early blight epidemics. The average temperature during the epidemic favoured the occurrence of conidium germination and increased the potential of *A. grandis* to cause infection and severe disease. The relative humidity during this study also favoured the disease epidemic. A trial conducted in the winter in another region showed that a relative humidity of 80% was sufficient to generate leaf wetness (Costa et al. 2002) because dew tends to form at a lower relative humidity in the winter than in the summer (Jones 1986). Evidently, the leaf wetness observed in this study was sufficient for the development of early blight epidemics during the experiments. Climatic conditions favourable to early blight and

the presence of an aggressive isolate of *A. grandis* in potato cultivars explained the rapid development of the disease under field conditions.

The potato grower must evaluate each characteristic of the cultivars available for planting and select the best cultivar to plant. By using a classification of potato cultivars resistant to foliar early blight, the grower will find it easier to choose the most suitable cultivars. To date, the most important early blight control measure is fungicide application. Obtaining potato cultivars resistant to early blight will reduce the production costs and result in less environmental contamination and fungicide residues on the tubers. As a consequence, the market competitiveness of the growers will increase, and a higher sustainability of the entire potato production chain should result.

## 5 Acknowledgements

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for providing the financial support and the student grants.

## 6 References

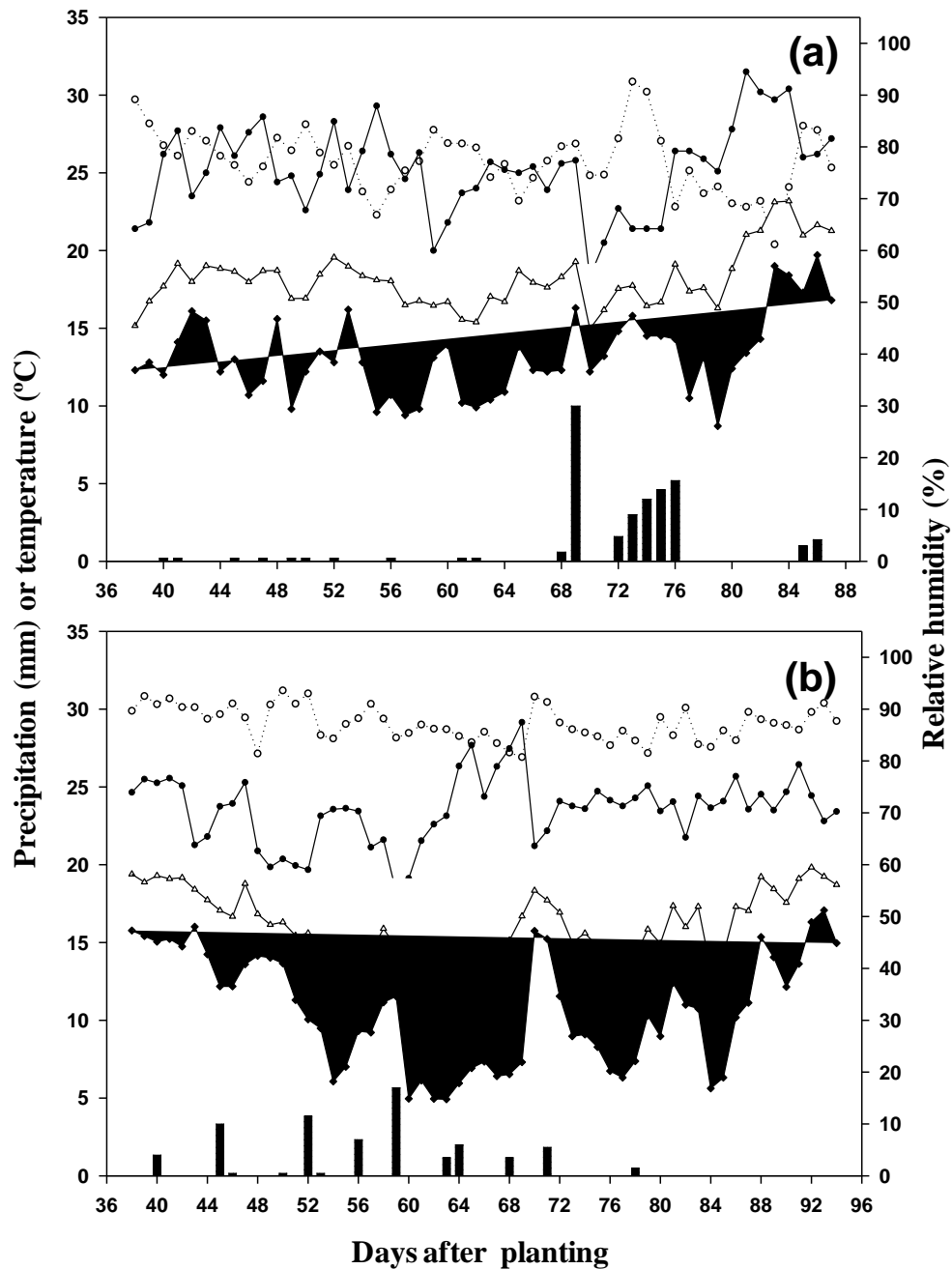
- Adams SS, Stevenson WR (1990) Water management, disease development and potato production. *Am J Pot Res* 67: 3-11
- Agrios GN (2005) *Plant Pathology*. 5<sup>a</sup> edicion. 922p.
- Batista DC, Lima MA, Haddad F, Maffia LA, Mizubuti ESG (2006) Validation of decision support systems for tomato early blight and potato late blight, under Brazilian conditions. *Crop Prot* 25: 664-670
- Boiteux LS, Reifschneider FJB, Fonseca MEN, Buso JA (1995) Search for sources of early blight (*Alternaria solani*) field resistance not associated with vegetative late maturity in tetraploid potato germplasm. *Euphytica* 83:63-70
- Brune S, Melo PE, Lima MF (1994) Resistência a *Alternaria solani*, características agronômicas e qualidade de fritura em clones de batata imunes a PVY e PVX. *Hortic Bras* 12:125-130
- Brune S, Reifschneider FJB (1989) Avaliação da resistência de batata à pinta-preta. *Hortic Bras* 7:20-21
- Brune S, Reifschneider FJB (1992) Identificação de fontes de resistência à pinta-preta em batata. *Hortic Bras* 10:100-102

- Brune S, Reifschneider FJB, Buso JA (1990). Resistência de genótipos de batata (*Solanum tuberosum*) à pinta-preta (*Alternaria solani*). Fitopatol Bras 15:297-299
- Bussey MJ, Stevenson WR (1991) A leaf disk assay for detecting resistance to early blight caused by *Alternaria solani* in juvenile potato plants. Plant Dis 75: 385-390
- Castro MEA, Zambolim L, Chaves GM, Cruz CD, Matsuoka K (2000) Variabilidade patogênica de *Alternaria solani*, agente causal da pinta-preta do tomateiro. Summa Phytopathol 8:24-28
- Christ BJ (1991) Effect of disease assessment method on ranking cultivars for resistance to early blight. Plant Dis 75: 353-356
- Christ BJ, Haynes KG (2001) Inheritance of resistance to early blight disease in a diploid potato population. Plant Breed 120:169-172
- Dita-Rodríguez MA, Brommonschenkel SH, Matsuoka K, Mizubuti ESG (2006) Components of resistance to early blight in four potato cultivars: effect of leaf position. J of Phytopathol 154:230-235
- Douglas DR, Pavek JJ (1972) Screening potatoes for field resistance to early blight. Am J Pot Res 49:1-6
- Duarte HSS, Zambolim L, Mizubuti ESG, Pádua JG, Ribeiro Júnior JI, Carmo EL, Nogueira Júnior AF (2010) Proposal of a classification system for assessing resistance of potato cultivars to late blight. In: III Workshop de Epidemiologia de Doenças de Plantas, 2010, Bento Gonçalves-RS. III Workshop de Epidemiologia de Doenças de Plantas, 2010. v. 1. p. 72-72.
- Duarte HSS, Zambolim L, Nogueira Junior AF, Rosado AWC, Mizubuti ESG, Capucho AS, Cardoso CR (2010) Development and validation of a diagrammatic scale for assessment of potato early blight. In: 43º Congresso Brasileiro de Fitopatologia, 2010, Cuiabá-MT. Tropical Plant Pathol 35:131-131.
- Fao (2012) Food and Agriculture Organization. Disponível em: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>. Acesso em 27 de setembro de 2012.
- Guenther JF Wiese MV, Pavlista AD, Sieczka JB, Wyman J (1999) Assessment of pesticide use in the US potato industry. Am J Pot Res 76:25-29
- Harrison MO, Livingston CH, Oshima M (1965) Epidemiology of potato early blight in Colorado. 1. Initial infection, disease development and the influence of environmental factors. Am J Pot Res 42:279-291

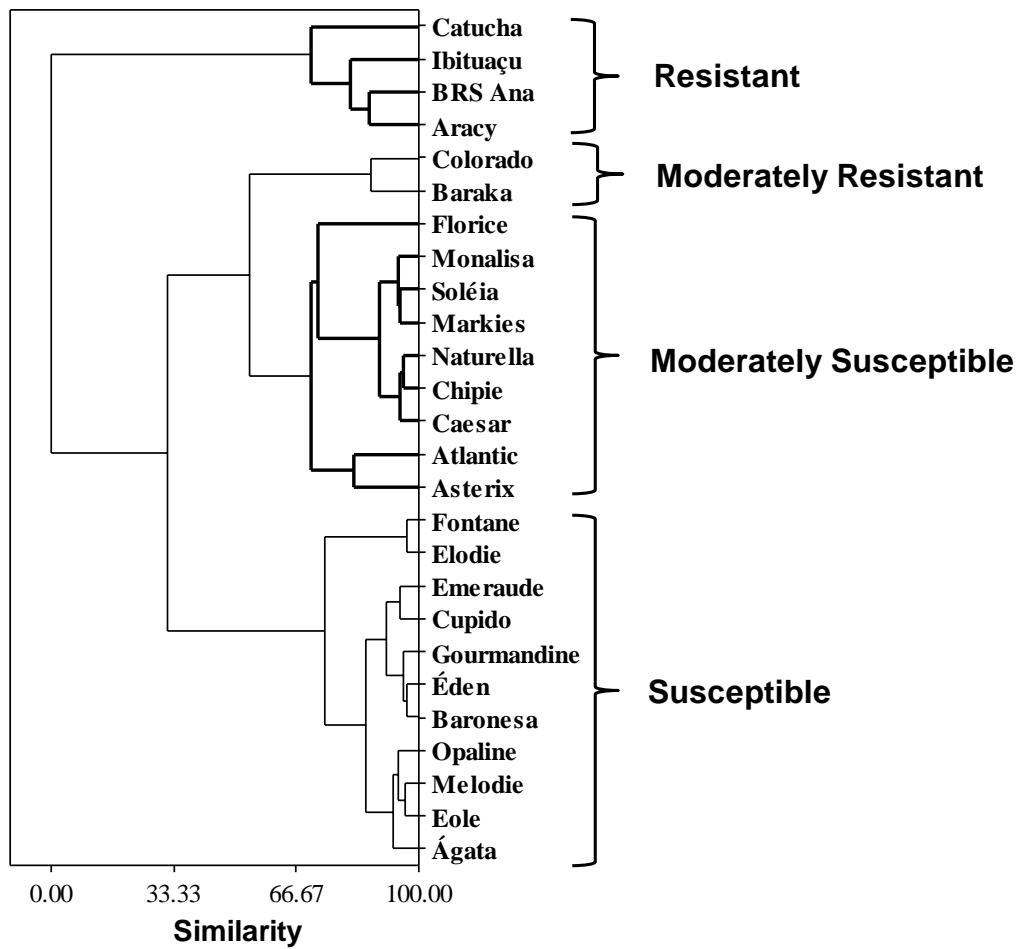
- Herriott AB, Haynes Jr FL, Shoemaker PB (1990) Inheritance of resistance to early blight disease in tetraploid x diploid crosses of potatoes. *Hortscience* 25:224-226
- Holley JE, Hall R, Hofstra G (1983) Identification of rate-reducing resistance to early blight in potato. *Can J Plant Pathol* 5:111-114
- Johnson KB, Teng PS (1990) Coupling a disease progress model for early blight to a model of potato growth. *Phytopathology* 80:416-425
- Jones JB, Stall RE, Zitter TA (1991) *Compendium of tomato disease*. St. Paul. APS Press.
- Kaiser HF (1958) The varimax criterion for analytic rotation in factor analysis. *Psychometrika* 23:187-200
- Madden LV, Hughes G, Van Den Bosh F (2007) *The Study of Plant Disease Epidemics*. St. Paul: APS Press.
- Mendoza HA., Martin, C, Vallejo, R.L. and Espinoza, J., 1986. Breeding resistance to early blight (*Alternaria solani*). *Am Pot J* 63: 444- 445.
- Nazareno NRX, Scotti CA, Mafioletti, RL, Boschetto N (1999) Controle da requeima da batata através do monitoramento das variáveis climáticas. *Fitopatol Bras* 24:170 - 174
- Pelletier JR, Fry WE (1990) Characterization of resistance to early blight in three potato cultivars: receptivity. *Phytopathology* 80:511–517
- Pscheidt JW, Stevenson WR (1986) Comparison of forecasting methods for controlo f potato early blight in Wiscosin. *Plant Dis* 70:361-366
- Reifschneider FJB, Furumoto O, Filgueira FAR (1984) Illustrated key for the evaluation of early blight of potatoes. *FAO Plant Protect B* 32:91-94
- Rich AE (1983) *Potato diseases*. Ac Press, NY. 238p.
- Rodrigues TTMS, Berbee ML, Simmons EG, Cardoso CR, Reis A, Maffia LA., Mizubuti ESG (2010) First report of *Alternaria tomatophila* and *A. grandis* causing early blight on tomato and potato in Brazil. *New Dis Report* 22: 28-28
- Rotem J (1994) *The genus Alternaria: Biology, epidemiology and pathogenicity*. St Paul: APS Press.
- Shuman JL, Christ BJ (2005) Integrating a host-resistance factor into the fast system to forecast early blight of potato. *Am J Pot Res* 82:9-19
- Simon AG, Pinto CABP, Lambert ES, Andreu MA (2009) Seleção de clones de batata resistentes à pinta preta e tolerantes ao calor. *Revista Ceres* 56:031-037.

- Simmons EG (2000) *Alternaria* themes and variations (244-286) species on solanaceae. Mycotaxon 75:1-115
- Zachmann R (1982) Early blight of potato (*Alternaria solani*). Lima, CIP, 14p. Technical Information Bulletin, 17.
- Zhang, R. 2004. Genetic characterization and mapping of partial resistance to early blight in diploid potato. Thesis. 158 pag.

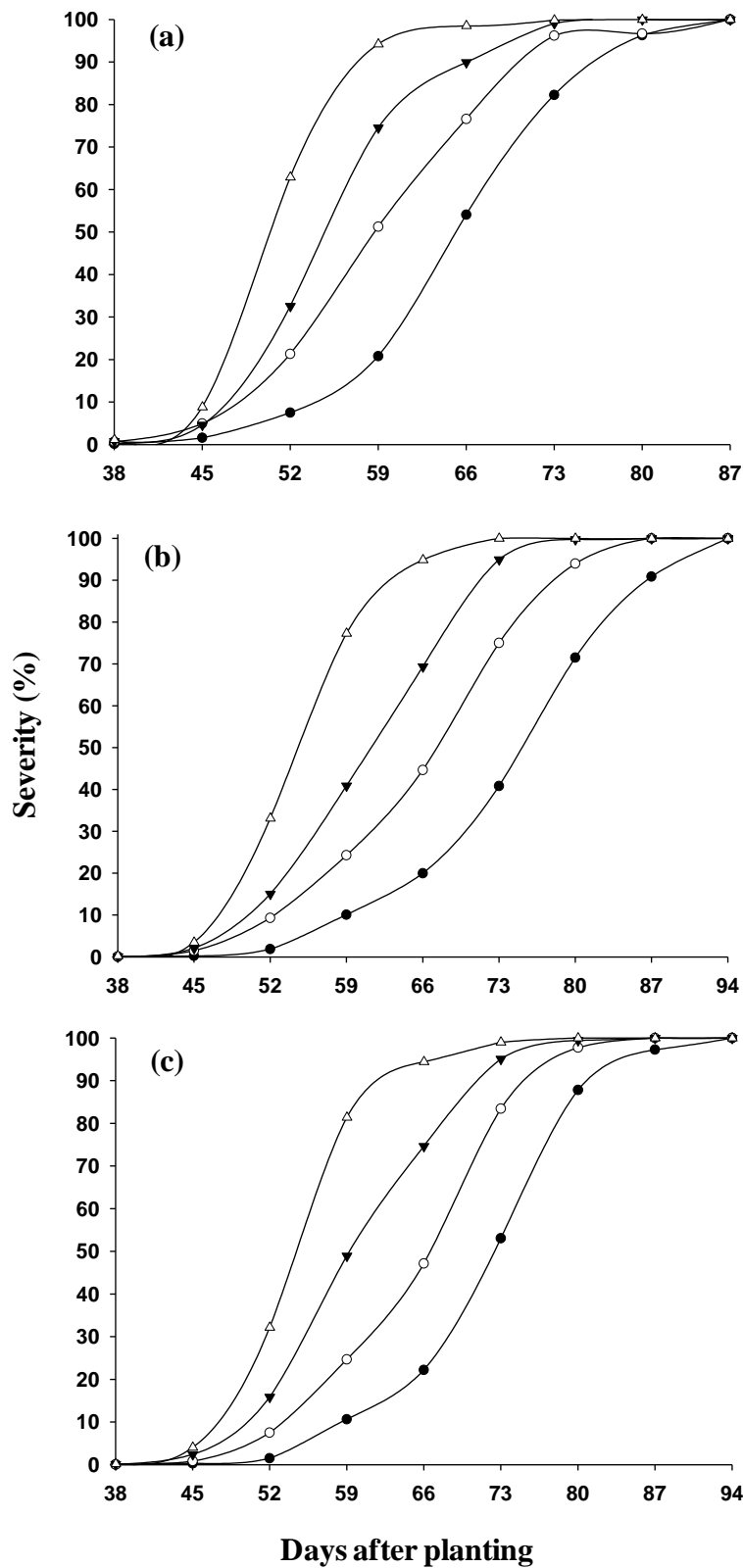
## 7 Figures



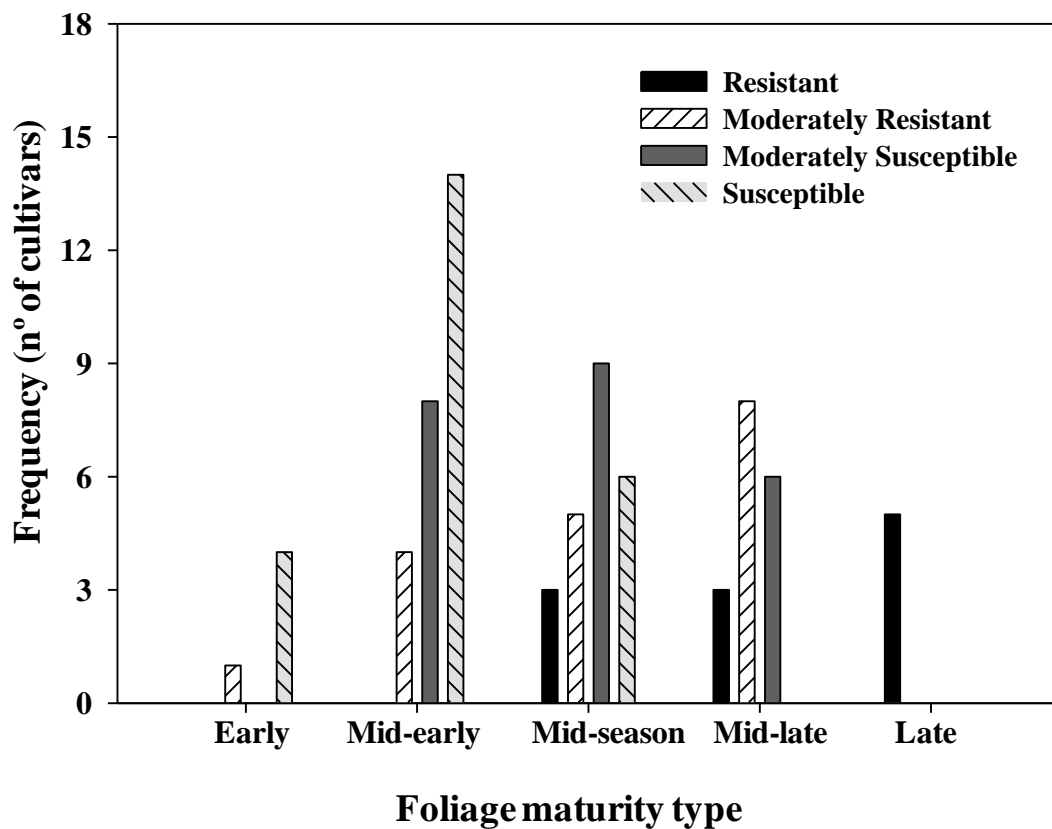
**Figure 1** Values of meteorological variables during the epidemic for experiments 1 (a), 2 and 3 (b). Relative humidity is represented by a dotted line with empty circles, pluviometric precipitation by vertical bars and temperature by a solid line. Maximum, medium and minimum temperatures are represented by  $\bullet$ ,  $\triangle$  and  $\diamond$ , respectively.



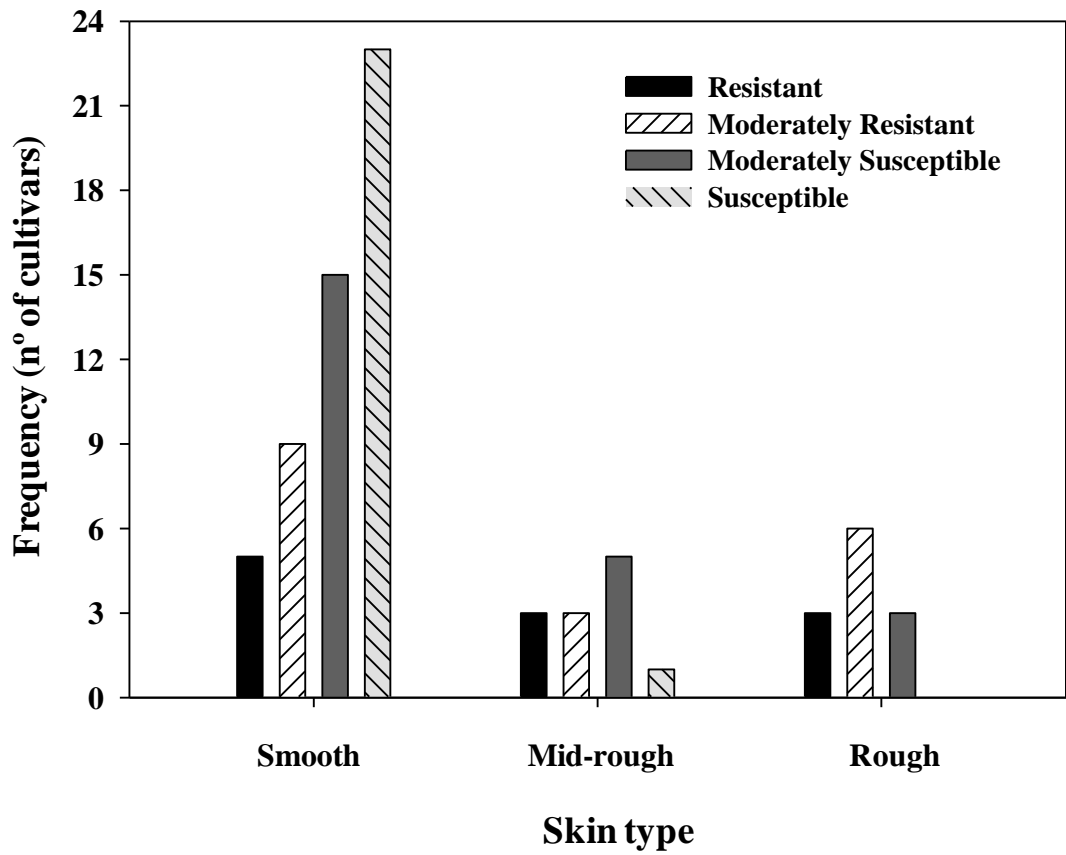
**Figure 2** Dendrogram of potato cultivars with their respective resistance levels to early blight (*Alternaria grandis*).



**Figure 3** Early blight curves of each level of resistance for experiments 1 (a), 2 (b) and 3 (c). Resistant, Moderately Resistant, Moderately Susceptible and Susceptible are represented by ●, ○, ▼ and △, respectively.



**Figure 4** The relationship between resistance to early blight in potato and foliage maturity type for the cultivars of experiments 1, 2 and 3.



**Figure 5** The relationship between resistance to early blight in potato and skin type for the cultivars of experiments 1, 2 and 3.

## 8 Tables

**Table 1** Origins, foliage maturity type and skin type of potato cultivars evaluated in experiments 1 and 2.

<b>Cultivars</b>	<b>Origins</b>	<b>Maturity<sup>a</sup></b>	<b>Skin type</b>
Ágata	Netherlands	Early	Smooth
Aracy	Brazil	Late	Rough
Asterix	Netherlands	Mid-late	Mid-rough
Atlantic	EUA	Mid-season	Rough
Baraka	Netherlands	Mid-late	Rough
Baronesa	Brazil	Mid-season	Smooth
BRS Ana	Brazil	Mid-late	Mid-rough
Caesar	Netherlands	Mid-late	Smooth
Catucha	Brazil	Mid-season	Smooth
Chipie	France	Mid-season	Mid-rough
Colorado	France	Mid-late	Rough
Cupido	Netherlands	Mid-early	Smooth
Éden	France	Mid-season	Smooth
Elodie	France	Mid-early	Smooth
Emeraude	France	Mid-early	Smooth
Eole	France	Mid-early	Smooth
Florice	France	Mid-early	Smooth
Fontane	Netherlands	Mid-season	Smooth
Gourmandine	France	Mid-season	Smooth
Ibituaçu	Brazil	Late	Smooth
Markies	Netherlands	Mid-late	Smooth
Melody	Netherlands	Mid-early	Smooth
Monalisa	Netherlands	Mid-early	Smooth
Naturella	France	Mid-late	Smooth
Opaline	France	Mid-early	Smooth
Soléia	France	Mid-season	Smooth

### Footnotes

<sup>a</sup> The foliage maturity type is based on the number of days from planting until maturity. Early= < 90 days; Mid-early = 91-100 days; Mid-season = 101-110 days; Mid-late = 111-120 days; Late = > 121days.

**Table 2** Origins, foliage maturity type and skin type of potato cultivars evaluated in experiments 3.

<b>Cultivars</b>	<b>Origins</b>	<b>Maturity type<sup>a</sup></b>	<b>Skin type</b>
Ambra	Netherlands	Early	Smooth
Amorosa	Netherlands	Mid-early	Smooth
Annabelle	Netherlands	Early	Smooth
Aracy Ruiva	Brazil	Late	Rough
Armada	Netherlands	Mid-early	Mid-rough
BRS Elisa	Brazil	Mid-season	Smooth
Canelle	France	Mid-early	Smooth
Catucha (resistant standard)	Brazil	Mid-season	Smooth
Elodie (susceptible standard)	France	Mid-early	Smooth
Fabula	Netherlands	Mid-season	Smooth
Gredine	France	Mid-early	Smooth
IAPAR Cristina	Brazil	Mid-late	Rough
Innovator	Netherlands	Mid-early	Rough
Itararé	Brazil	Mid-late	Mid-rough
Madeleine	Netherlands	Mid-early	Smooth
Maranca	Netherlands	Mid-season	Mid-rough
Marlen	Netherlands	Mid-season	Mid-rough
Matador	Netherlands	Mid-early	Smooth
Mozart	Netherlands	Mid-season	Smooth
Rodeo	Netherlands	Mid-season	Mid-rough
Sinora	Netherlands	Mid-early	Mid-rough
Victoria	Netherlands	Mid-late	Rough
Vivaldi	Netherlands	Mid-early	Smooth
Voyager	Netherlands	Early	Smooth

### Footnotes

<sup>a</sup> The foliage maturity type is based on the number of days from planting until maturity. Early= < 90 days; Mid-early = 91-100 days; Mid-season = 101-110 days; Mid-late = 111-120 days; Late = > 121days.

**Table 3-**Averages of epidemiological variables for each resistance level to early blight.

Resistance levels	Variables <sup>a</sup>			
	RAUDPC	Y <sub>50</sub>	<i>r</i>	T <sub>0.5</sub>
<b>Experiment 1</b>				
<b>R</b>	0.45	35.06	0.20	5.60
<b>MR</b>	0.53	53.09	0.22	2.71
<b>MS</b>	0.66	83.35	0.34	1.60
<b>S</b>	0.74	96.05	0.46	1.12
<b>Experiment 2</b>				
<b>R</b>	0.35	19.98	0.17	10.58
<b>MR</b>	0.50	48.96	0.20	3.53
<b>MS</b>	0.58	64.69	0.22	2.69
<b>S</b>	0.70	95.21	0.30	1.50
<b>Experiment 3</b>				
<b>R</b>	0.39	22.21	0.21	10.45
<b>MR</b>	0.50	47.14	0.22	6.19
<b>MS</b>	0.60	74.65	0.24	3.39
<b>S</b>	0.69	94.40	0.33	1.97

**Footnotes**

R = resistant; MR = moderately resistant; MS = moderately susceptible and S = susceptible

<sup>a</sup>RAUDPC= relative area under the disease progress curve; Y<sub>50</sub>= severity in the middle of the epidemic; *r* = disease progression rate and T<sub>0.5</sub> = time in days from the onset of the first symptoms in the experiment until 0.5% severity.

**Table 4-** Resistance levels of cultivars for experiment 1, PC1 scores obtained from the four epidemiological variables, standardized values, and percentages of the cultivar standardized values in relation to the standardized value of Elodie cultivars and ranges for each resistance level to early blight.

Cultivars	Resistance levels <sup>a</sup>	Score PC1 <sup>b</sup>	Standardized values	% <sup>c</sup>	Range
Catucha		4.64	0.00	0.00	
Aracy	R	3.40	-1.25	18.18	0.00-24.99
BRS Ana		3.28	-1.36	19.81	
Ibituaçu		3.07	-1.58	22.96	
Colorado	MR	1.64	-3.01	43.85	25.00-54.99
Baraka		0.97	-3.67	53.53	
Asterix		0.78	-3.86	56.29	
Florice		0.17	-4.48	65.25	
Caesar		0.07	-4.57	66.62	
Chipie		-0.19	-4.83	70.44	
Atlantic	MS	-0.21	-4.85	70.71	55.00-79.99
Naturella		-0.22	-4.87	70.96	
Monalisa		-0.41	-5.05	73.63	
Soléia		-0.53	-5.17	75.36	
Markies		-0.65	-5.29	77.20	
Emeraude		-0.86	-5.50	80.25	
Cupido		-1.01	-5.65	82.35	
Gourmandine		-1.14	-5.78	84.28	
Baronesa		-1.23	-5.87	85.62	
Éden		-1.30	-5.95	86.71	
Ágata	S	-1.41	-6.05	88.22	>80.00
Eole		-1.42	-6.06	88.41	
Melody		-1.49	-6.13	89.44	
Opaline		-1.71	-6.35	92.57	
Fontane		-2.03	-6.67	97.30	
Elodie		-2.22	-6.86	100.00	

#### Footnotes

<sup>a</sup> Cultivar resistance levels to early blight: R = resistant; MR = moderately resistant; MS = moderately susceptible and S= susceptible

<sup>b</sup> PC1 scores obtained from the four selected response variables.

<sup>c</sup> Percentages of standardized values of cultivars in relation to Elodie cultivar standardized values.

**Table 5** - PC1 scores obtained for the four epidemiological variables, the percentages of cultivar standardized values in relation to the standardized values of Elodie cultivar and resistance levels to early blight for experiments 1, 2 and 3.

<b>Cultivars</b>	<b>Resistance Exp. 1<sup>a</sup></b>	<b>PC1 Exp. 2<sup>b</sup></b>	<b>%<sup>c</sup></b>	<b>Resistance Exp. 2<sup>a</sup></b>	<b>Cultivars</b>	<b>PC1 Exp. 3<sup>b</sup></b>	<b>%<sup>c</sup></b>	<b>Resistance Exp. 3<sup>a</sup></b>
Ágata	S	-2.81	98.03	S	Ambra	-1.65	82.98	S
Aracy	R	2.61	17.52	R	Amorosa	0.99	42.44	MR
Asterix	MS	1.63	32.14	MR	Annabelle	1.22	38.99	MR
Atlantic	MS	0.75	45.16	MR	Aracy Ruiva	2.24	23.31	R
Baraka	MR	0.51	48.76	MR	Armada	1.21	39.15	MR
Baronesa	S	-1.74	82.07	S	BRS Elisa	1.53	34.30	MR
BRS Ana	R	2.87	13.74	R	Canelle	-2.44	94.93	S
Caesar	MS	-0.24	59.91	MS	Catucha	3.77	0.00	R
Catucha	R	3.79	0.00	R	Elodie	-2.77	100.00	S
Chipie	MS	-0.61	65.28	MS	Fabula	1.32	37.41	MR
Colorado	MR	1.33	36.48	MR	Gredine	-2.09	89.70	S
Cupido	S	-1.82	83.29	S	IAPAR Cristina	0.92	43.59	MR
Éden	S	-0.93	70.04	MS	Innovator	-0.29	62.10	MS
Elodie	S	-2.94	100.00	S	Itararé	2.69	16.54	R
Emeraude	S	-0.68	66.43	MS	Madeleine	-0.31	62.43	MS

Eole	S	-0.11	57.89	MS	Maranca	1.63	32.73	MR
Florice	MS	1.24	37.93	MR	Marlen	-1.84	85.77	S
Fontane	S	-0.46	63.10	MS	Matador	-0.98	72.73	MS
Gourmandine	S	-0.66	66.06	MS	Mozart	0.41	51.31	MR
Ibituaçu	R	2.61	17.52	R	Rodeo	-0.39	63.56	MS
Markies	MS	0.10	54.74	MR	Sinora	-0.43	64.18	MS
Melody	S	-2.08	87.24	S	Victoria	0.11	55.99	MS
Monalisa	MS	-1.20	74.05	MR	Vivaldi	-3.02	103.80	S
Naturella	MS	1.69	31.12	MR	Voyager	-1.84	85.82	S
Opaline	S	-2.73	96.75	S	-	-	-	-
Soléia	MS	-0.12	58.05	MS	-	-	-	-

### Footnotes

Exp = experiment

<sup>a</sup> Cultivar resistance levels to early blight: R = resistant; MR = moderately resistant; MS = moderately susceptible and S= susceptible

<sup>b</sup> PC1 scores obtained for the four epidemiological variables.

<sup>c</sup> Percentages of standardized values of cultivars in relation to Elodie cultivar standardized values.

## ARTICLE 4

### **Comparative epidemiology of late blight and early blight of potatoes under different environmental conditions and fungicide applications in Brazil**

#### **Abstract**

Among the diseases that affect potatoes, late blight caused by *Phytophthora infestans* (Mont.) de Bary and early blight caused by *Alternaria grandis* are considered of great importance due to the losses they cause in potato crops. Aiming to compare late blight and early blight potato epidemics under different environmental conditions and fungicide applications, three experiments were carried out. The 'Ágata' cultivar, which is susceptible to both late blight and early blight, was used. Each experiment consisted of two side-by-side trials, and each trial was set in a randomised block design with 5 treatments and 5 replications. At 30 days after inoculation, the potato plants of trial 1 were artificially inoculated with the A2 mating type isolate of *P. infestans* (200 sporangia/mL) and the potato plants of trial 2 were artificially inoculated with isolates of *A. grandis* (200 conidia/mL). Fungicide applications started seven days after inoculation. The late blight severity and the early blight severity were quantified every two days, and the disease severity data were used to calculate the area under the disease progress curve (AUDPC). The yield and its loss were also measured. The late blight was more aggressive than the early blight under different environmental conditions, and this was reflected in the yields. The late blight reached yield losses of 82.4%, while the early blight reached yield losses of 44.9%. Fungicide efficiency changed under the different environmental conditions, showing the importance of using tools that monitor the weather, such as forecast systems, for effective disease control.

**Keywords:** *Alternaria grandis*; *Phytophthora infestans*; *Solanum tuberosum*.

#### **1 Introduction**

The potato (*Solanum tuberosum* L.) is the world's fourth largest food crop (FAO, 2012). It is cultivated in more than one hundred countries. Potato production is limited by several factors, including diseases. Among the diseases that affect

potatoes, late blight, caused by *Phytophthora infestans* (Mont.) de Bary, and early blight, caused by *Alternaria grandis* are considered of great importance due to the losses they cause in potato crops.

Late blight is favoured by high humidity and moderate temperatures (Harrison 1992), conditions found in all the Brazilian regions that cultivate potatoes. When control measures fail, late blight epidemics may destroy an entire production field in a few days. Temperatures over 30°C can slow or stop disease development and the pathogen may remain alive in the host's tissues, it may spur and cause damage as soon as the climatic conditions become favourable (Lima et al. 2009). The disease causes lesions on the leaves, stems and tubers, which can be observed at any stage of the plant's development (Zambolim et al. 2011).

The population of *P. infestans* in Brazil consists of two clonal lineages: US-1 is associated with tomatoes (A1 mating type) and BR-1 (A2 mating type) is associated with potatoes. Isolates of the BR-1 clonal lineages are more aggressive to potato cultivars than to tomato cultivars, indicating a significant host-specificity (Suassuna et al. 2004).

Early blight is favoured by high humidity and high temperatures; it usually destroys the crop completely if control measures are not implemented (Adams and Stevenson, 1990). The disease causes necrotic lesions on leaves, stems and tubers (Rotem 1994). These lesions can be observed at any stage of the plant's development. The damage is most severe in mature and senescent tissues (Dita Rodriguez et al. 2006; Johnson and Teng 1990). At high severity, the disease destroys the leaves, causing defoliation, and thereby decreasing the yield and quality of the tubers (Castro et al. 2000).

The mitosporic fungus *Alternaria solani* Sorauer has been named as the causal agent of the disease (Rotem 1994). However, in a review of the genus *Alternaria* supported by morphological studies, *A. tomatophila* Simmons was reported to be the causal agent of early blight in tomatoes and *A. solani* was the causal agent of early blight in potatoes (Simmons 2000). Recent studies in Brazil have revealed the widespread occurrence of *A. grandis* and *A. tomatophila* causing early blight in potatoes and tomatoes, respectively. Interestingly, in the same study, isolates of *A. solani* were not detected causing early blight in potatoes (Rodrigues et al. 2010). Nevertheless, the symptoms of early blight caused by *A. grandis* are the same to those caused by *A. solani*.

Despite the impact of late blight and early blight on potato crops, there has been no comparative epidemiological analysis of these diseases under different environmental conditions. Comparative epidemiology aim to find similarities and differences among epidemics (Kranz 1988). These studies tend to emphasise the temporal dynamics of plant diseases, probably because the disease progress is often the most easily viewed manifestation.

When performing temporal analyses of plant diseases, the progress curve is the best representation of an epidemic. Interpreting the shape of these curves and determining certain parameters, such as the area under disease progress curve, the rate of the disease progression, and disease severity at the middle and at the end of the epidemic, are fundamental to understanding the epidemic behaviour and thus, disease management.

Among late blight and early blight control measures, the regular application of protectors and systemic fungicides is the most important and is considered to be effective (Aylor et al. 2001; Guenther et al. 1999, Mizubuti 2001, Töfoli et al. 2003, Shuman and Christ, 2005). Normally, fungicide applications to control these diseases follow a calendar schedule with predefined application intervals. To determine if the schedule is appropriate, it would be useful to know the effectiveness of different fungicides applied under different environmental conditions at controlling early blight and late blight of potatoes.

Damage caused by potato late blight is known to be greater when the moderate temperatures and potato early blight causes greater damage under hotter conditions. In recent years, early blight severity in potato fields has increased and currently, the potential damage from each disease under different environmental conditions still unknown.

The objective of this study was to compare epidemics of late blight and early blight of potatoes under different environmental conditions and fungicide applications programs using comparative epidemiology.

## **2 Material and Methods**

### **2.1 General aspects**

The three experiments were carried out in an experimental field at the Universidade Federal de Viçosa in Viçosa, which is in the southeast of the state of Minas Gerais, Brazil, at 20°44'44"S, 42°50'59"W and 661 m above sea level.

Plants were fertilised with ammonium sulphate, superphosphate and potassium chloride at planting time at the rate of 38-420-150 N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O Kg ha<sup>-1</sup>. The plants were hilled and fertilised again with ammonium sulphate (152 Kg N ha<sup>-1</sup>) 25 days after planting (DAP). The tubers were planted at distances of 0.19 to 0.44 m, depending on the diameter of the tuber, to obtain 15 stems/m<sup>2</sup>. Sprinkle irrigation was used over the entire experimental area. The herbicide metribuzin (Sencor) was applied at planting with a backpack sprayer with a fan tip at the rate of 0.30 Kg of a.i ha<sup>-1</sup>, using a pressure-regulating valve to obtain a 30 lb/inch<sup>2</sup> working pressure. The insecticides cipermetrina + profenofós (Polytrin) and abamectina (Vertimec 18 CE) were applied in the foliage at the rate of 0.35 Kg of a.i ha<sup>-1</sup> and of 58 g of a.i ha<sup>-1</sup>, respectively, with a backpack sprayer with a conical tip, using a pressure-regulating valve to obtain a 30 lb/inch<sup>2</sup> working pressure.

The climate variables measured included: precipitation (rain plus irrigation); maximum, average, and minimum relative humidity (RH); and maximum, average and minimum temperature. The measures were taken using a meteorological station (Squitter) located inside the experimental potato field that collected the data every 15 minutes. The temperature and relative humidity sensors were located above the potato canopy at 1.50 m. The percentage of time that was climatically favourable to late blight and early blight during the epidemic was calculated for each experiment. The climate was considered favourable to late blight when the temperature varied from 10 to 22°C and the relative humidity was above 85%. For early blight, favourable conditions were temperature ranging from 18 to 30°C and relative humidity above 85%.

## **2.2 Experiment 1**

This experiment was carried out from April 4th to June 29th of 2011 from early autumn to early winter.

The 'Ágata' cultivar, which is susceptible to both late blight and early blight (Duarte et al. 2011; Duarte et al. 2012), was used. The 'Ágata' is an early-foliage-

maturity cultivar type. Each plot consisted of three rows measuring 4.0 m x 2.4 m; the plots were 2.5 m apart.

This experiment consisted of two side-by-side trials, with each trial set in a randomised block design with 5 treatments and 5 replications.

In trial 1, the treatments were: 1) untreated check (without fungicide application), 2) weekly application of chlorotalonil (Daconil BR) at the rate of 1.3 Kg of a.i ha<sup>-1</sup>, 3) biweekly application of metalaxyl + mancozeb (Ridomil Gold MZ) at the rate of 0.1 + 1.6 Kg of a.i ha<sup>-1</sup>, respectively, 4) weekly alternated application of metalaxil + mancozeb and chlorotalonil in the rates described in treatments 2 and 3, and 5) treated check- application of dimetomorph + chlorotalonil (Forum Plus) at the rate of 0.2 + 1.0 Kg of a.i ha<sup>-1</sup>, respectively, every two days and tebuconazole (Folicur) at the rate of 0.3 Kg of a.i ha<sup>-1</sup> every three days, to obtain healthy plants and thus, to know the potato yield in the absence of late blight and early blight. The fungicide applications started seven days after inoculation (37 DAP), except for treatment 5, in which fungicide applications started at 30 DAP. The fungicide applications finished when the plants reached maturity. Potato plants on the plots, except for the plants in treatment 5, were artificially inoculated at 30 DAP with A2 mating type isolates (200 sporangia/mL) of *P. infestans* collected from Maria da Fé city, Minas Gerais state. The inoculation was performed with a backpack sprayer with a conical tip, using a pressure-regulating valve to obtain a 30 lb/inch<sup>2</sup> working pressure. The isolates of *P. infestans* were characterised as the A2 mating type and mitochondrial haplotype (mtDNA) Ib (Oliveira 2010). The plants from the treatments 1, 2, 3 and 4 were sprayed with tebuconazole (Folicur) at the rate of 0.3 Kg of a.i ha<sup>-1</sup> every three days because this fungicide has an effect only for early blight. Thus, only late blight developed on plants from this trial.

In trial 2, the treatments were: 1) untreated check (without fungicide application), 2) weekly application of chlorotalonil (Daconil BR) at the rate of 1.3 Kg of a.i ha<sup>-1</sup>, 3) biweekly application of tebuconazole (Folicur) at the rate of 0.3 Kg of a.i ha<sup>-1</sup>, 4) weekly alternated application of tebuconazole and chlorotalonil in the rates described in treatments 2 and 3, and 5) treated check-application of dimetomorph + chlorotalonil (Forum Plus) at the rate of 0.2 + 1.0 Kg of a.i ha<sup>-1</sup>, respectively, every two days and tebuconazole (Folicur) at the rate of 0.3 Kg of a.i ha<sup>-1</sup> every three days to obtain healthy plants and to determine potato yield in the absence of late blight and early blight. The fungicide applications started seven days

after inoculation (37 DAP), except in treatment 5 to which fungicide applications started at 30 DAP. Potato plants on the plots, except for the plants in treatment 5, were artificially inoculated at 30 DAP with four isolates (200 conidia/mL) of *A. grandis* collected from different potato regions of Brazil (Camanducaia-MG, Cristalina-GO, Bom Repouso-MG, Bueno Brandão-MG). The inoculation was performed with a backpack sprayer with a conical tip, using a pressure-regulating valve to obtain a 30 lb/inch<sup>2</sup> working pressure. The plants from treatments 1, 2, 3 and 4 were sprayed with dimetomorph (Forum) at the rate of 0.4 Kg of a.i ha<sup>-1</sup> every two days because this fungicide has an effect on late blight. Thus, only early blight developed in this trial.

At 10, 15 and 20 DAP, chlorotalonil (Daconil BR) fungicide was applied at the rate of 1.3 Kg of a.i ha<sup>-1</sup> in the experiment to prevent late blight and early blight lesions before the inoculation.

### **2.3 Experiment 2 and 3**

Experiment 2 was carried out from May 30 to September 3rd of 2011, from late autumn to late winter, and used the same methodology as experiment 1. Experiment 3 was carried out from August 3rd to October 28th of 2011, from mid-winter to mid-spring, using the same methodology as experiment 1.

### **2.5 Disease assessments and yield determination**

Severities of late blight and early blight on plants for each plot were quantified every two days, from 30 to 70 DAP, using a descriptive key (James 1971) modified by Fry et al. (1977) and by Duarte et al. (2011).

Potato yield was determined by weighing the tubers of all the plants from each plot after the plants matured. The yield data were converted into Kg.ha<sup>-1</sup>.

### **2.6 Data analysis**

Severity data were plotted to obtain disease progress curves and then the area under disease progress curves (AUDPC) was calculated as proposed by Shaner and Finney (1977). The 95% confidence interval was then determined for each treatment.

The percentage of control (PC) for each treatment and disease in the three experiments was calculated as:

$$PC (\%) = (AUDPC_{\text{Untreated Check}} - AUDPC_{\text{Treatment}}) / AUDPC_{\text{Untreated Check}} \times 100$$

The 95% confidence interval for the yield was determined for each treatment and disease. The percent yield loss (PYL) was calculated as:

$$PYL (\%) = (YIELD_{\text{Treated Check}} - YIELD_{\text{Treatment}}) / YIELD_{\text{Treated Check}} \times 100$$

### **3 Results**

#### **3.1 Meteorological data**

In experiment 1, the temperature during the epidemic ranged from 6.2°C to 28.3°C with an average temperature of 16.7°C (Fig. 1a). The relative humidity ranged from 38.6 to 98.5% (data not shown) with an average humidity of 86.2% (Fig. 1a). The total precipitation was 102.8 mm (Fig. 1a). The percentage of time that was climatically favourable to late blight was 57.5%, compared with early blight (7.1%), during the epidemic.

In experiment 2, the temperature during the epidemic ranged from 4.1°C to 30.1°C with an average temperature of 15.5°C (Fig. 1b). The relative humidity ranged from 36.0 to 98.4% (data not shown) with an average humidity of 85.6% (Fig. 1b). The total precipitation was 119.0 mm (Fig. 1b). The percentage of time that was climatically favourable to late blight was 49.5%, compared with early blight (2.34%), during the epidemic.

In experiment 3, the temperature during the epidemic ranged from 5.0°C to 32.6°C with an average temperature of 18.4°C (Fig. 1c). The relative humidity ranged from 28.9 to 97.5% (data not shown) with an average humidity of 77.6% (Fig. 1c). The total precipitation was 365.8 mm (Fig. 1c). The percentage of time that was climatically favourable to late blight was 38.5%, compared with early blight (6.2%) during the epidemic.

#### **3.2 Disease progress**

In experiment 1, the late blight and early blight epidemics started at 34 and 36 DAP, respectively. At 50 DAP, late blight severity in the plots on which fungicide

was not applied reached 100%, and at 70 DAP, early blight severity on plants from plots without fungicide applications reached 82%. For the environmental conditions of this experiment, the late blight was more aggressive than the early blight. In the plots on which fungicides were applied to control the late blight, the severity reached 100% at 52, 54 and 56 DAP for treatments 2, 3 and 4, respectively. However, in the plots on which fungicides were applied to control the early blight, the severity at 70 DAP was 38, 29 and 18% for treatments 2, 3 and 4, respectively. At 87 DAP, the plants in the treatment 5 plots (the healthy crop) reached maturity.

In experiment 2, the late blight and early blight epidemics started at 36 DAP. At 58 DAP, the severity of the late blight in the plots without fungicides spray reached 100% and at 70 DAP, the severity of the early blight in the plots that received no fungicide applications reached 39%. For the environmental conditions of this experiment, the late blight was more aggressive than the early blight. In plots on which fungicides were applied to control the late blight, the severity at 70 DAP was 98.6, 99.8 and 94% for treatments 2, 3 and 4, respectively. However, in plots on which fungicides were applied to control the early blight, the severity at 70 DAP was 12.2, 10.4 and 6.8% for treatments 2, 3 and 4, respectively. At 96 DAP, the plants of the treatment 5 plots (the healthy crop) reached maturity.

In experiment 3, the late blight and early blight epidemics started 40 DAP. At 64 DAP, the severity of the late blight in the plots without fungicides application reached 100% and at 70 DAP the severity of early blight in plots without fungicides application reached 81%, indicating that for the environmental conditions of this experiment, the late blight was more aggressive than the early blight. In plots on which fungicides were applied to control the late blight, the severity at 70 DAP was 0.8%, 0.9% and 0.7% for treatments 2, 3 and 4, respectively. However, in the plots on which fungicides were applied to control the early blight, the severity at 70 DAP was 20.8%, 12.6% and 7.6% for treatments 2, 3 and 4, respectively. At 86 DAP, the plants of the plots of treatment 5 (the healthy crop) reached maturity.

### **3.3 Area under the disease progress curve (AUDPC) and yield for late blight and early blight under different environmental conditions and different fungicide applications programs**

Under the environmental conditions of experiment 1, the AUDPC values for plants in the plots on which the fungicides were not applied to control the late blight (treatment 1, trial 1) was greater than the AUDPC values in the plots on which the fungicides were not applied to control the early blight (treatment 1, trial 2) (Table 1), indicating that the late blight was more aggressive than the early blight, which was reflected in yield. The late blight caused yield losses of 82.4, while the early blight caused yield losses of 44.9% (Table 2). In trial 1, despite the fact that the AUDPC values of treatments 2, 3 and 4 were lower than the AUDPC values of treatment 1, the percentage of control of the late blight was very low (<6.3%) (Table 1), indicating that the applied fungicides were not effective at controlling late blight, which is confirmed by the yield in the treatments, where treatments 2, 3 and 4 had the same yield as treatment 1, and the losses were greater than 80.7% (Table 2). In trial 2, the AUDPC values in the treatments 2, 3 and 4 were lower than that of treatment 1, and the percentage of control greater than 61.6%, indicating that the applied fungicides were moderately effective at controlling the early blight. Treatment 4 was the most efficient at controlling the early blight and showed a lower AUDPC value and the highest control percentage, followed by treatment 3 and then by treatment 2 (Table 1). The yields in the treatments 2, 3 and 4 were higher than the yield of treatment 1, and the losses were less than 34.9% (Table 2).

Under the environmental conditions of experiment 2, the AUDPC values in the plots on which fungicides were not applied to control the late blight (treatment 1, trial 1) were greater than the AUDPC values in the plots on which fungicides were not applied to control the early blight (treatment 1, trial 2) (Table 1), indicating that the late blight was more aggressive than the early blight, and this was reflected in the yield. The late blight caused losses of 76.1% in the yield while the early blight caused losses of 30.5% (Table 2). In trial 1, the AUDPC values for treatments 2, 3 and 4 were lower than for treatment 1, and the percentage of control obtained was less than 35.6% (Table 1), indicating that the fungicides applied were not very effective at controlling the late blight. The yields did not differ among treatments 2, 3 and 4 and were higher than the yield of treatment 1, and the losses were greater than 53.2% (Table 2). In trial 2, the AUDPC values of treatments 2, 3 and 4 were lower than the value for treatment 1, and the percentage of control obtained was greater than 52.8% (Table 1), indicating that the fungicides applied were moderately effective at controlling the early blight. Treatment 4 was the most effective at

controlling the early blight, as indicated by its lower AUDPC value; it has the highest value for percentage of control, followed by treatment 3 and then by treatment 2 (Table 1). The yields for treatments 2, 3 and 4 were higher than the yield for treatment 1, and the losses were less than 19% (Table 2).

Under the environmental conditions of experiment 3, the AUDPC values in the plots on which the fungicides were not applied to control the late blight (treatment 1, trial 1) were greater than the AUDPC values in the plots on which the fungicides were not applied to control the early blight (treatment 1, trial 2) (Table 1), indicating that the late blight was more aggressive than the early blight, which was reflected in the yield. The late blight caused yield losses of 47.5%, while the early blight caused yield losses of 38.1% (Table 2). In trial 1, the AUDPC and the yield values did not differ among treatments 2, 3 and 4, but they differed from the values for treatment 1. The percentages of control of the late blight in treatments 2, 3 and 4 were higher than 99%, and losses were less than 13.3% (Table 1 and 2), indicating that the fungicides applied were effective at controlling the late blight. The yields did not differ among treatments 2, 3 and 4 and were higher than the yield of treatment 1, and the losses were less than 13.3% (Table 2). For trial 2, the AUDPC values of treatments 2, 3 and 4 were lower than the values for treatment 1, and the percentage of control obtained was greater than 81.4% (Table 1), indicating that the fungicides applied were very effective at controlling the early blight. Treatment 4 was the most effective at controlling the early blight, as indicated by the lower value of the AUDPC and the highest percentage of control, followed by treatment 3 and then by treatment 2 (Table 1). The yields did not differ among treatments 2, 3 and 4 and were higher than the yield of treatment 1, and the losses were less than 16.1% (Table 2).

#### **4 Discussion**

The results from the present study are consistent with those reported in the literature on how climatic conditions affect the development of disease epidemics. In the three climatic conditions evaluated, the AUDPC values indicated that late blight epidemics developed faster than the early blight epidemics, showing that the conditions were more favourable to the late blight than the early blight. This relationship between the climatic conditions and the development of the disease epidemics was also observed when comparing each disease under different

environmental conditions. The conditions in experiment 1 were most favourable to the development of late blight, followed by those of experiment 2 and then those of experiment 3; the AUDPC values in the plots that did not receive the fungicide applications were 2689.4, 2064.6 and 1251, respectively (Table 1). For the early blight, the conditions were more favourable in experiment 1, followed by experiment 3 and then by experiment 2; the AUDPC values in the plots that did not receive the fungicide applications were 946.0, 197.3 and 587.6, respectively (Table 1).

The temperature ranges and the RH threshold used to calculate the percentage of time that was climatically favourable to late blight and early blight during the epidemic for each experiment were appropriate for evaluating the different climatic conditions. The percentage of time that was climatically favourable to late blight was 57.5%, 49.5% and 38.5% for experiments 1, 2 and 3, respectively, showing the same trend as the AUDPC values, which indicate that the conditions were most favourable to late blight in experiment 1, followed by experiment 2 and then by the experiment 3. The percentage of time that was climatically favourable to early blight was of 7.1%, 2.34% and 6.2% for experiments 1, 2 and 3, respectively, showing the same trend as the AUDPC values, which show that the conditions were most favourable to early blight in experiment 1, followed by experiment 3 and then by experiment 2. The temperature range, from 10 to 22°C, used to calculate the percentage of time that was favourable to the development of late blight was obtained by Maziero et al. 2009. In this temperature range, bigger lesions were observed when using mating type A2 isolates of *P. infestans*. The temperature range from 18 to 30°C used to evaluate the favourability to early blight was obtained by Cardoso (2010). In this temperature range; bigger lesions were observed when using isolates of *A. grandis*. An RH of 85% for both diseases was used because is the threshold needed to estimate leaf wetness duration when the RH sensor is located above the potato canopy (Andrade-Piedra et al. 2005).

Due to fact that late blight is normally a more aggressive disease than early blight, it is a source of great concern to potato farmers. Late blight can lead to plant death in a few days and cause a total loss of production if the symptoms appear soon after the emergence of the plants and the environmental conditions are favourable. Therefore, farmers begin their fungicide applications soon after plant emergence. Fungicides to control late blight are formulated in three ways: a) protectant fungicide only, such as clorothalonil, mancozeb, etc.; b) systemic fungicide formulated with

protectant fungicide, such as dimetomorph + clorothalonil, metalaxyl-M + mancozeb, cimoxanil + mancozeb, mandipropamid + clorothalonil, etc.; and c) systemic fungicide only, such as dimetomorph, propamocarb hydrochloride, mandipropamid, metalaxyl-M, fluazinam, propineb, etc. Most farmers control late blight by using protectant fungicide, systemic fungicide formulated with protectant fungicide and systemic fungicides such as fluazinam and propineb, which are effective for both late blight and early blight, thus controlling early blight indirectly. As a result, early blight is less severe, increasing as the plant tissues get older because mature and senescent tissues become more susceptible to early blight (Dita Rodriguez et al., 2006; Johnson & Teng, 1990). The findings might suggest that early blight is a problem only at the end of crop. The results of the experiments performed in this study, indicated that the severity of early blight in the inoculated plants in the plots that do not receive fungicide application can reach high values even before the start of the process of senescence. When farmers use systemic fungicides that control only late blight, such as dimetomorph, propamocarb hydrochloride, mandipropamid and metalaxyl-M, etc., and do not use a product that specifically controls early blight, the severity of early blight can reach the high values as observed in the experiments of this study, or even higher values of severity. This is because the plants used in the experiments in this study were inoculated at 30 DAP, but in the field, early blight epidemics can start soon after plant emergence if the inoculum of *A. grandis* is present. If the farmers use overlapping cultivation, where new planting is done in areas close to crops in the final cycle, spores of *A. grandis* can cause lesions on the plants after emergence, thereby increasing the duration of the epidemic. Another problem that results from using only a systemic fungicide is that it increases the risk of selection of resistant isolates.

To compare late blight and early blight epidemics in different environmental conditions, the day of inoculation and the end of the evaluations were standardised. All the plants in the experiments were inoculated at 30 DAP. This is because the plants of the 'Ágata' cultivar reach the maximum number of stems per plant at approximately 30 DAP under mild or cold temperature conditions (Melo et al. 2003). All the plants in the experiments were evaluated until 70 DAP, the point at which, under conditions of mild temperature and cold temperature, the leaf area index and the number of leaves of the cultivar 'Ágata' decline rapidly due to the ageing of the

lower leaves (Melo et al. 2003). Because of this, it is difficult to differentiate the disease from senescence.

The experiments were carried out from March to October in a low altitude region with low to mild temperatures, where potato growth is more adapted. Potato cultivation in the period from October to February, when temperatures are higher, is usually done at high altitudes (> 1000 meters above sea level) where the temperatures are lower. A fourth experiment was carried out in the same area of the three experiments in this study (regions at low altitude) to determine if potatoes can be grown there in the October-February period. Planting was in late October; the plants reached maturity at 55 DAP due to periods of high temperature, indicating that they very rapidly became senescent. Thus, it was not possible to compare the late blight and early blight epidemics in this experiment.

In the three climatic conditions evaluated, the late blight caused greater yield losses than the early blight, because the late blight epidemics developed more rapidly than the early blight epidemics. Late blight can cause yield losses of 100% when the severity of the disease reaches high levels prior to tuberisation, as observed by Duarte et al. (2008). In this study, we observed yield losses of 82.4%, because the 'Ágata' cultivar plants begin the tuberisation process at approximately 35 DAP (Melo et al. 2003), when the levels of severity for the late blight were low due to the inoculation with *P. infestans* that occurred at 30 DAP. An early blight epidemic can reduce tuber yield by more than 20% (Johnson and Teng 1990; Shtienberg et al. 1996), and losses of approximately 58% have been reported in Brazil (Arana et al. 2007). In the present study, the maximum yield loss was 44.9%.

The efficiency of the fungicides, applied following a calendar schedule, at controlling the late blight and early blight was different for each environmental condition, as shown by the AUDPC values. For example, the AUDPC value when fungicides were used to control late blight was greater than 2519.1 for experiment 1, while in experiment 3, the AUDPC value was lower than 9.12 (Table 1), indicating that the effectiveness of control by the fungicides is influenced by the environmental conditions. When a calendar schedule is used, the intervals of fungicide applications are fixed independently of the environmental conditions, creating problems with controlling plant diseases effectively. When conditions favour rapid disease development, the pre-determined application interval might be longer than necessary to control the disease and this causes failure in its control. When conditions are less

favourable or unfavourable to development of the disease, the pre-determined application interval might be shorter than needed for effective disease control, with the result that farmers spend more on fungicides than necessary. Forecast systems can be used as tools to help manage the disease and rationalise fungicide use (Gleason et al. 1995; Hardwick 1998). Such systems are based on meteorological data and aim to identify periods that are favourable for disease development and, on that basis, determine when to begin applying fungicides and how often.

## 5 Acknowledgements

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for providing the financial support and the student grants.

## 6 References

- Adams SS, Stevenson WR (1990) Water management, disease development and potato production. *Am J Pot Res* 67: 3-11.
- Andrade-Piedra JL, Hijmans RJ, Juárez HS, Forbes GA, Shtienberg D, Fry W E (2005) Simulation of potato late blight in the Andes. II: validation of the LATEBLIGHT model. *Phytopathology* 95:1200-1208.
- Arana ROC, Zambolim L, Costa LC (2007) Potato early blight epidemics and comparison of methods to determine its initial symptoms in a potato field. *Rev.Fac.Nal.Agr.Medellín* 60:3877- 3890.
- Aylor DE, Fry WE, Mayton H, Andrade Piedra, JL (2001) Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology* 91:1189-1196
- Batista DC, Lima MA, Haddad F, Maffia LA, Mizubuti ESG (2006) Validation of decision support systems for tomato early blight and potato late blight, under Brazilian conditions. *Crop Protection* 25:664-670.
- Cardoso CR (2010) Agressividade de *Alternaria tomatophila*, *A. grandis* e *A. solani* em Batateira e Tomateiro. Dissertação de mestrado. Universidade Federal de Viçosa. 42 pag.

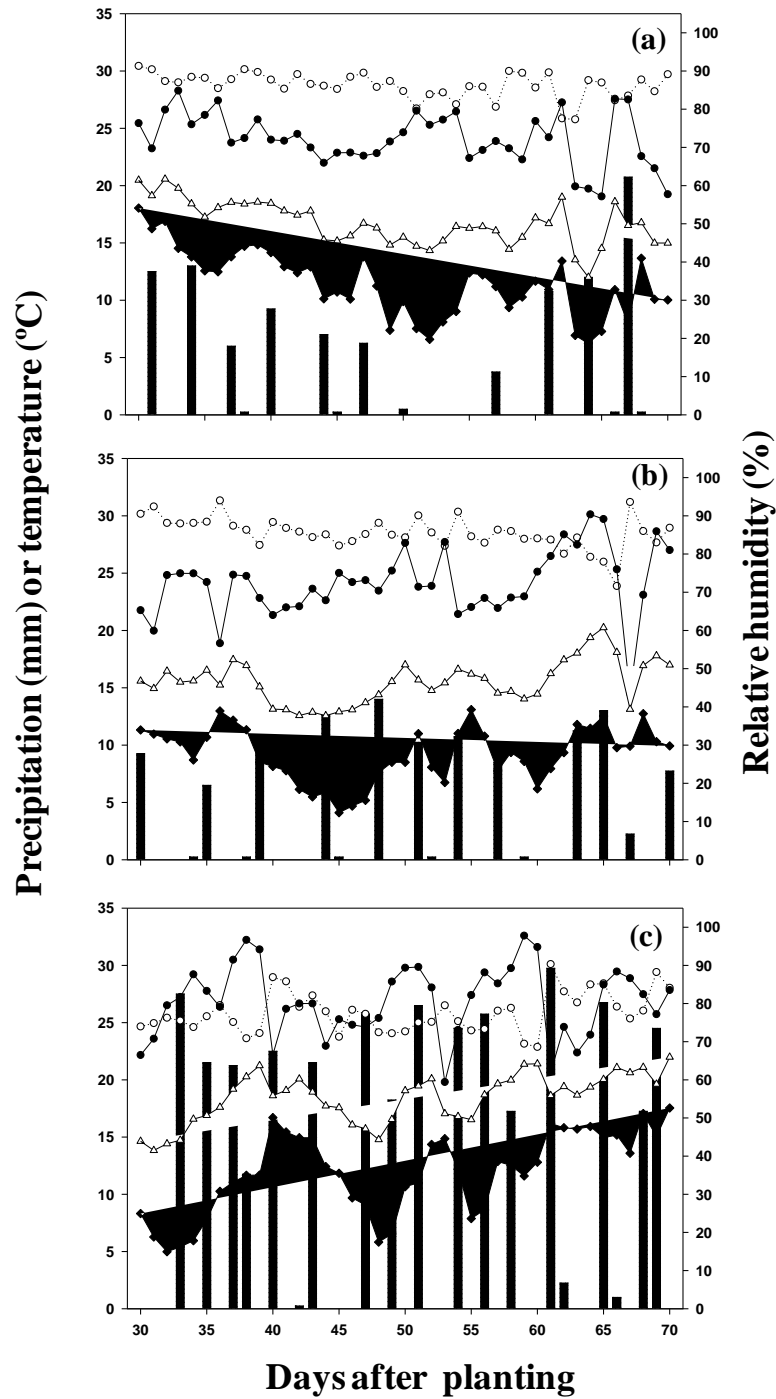
- Castro MEA, Zambolim L, Chaves GM, Cruz CD, Matsuoka K (2000) Variabilidade patogênica de *Alternaria solani*, agente causal da pinta-preta do tomateiro. *Summa Phytopathol* 8:24-28.
- Dita-Rodríguez MA, Brommonschenkel SH, Matsuoka K, Mizubuti ESG (2006) Components of resistance to early blight in four potato cultivars: effect of leaf position. *J Phytopathol* 154:230-235.
- Duarte HSS, Zambolim L, Mizubuti ESG, Nogueira Junior AF, Rosado AWC, Padua JG, Ribeiro Junior JI, Vaz, GRO (2011) Assessment of resistance level of potato cultivars to early blight (*Alternaria grandis*). In: XLIV Congresso Brasileiro de Fitopatologia, 2011, Bento Gonçalves. *Tropical Plant Pathology-Suplemento* 36:017-017.
- Duarte HSS, Zambolim L, Mizubuti ESG, Padua JG, Ribeiro Junior JI, Carmo EL, Nogueira Junior AF (2012) The field resistance of potato cultivars to foliar late blight and its relationship with foliage maturity type and skin type in Brazil. *Australasian Plant Pathol* 41:139-155.
- Duarte HSS, Zambolim L, Rodrigues FÁ, Rios JA (2008) Efeito do silicato de potássio isoladamente ou em mistura com fungicida no controle da requeima da batateira. *Summa Phytopathol*, 34:68-70.
- Fao (2012) Food and Agriculture Organization. Disponível em: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>. Acesso em 27 de setembro de 2012.
- Fry WE (1977) Integrated control of potato late blight: Effects of polygenic resistance and techniques of timing fungicide applications. *Phytopathology* 67:415-420.
- Gleason ML, MacNab AA, Pitblado RE, Ricker MD, East DA, Latin RX (1995) Disease-warning systems for processing tomatoes in eastern North America: are we there yet? *Plant Dis.* 79:113–121.
- Guenthner JF, Wiese MV, Pavlista AD, Sieczka JB, Wyman J (1999) Assessment of pesticide use in the US potato industry. *Am J Pot Res* 76: 25-29.
- Hardwick NV (1998) Disease forecasting. In: Gareth Jones, D. (Ed.), *The Epidemiology of Plant Diseases*. Kluwer Academic Publishers, Dordrecht, pp. 207–230.
- Harrison JG (1992) Effects of the aerial environment on late blight of potato foliage – a review. *Plant Pathol.* 41:384-416.

- James WC (1971). An illustrated series of assessment keys for plant disease, their preparation and usage. *Canadian Plant Disease Survey* 51: 39-65.
- Johnson KB, Teng PS (1990) Coupling a disease progress model for early-blight to model of potato growth. *Phytopathology*. 80:416-425.
- Kranz J (1988) The methodology of comparative epidemiology. In: Kranz, J. & Rotem, J. (Eds.) *Experimental Techniques in Plant Disease Epidemiology*. Heidelberg. Springer-Verlag. pp.279-290.
- Lima MA, Maffia LA, Barreto RW, Mizubuti ESG (2009) *Phytophthora infestans* in a subtropical region: survival on tomato debris, temporal dynamics of airborne sporangia and alternative hosts. *Plant Pathol* 58: 87-99
- Maziero JMN, Maffia LA, Mizubuti ESG (2009) Effects of temperature on events in the infection cycle of two clonal lineages of *Phytophthora infestans* causing late blight on tomato and potato in Brazil. *Plant Dis*. 93:459-466.
- Melo PCT, Granja NP, Miranda Filho HS, Sugawara AC, Oliveira RF (2003) Análise do crescimento da cultivar de batata Ágata. *Batata Show* 8:16 – 17.
- Mizubuti ESG (2001) Requeima ou mela da batata e do tomate. In: Luz EDN, Santos AF, Matsuoka, K, Bezerra JL (Eds) *Doenças causadas por Phytophthora no Brasil*. Campinas, BR: Livraria Editora Rural, pp 100-174.
- Oliveira SAS (2010) Genetic structure, aggressiveness and fungicide sensitivity of *Phytophthora infestans* populations from the south and southeast regions of Brazil. PhD Thesis. Viçosa Federal University.
- Rodrigues TTMS, Berbee ML, Simmons EG, Cardoso CR, Reis A, Maffia LA, Mizubuti ESG (2010) First report of *Alternaria tomatophila* and *A. grandis* causing early blight on tomato and potato in Brazil. *New Dis Reports* 22: 28-28.
- Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* 67:1051-1056.
- Shtienberg D, Blachinsky D, Ben-Hador G, Dinour A (1996) Effects of growing season and fungicide type on the development of *Alternaria solani* and on potato yield. *Plant Dis* 80: 994-998.
- Shuman JL, Christ BJ (2005) Integrating a host-resistance factor into the Fast system to forecast early blight of potato. *Am J Pot Res* 82: 9-19.
- Suassuna ND, Maffia LA, Mizubuti ESG (2004) Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. *Plant Pathol* 53:405-413

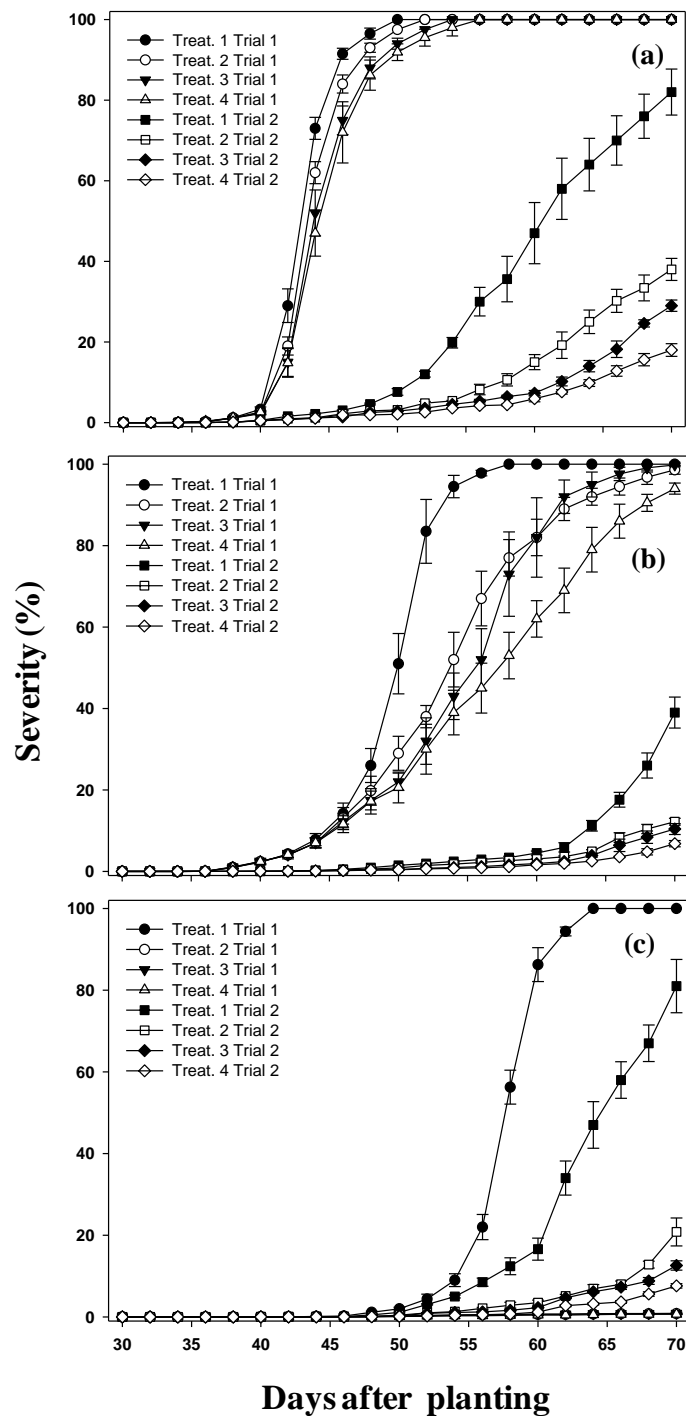
Töfoli JG, Domingues RJ, Garcia Junior O, Kurozawa C (2003) Controle da pinta preta do tomateiro por fungicidas e seus impactos na produção. *Summa Phytopathol* 29: 225-233.

Zambolim L, Duarte HSS, Zambolim EM (2011) Medidas integradas de controle das doenças fúngicas da batata. In: Zambolim L (Ed). *Produção integrada da batata*. v.1. p.411-438.

## 7 Figures



**Fig. 1** Values of meteorological variables recorded during the epidemic in experiments 1 (a), 2 (b) and 3 (c). Average relative humidity is represented by a dotted line with empty circles, pluviometric precipitation by vertical bars and temperature by a solid line. Maximum, medium and minimum temperatures are represented by  $\bullet$ —,  $\triangle$ — and  $\blacklozenge$ —, respectively.



**Fig. 2** Progression curves of each treatment in experiments 1 (a), 2 (b) and 3 (c). Trial 1 (late blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- metalaxyl + mancozeb biweekly and 4- metalaxil + mancozeb and chlorotalonil weekly alternate). Trial 2 (early blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- tebuconazole biweekly and 4- tebuconazole and chlorotalonil weekly alternate). Vertical bars represent the standard deviation of the mean.

## 8 Tables

**Table 1.** Area under disease progress curve (AUDPC), confidence interval (CI) for each treatment and percentage of control of each treatment for experiments 1, 2 and 3.

Trials*	Treat- ments	Experiment 1			Experiment 2			Experiment 3		
		AUDPC**	CI (95%)	% of control	AUDPC**	CI (95%)	% of control	AUDPC**	CI (95%)	% of control
<b>Trial 1</b> (late blight)	<b>1</b>	2689.4	2672.3-2705.9	0.0	2064.6	1997.7-2131.5	0.0	1251.5	1214.0-1289.0	0.0
	<b>2</b>	2618.6	2604.0-2633.2	2.6	1627.8	1518.9-1736.7	21.2	12.2	10.8-13.6	99.0
	<b>3</b>	2550.2	2519.5-2580.9	5.2	1563.4	1427.5-1699.3	24.3	12.9	5.9-19.9	99.0
	<b>4</b>	2519.1	2462.8-2575.4	6.3	1328.8	1199.6-1458.0	35.6	9.0	7.0-11.0	99.3
<b>Trial 2</b> (early blight)	<b>1</b>	946.0	837.1-1054.9	0.0	197.3	175.8-218.8	0.0	587.6	532.7-642.5	0.0
	<b>2</b>	363.4	321.2-405.6	61.6	93.0	85.3-100.7	52.8	109.2	97.6-120.8	81.4
	<b>3</b>	235.6	227.8-243.4	75.1	67.9	55.7-80.1	65.6	81.4	78.4-84.4	86.1
	<b>4</b>	167.2	155.0-179.4	82.3	44.5	40.8-48.2	77.4	44.4	41.0-47.8	92.4

\*Trial 1 (late blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- metalaxyl + mancozeb biweekly and 4- metalaxil + mancozeb and chlorotalonil weekly alternate). Trial 2 (early blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- tebuconazole biweekly and 4- tebuconazole and chlorotalonil weekly alternate).

\*\* Mean of five replication.

**Table 2.** Yield and its confidence interval (CI) for each treatment, and yield loss of each treatment for experiments 1, 2 and 3.

Trials*	Treatments	Experiment 1			Experiment 2			Experiment 3		
		Yield** (Kg/ha)	CI (95%) (Kg/ha)	Loss (%)	Yield** (Kg/ha)	CI (95%) (Kg/ha)	Loss (%)	Yield** (Kg/ha)	CI (95%) (Kg/ha)	Loss (%)
<b>Trial 1 (late blight)</b>	<b>1</b>	6191	4413-7969	82.4	14688	11374-18002	76.1	26153	23382-28924	47.5
	<b>2</b>	6685	5340-8030	81.0	25729	24388-27071	58.1	43152	39942-46361	13.3
	<b>3</b>	6761	5959-7563	80.7	22917	19370-26464	62.7	44257	39663-48851	11.1
	<b>4</b>	6497	5493-7501	81.5	28750	24594-32906	53.2	46095	41046-51146	7.4
	<b>5</b>	35169	33094-37244	0.0	61452	57735-65169	0	49802	43798-55808	0
<b>Trial 2 (early blight)</b>	<b>1</b>	19911	18672-21150	44.9	41983	38596-45370	30.5	29602	25142-34061	38.1
	<b>2</b>	23529	22406-24652	34.9	48958	45564-52352	19.0	40152	34267-46036	16.1
	<b>3</b>	26978	24422-29534	25.3	50417	47731-53103	16.5	42902	37462-48341	10.3
	<b>4</b>	27450	24901-29999	24.0	55942	52682-59202	7.4	43471	36365-50576	9.1
	<b>5</b>	36124	32962-39285	0.0	60417	54772-66062	0	47850	43235-52465	0

\*Trial 1 (late blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- metalaxyl + mancozeb biweekly and 4- metalaxil + mancozeb and chlorotalonil weekly alternate). Trial 2 (early blight), the treatments were: 1- without fungicide application; 2- chlorotalonil weekly; 3- tebuconazole biweekly and 4- tebuconazole and chlorotalonil weekly alternate).

\*\* Mean of five replication.

## **GENERAL CONCLUSIONS**

### **ARTICLE 1**

- 1) The majority of potato cultivars were susceptible to foliar late blight.
- 2) The cultivars with the highest levels of resistance to foliar late blight (resistant and moderately resistant) were later maturity. Most cultivars that have been classified as moderately susceptible or susceptible were earlier maturity.
- 3) In general, cultivars that are more resistant to foliar late blight had a rougher skin, whereas the skins of the more susceptible cultivars had smoother.

### **ARTICLE 2**

- 1) The Standard area diagram (SAD) developed and validated to quantify the severity of early blight on leaves of potato and to providing assistance in the use of the Field key (FK) developed and validated to quantify the severity of early blight in potato plants in experimental plots was a useful tool for improving the accuracy, precision and reliability of estimates.

### **ARTICLE 3**

- 1) The majority of potato cultivars were susceptible and moderately susceptible to foliar early blight.
- 2) The resistance levels of some cultivars to early blight changed according to the environmental conditions.
- 3) The resistant cultivars to early blight had mid-season, mid-late or late maturity. No cultivars susceptible to early blight were observed to have later maturity (mid-late or late maturity). In most cases, the cultivars found to be susceptible to early blight had earlier maturity (early or mid-early maturity).
- 4) The cultivars found to be resistant had a rough, mid-rough or smooth skin. Cultivars with rough skin and susceptible to early blight were not observed. In most cases, the cultivars with smooth skin that were found to be susceptible or moderately susceptible to early blight.

## **ARTICLE 4**

- 1) Based on AUDPC, late blight was more aggressive than early blight under all three environmental conditions, and this was reflected in yield.
- 2) Fungicide effects on late blight and early blight and associated yield losses varied among environments, suggesting the importance of using tools such as forecast systems to guide fungicide application decisions.
- 3) Late blight caused yield losses as high as of 82.4%, while early blight caused yield losses of up to 44.9%.