JÚLIA MARQUES OLIVEIRA

SPATIAL PROGRESS OF BACTERIAL BLIGHT IN COFFEE NURSERY AND ENVIRONMENTAL VARIABLES FOR "*in vitro*" CULTIVATION OF *Pseudomonas syringae* pv. *garcae*

LAVRAS – MG
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Prof. Dr. Edson Ampélio Pozza
Orientador

Prof. Dr. Leônidas Leoni Belan
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PROGRESSO ESPACIAL DA MANCHA AUREOLADA EM MUDAS DE CAFÉ E VARIÁVEIS AMBIENTAIS PARA CULTIVO 'in vitro' de *Pseudomonas syringae* pv. *garcae*

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitopatologia, para a obtenção do título de Doutor.

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2022

À minha avó Rubina exemplo de resiliência e honestidade.

Com muito amor,

DEDICO

À minha avó Levanir (in memorian) exemplo de fé, bondade, perseverança e amor.

Com muita saudade,

HOMENAGEIO

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“ *Como sou pouco e sei pouco, faço pouco que me cabe. Me dando por inteiro*. ” (Ariano Suassuna)

RESUMO

A mancha aureolada, cujo agente etiológico é a bactéria *Pseudomonas syringae* pv. *garcae* tem ocorrido em viveiros e lavouras de *Coffea arabica*, causando danos a cultura. Os objetivos com essa tese foram: comparar a eficiência de métodos de inoculação, com e sem ferimento, e sua interação com diferentes concentrações de bactéria *P. syringae* pv. *garcae* na intensidade da mancha aureolada do cafeeiro; verificar a interação de diferentes temperaturas e fotoperíodos na concentração de inóculo de *P. syringae* pv. *garcae* e na expressão dos sintomas da mancha aureolada em folhas de cafeeiro; e, caracterizar o progresso espaço-temporal da mancha aureolada (*P. syringae* pv. *garcae)* em mudas de cafeeiro (*C. arabica*) no viveiro a partir de inóculo inicial pré-definido. Os métodos de inoculação de *P. syringae* pv. *garcae* por ferimento no limbo foliar de cafeeiro proporcionaram maiores incidência e severidade da mancha aureolada. Dentre esses, os métodos de inoculação que proporcionaram maiores valores de incidência e severidade da doença foram via injeção de inóculo, ferimento com conjunto multiagulhas e jato-de-areia. Para esses métodos, as concentrações de inóculo $1{,}3x10^9$, $1{,}6x10^9$ e $8{,}5x10^8$ UFC mL^{-1}, respetivamente, proporcionaram máxima intensidade da doença. A maior concentração média de inóculo foi de $1{,}22x10^9$ UFC/ml obtida no fotoperíodo de 12 horas. Nessas condições à 23,3°C houve a maior produção de inóculo de $2{,}40x10^9$ e $2{,}11x10^9$ UFC/ml. Embora ocorreu diferença quanto ao fotoperíodo na produção de inóculo, apenas a temperatura influenciou na AACPS sendo maior a 23,7°C. A correlação positiva da temperatura com a concentração de inóculo e dessa última com a AACPS comprovou a importância das variáveis ambientais, principalmente a temperatura, na obtenção do maior número de células bacterianas para a expressão dos sintomas. Maiores dimensões das células bacterianas ocorreram quando a incubação foi realizada nas condições de fotoperíodo de luz contínua na temperatura de 28°C e escuro contínuo na temperatura de 26°C. No viveiro de mudas de cafeeiro, houve epidemia da mancha aureolada. O início da epidemia ocorreu aos 15 dias após a introdução do inóculo inicial, e duração da epidemia foi de oito semanas. Houve dependência espacial das mudas doentes em relação ao inóculo inicial. A partir dessas plantas infectadas, o patógeno foi disseminado a mais de 47 cm de raio da fonte de inóculo, em 37 dias, com dependência espacial. Essa distância aumentou com o incremento do número de plantas doentes no foco principal e/ou focos secundários.

Palavras-chave: Mancha aureolada. Inoculação. Concentração de inóculo. Geoestatística.

ABSTRACT

The Bacterial blight, whose etiologic agent is the bacterium *Pseudomonas syringae* pv. *garcae* has occurred in nurseries and crops of *Coffea arabica*, causing damage to the culture. The objectives of this thesis were to: compare the efficiency of inoculation methods, with and without injury, and their interaction with different concentrations of *P. syringae* pv. *garcae* on the intensity of the bacterial blight of coffee; to verify the interaction of different temperatures and photoperiods on the inoculum concentration of *P. syringae* pv. *garcae* and the expression of the symptoms of the Bacterial blight on coffee leaves; and, to characterize the spatio-temporal progress (*P. syringae* pv. *garcae*) in coffee seedlings (*C. arabica*) in the nursery using inoculum pre-set start. According to the results, the methods of inoculation by wounding the leaf of coffee plants to inoculate *P. syringae* pv. *garcae* provided higher incidence and severity of Bacterial blight. Among these, the inoculation methods with the highest incidence and severity of the disease are via inoculum injection, wound with a multi-needle set and sandblasting. For these methods, inoculum concentrations of $1.3x10^9$, $1.6x10^9$ and $8.5x10^8$ CFU/ml, respectively, provided maximum disease severity. The highest mean inoculum concentration was $1.22x10^9$ CFU/ml obtained in the 12-hour photoperiod. Under these conditions at 23.3°C there was the highest inoculum production of $2.40x10^9$ and $2.11x10^9$ CFU/ml. Although there was a difference regarding the photoperiod in the production of inoculum, only the temperature influenced the AACPS, being higher at 23.7°C. The positive correlation of temperature with inoculum concentration and the latter with AACPS proved the importance of environmental variables, especially temperature, in obtaining the highest number of bacterial cells for the expression of symptoms. Larger bacterial cell dimensions occurred when incubation was performed under photoperiod conditions of continuous light and temperature of 28°C or continuous dark and temperature of 26°C. In the coffee seedling nursery, there was an epidemic of the Bacterial blight. The start was 15 days after the introduction of the initial inoculum in the pond, lasting eight weeks. There was spatial dependence of the diseased seedlings in relation to the initial inoculum, that is, the space-time progress of the disease was related to the introduction of infected plants. From these infected plants, the pathogen was disseminated more than 40 cm from the inoculum source in less than 40 days, with spatial dependence. This distance increased with the increase in the number of diseased plants in the main focus and/or secondary focus.

Keywords: Bacterial blight. Inoculation. Inoculum concentration. Geostatistics.

Impactos sociais, tecnológicos, econômicos e culturais

A mancha aureolada, cujo agente etiológico é a bactéria *Pseudomonas syringae* pv. *garcae* (*Psg*) tem ocorrido em viveiros e lavouras de *Coffea arabica*, causando danos a cultura. A pesquisa apresenta impactos relevantes nos âmbitos econômico, tecnológico e ambiental. No âmbito econômico, Brasil é o maior produtor, exportador e o segundo maior consumidor mundial de café. A mancha aureolada tem ocorrido com frequência, causando perdas desde viveiros de mudas até lavouras das principais regiões cafeeiras do país. Embora a doença seja importante por causar danos e perdas significativas, são necessários estudos para elaborar e propor estratégias sustentáveis de manejo para esse patossistema. Para isso são necessárias pesquisas sobre a epidemiologia, a etiologia e o manejo da mancha aureolada. Para essas pesquisas, é necessário obter inóculo viável e inocular com eficiência o cafeeiro. Com essa tese obteve-se: identificação do melhor método de inoculação da bactéria *Psg* na intensidade da mancha aureolada do cafeeiro; verificação da interação de diferentes temperaturas e fotoperíodos na concentração de inóculo de *Psg* e na expressão dos sintomas da mancha aureolada em folhas de cafeeiro; e, caracterização do progresso espaço-temporal da mancha aureolada em mudas de cafeeiro (*C. arabica*) no viveiro a partir de inóculo inicial pré-definido. No âmbito tecnológico, foram obtidos três artigos científicos, dois deles já publicados. Diante desse cenário atual, o controle da doença é essencial, sendo empregados antibióticos, produtos cúpricos, bacteriostáticos, aplicados em pulverizações. Quando a doença está instalada na lavoura, o controle químico fica pouco eficiente e existem poucas cultivares resistentes. Para reduzir o uso dessas moléculas e aumentar a sustentabilidade ambiental e financeira do produtor, o princípio da exclusão pode ser utilizado. Baseado na redução do inóculo inicial, com o uso de mudas sadias e permitir somente a entrada de maquinários limpos e desinfestados. como de forma de evitar a disseminação do patógeno para áreas livres da doença.

Social, technological, economic and cultural impacts

The Bacterial blight, whose etiologic agent is the bacterium *Pseudomonas syringae* pv. *garcae* (*Psg*) has occurred in nurseries and crops of *Coffea arabica*, causing damage to the culture. The research presents relevant impacts in the economic, technological and environmental spheres. In economic terms, Brazil is the largest producer, exporter and second largest consumer of coffee in the world. Bacterial blight has occurred frequently, causing losses ranging from seedling nurseries to crops in the country's main coffee-growing regions. Although the disease is important because it causes significant damage and losses, studies are needed to develop and propose sustainable management strategies for this pathosystem. This requires research into the epidemiology, etiology, and treatment of Bacterial blight. For this research, it is necessary to obtain viable inoculum and efficiently inoculate the coffee plant. This thesis obtained: identification of the best method of inoculation of the *Psg* bacteria in the intensity of the Bacterial blight of the coffee tree; verification of the interaction of different temperatures and photoperiods on the inoculum concentration of *Psg* and the expression of the symptoms of the Bacterial blight on coffee leaves; and, characterization of the spatio-temporal progress (*P. syringae* pv. *garcae*) in coffee seedlings (*C. arabica*) in the nursery using inoculum pre-set start. In the technological area, three scientific articles were obtained, two of then already been published. Given this current scenario, disease control is essential, with antibiotics, copper products, and bacteriostatics being used in sprays. When the disease is present in the crop, chemical control is not very efficient and there are few resistant cultivars. To reduce the use of these molecules and increase the environmental and financial sustainability of the producer, the exclusion principle can be used. Based on the reduction of the initial inoculum, use of healthy seedlings and allowing only clean and disinfestate machinery to enter. As a way to prevent the spread of the pathogen to disease-free areas.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O café é uma das principais 'commodities' comercializadas no mundo. O Brasil é o maior produtor, exportador e o segundo maior consumidor mundial de café (USDA, 2021). Nos últimos quatro anos, foram produzidos, em média, mais de 41 milhões de sacas de 60 Kg/ano de café arábica no estado de Minas Gerais, o maior produtor de café arábica do país (CONAB, 2021). No entanto, vários fatores podem reduzir essa produtividade e comprometer tanto o fornecimento no mercado interno quanto as exportações. Dentre eles, citam-se as adversidades climáticas, as pragas e as doenças (Pozza et al., 2010). Entre esses, destaca-se a bactéria *Pseudomonas syringae* pv. *garcae* (Young, Dye & Wilkie 1978) (Psg), causadora da mancha aureolada. Essa bactéria tem ocorrido com frequência, causando perdas desde viveiros de mudas até lavouras das principais regiões cafeeiras do país (Belan et al., 2014).

A disseminação do patógeno é rápida, a doença causa desfolha, seca de ramos e é de difícil controle, causando perdas significativas desde o viveiro até o campo, principalmente em regiões com temperaturas entre 18°C a 20°C, expostas ao vento sul e com duração do molhamento foliar superior a 10 horas. Ou então em lavouras submetidas a colheita mecanizada, mesmo em condições adversas de ambiente para a bactéria (POZZA, 2021). Embora a doença seja importante por causar danos e perdas significativas, são necessários estudos para elaborar e propor estratégias sustentáveis de manejo para esse patossistema. Para isso são necessárias pesquisas sobre a epidemiologia, a etiologia e o manejo da mancha aureolada. Para essas pesquisas, é necessário obter inóculo viável e inocular com eficiência o cafeeiro.

Na literatura foram descritos vários métodos de inoculação artificial de bactérias fitopatogênicas em plantas. Para Psg foram descritos métodos sem e com ferimentos. Oliveira e Romeiro (1990), Ithuri et al. (2013) e Rodrigues et al. (2017) utilizaram como um dos métodos de inoculação sem ferimento a pulverização da suspensão bacteriana nas concentrações $1{,}1x10^9$ UFC/ml, 10^8 UFC/ml e $3x10^8$ UFC/ml respectivamente. Já, entre os métodos de inoculação com ferimento, a injeção da suspensão bacteriana de Psg com seringa e agulha é um dos mais utilizados. Ithuri et al. (1970), Oliveira e Romeiro, (1990), Ithuri et al. (2013) e Rodrigues et al. (2017) utilizaram esse método nas concentrações de 10^9UFC/ml, $1{,}1x10^9$ UFC/ml, 10^8 UFC/ml e $3x10^8$ UFC/ml respectivamente. Além do método de injeção da suspensão bacteriana, outros

métodos com ferimento estão descritos, entre eles, o de abrasão com carborundum (Costa et al. 1957; Robbs 1979), corte com lâmina esterilizada com posterior pulverização da suspensão bacteriana (Ithiru et al. 2013), e abrasão com lixa e ferimento com multiagulhas (Rodrigues et al. 2017). Porém, ainda não se chegou a um consenso sobre o método mais eficiente para inocular *P. syringae* pv. *garcae* em plantas de cafeeiro, e reprodução dos sintomas da doença. Além disso, também não foi determinada qual concentração de inóculo proporciona maior intensidade da doença em determinado método de inoculação, com ou sem possibilidade de realizar ferimentos nas plantas, de forma a criar porta de entrada para a bactéria.

No campo, a temperatura e a luminosidade oscilam diuturnamente, mas quando constantes, em condições artificiais, permitem avaliar o efeito na biologia do patógeno (ANDRADE et al. 2007). Conhecer a influência dos fatores do ambiente *in vitro* e a infectividade do patógeno são fundamentais para produzir inóculo e determinar as condições ideais de inoculações artificiais (MUELLER; BUCK, 2003). Assim, como a ocorrência da doença depende de fatores ambientais, a morfologia e a fisiologia do patógeno também são influenciadas por essas condições. Tal fato pode alterar a capacidade infectiva do patógeno e consequentemente a intensidade da doença (AGRIOS, 2005). Logo, a temperatura e o fotoperíodo durante o período de incubação podem alterar as características morfológicas e, possivelmente, a infectividade de Psg, além da produção do seu inóculo.

A doença ocorre principalmente em tecidos novos de mudas em viveiros e lavouras recém-implantadas, causando sintomas em folhas, ramos e frutos, podendo causar também seca de ramos, reduzindo a produtividade da cultura nas regiões produtoras de café do Brasil como Minas Gerais, São Paulo e Paraná (Godoy et al. 1997; Pozza et al, 2010; Pozza, 2021; Rodrigues et al., 2013; Belan et al. 2014; Marin et al. 2018; Fernandes et al. 2020). Porém, com o advento da colheita mecanizada, esses sintomas têm se intensificado em ramos já lignificados, devido aos ferimentos causados por esse maquinário, introduzindo o patógeno dentro dos tecidos tanto de ramos plagiotrópicos quanto ortotrópicos. Diante desse cenário atual, o controle da doença é essencial, sendo empregados antibióticos e produtos cúpricos, bacteriostáticos, em pulverizações. Quando está instalada na lavoura, aliada simultaneamente a outras espécies ou patovares, como por exemplo a Mancha Bacteriana (*Pseudomonas syringae* pv. *tabaci*), o controle químico fica pouco eficiente e há poucas cultivares resistentes (Marin et al. 2018; Fernandes et al. 2020; Pozza, 2021). Para reduzir o emprego dessas moléculas e aumentar a sustentabilidade ambiental e financeira do produtor, pode-se empregar o princípio da exclusão, baseado na redução do inóculo inicial, utilizando-se mudas sadias e

permitir somente a entrada de maquinários limpos e desinfestados na lavoura (Belan et al., 2016), de forma a evitar a disseminação do patógeno para áreas livres da doença.

Ambiente favorável para epidemias de mancha aureolada ocorre frequentemente nos viveiros em função do adensamento de plantas, irrigação constante e atrito entre folhas de plantas adjacentes. Assim, em viveiros, é comum observar reboleiras de mudas infectadas (Rodrigues et al., 2013, Belan et al., 2014), formando gradiente de doença a partir de um foco central, capazes de aumentar ao longo do tempo (Belan et al. 2018). Essa variação na quantidade da mancha aureolada, a partir de um foco ou fonte de inóculo, pode ser estudada com as técnicas de geoestatística, uma ferramenta usada para analisar os aspectos epidemiológicos das doenças de plantas e modelar o progresso espacial (Alves et al. 2010; Alves et al. 2009; Alves et al. 2006; Leal et al. 2010; Mouen Bedimo et al. 2007; Roham et al. 2014; Belan et al. 2018). Belan et al. (2018) utilizaram a geoestatística para modelar o progresso espacial da mancha aureolada em um viveiro comercial de mudas de café, com aplicação de agroquímicos, porém avaliando focos iniciais aleatórios, de ocorrência natural.

Diante do exposto, os objetivos dessa tese foram: comparar a eficiência de métodos de inoculação, com e sem ferimento, e sua interação com diferentes concentrações de *P. syringae* pv. *garcae* na intensidade da mancha aureolada do cafeeiro; verificar a influência da interação entre temperatura e fotoperíodo durante período de incubação na produção de inóculo de *Psg,* tamanho das células e eficiência desse inóculo na expressão dos sintomas da mancha aureolada em folhas de cafeeiro; caracterizar o progresso espaço-temporal da mancha aureolada (*P. syringae* pv. *garcae)* em mudas de cafeeiro (*C. arabica*) no viveiro a partir de inóculo inicial pré-definido.

2 REFERENCIAL TEÓRICO

2.1 A mancha aureolada do cafeeiro

A mancha aureolada foi identificada a primeira vez no Brasil no município de Garça, estado de São Paulo. Em seguida, foi relatada sua ocorrência também nos estados de Minas Gerais e Paraná (Kimura et al., 1976; Mohan et al., 1976). Há relatos de ocorrência também em outros países, como na República do Quênia (Ramos; Shavdia, 1976), Etiópia (Korobko; Wondimagegne, 1997), Uganda e China (Chen, 2002). Nos anos seguintes à sua constatação no Brasil, a doença não foi considerada de importância econômica (Costa e Silva, 1960). Cerca de 17 anos depois do seu aparecimento, apenas casos isolados foram identificados (Mohan, 1976). Na região do Alto Paranaíba, Minas Gerais, os primeiros relatos datam da década de 1980 no município de São Gotardo, provocando seca de ramos (Vale; Zambolim, 1997). No entanto, a partir de 2010, essa doença passou a ser considerada de grande importância na economia cafeeira, por se tornar fator limitante para a produção de mudas e cultivo do café em algumas regiões. Ela se manifestou em viveiros de café nos estados do Paraná, São Paulo e especialmente no Sul de Minas Gerais, Triângulo Mineiro e Alto do Paranaíba (Sera et al., 2004; Zoccoli et al., 2011; Belan, 2014; Raimundi, 2014; Pozza, 2021).

A bactéria *Pseudomonas syringae* pv. *garcae* (Young, Dye & Wilkie 1978), agente etiológico da mancha aureolada (Amaral et al., 1956), é Gram negativa, suas células apresentam formato de bastonetes retos ou levemente curvados e podem ter um ou vários flagelos polares. É pertencente ao filo Proteobacteria, classe Gamma Proteobacteria, ordem Pseudomonadales, família Pseudomonadaceae, gênero *Pseudomonas*, espécie *P. syringae*, patovar *garcae* (Rodrigues et al. 2013). Produz pouca quantidade de pigmento fluorescente em meio de cultura King B (KB) (King et al., 1954) e, em meio de cultura, como Batata Dextrose Agar (BDA) e Nutriente Agar (NA), produzem pigmento marrom, denominado melanina, como citado por Barta e Willis (2005). Os isolados brasileiros produzem em meio King B pouco pigmento amarelo fluorescente, mas sim um pigmento marrom não fluorescente difusível neste meio, causando o escurecimento do mesmo (Kairu, 1997; Barta e Willis, 2005). Este pigmento fluorescente é produzido principalmente por isolados originados do Quênia (Kairu, 1997; Barta e Willis, 2005). A maioria dos isolados de *P. syringae* pv. *garcae* produzem tabtoxina, porém, alguns podem não a produzir (Barta; Willis, 2005).)

A bactéria infecta principalmente tecidos novos, como mudas em viveiros e plantas novas no campo, incidindo sobre folhas, ramos e frutos (Costa e Silva, 1960; Godoy et al. 1997;

Zambolim et al., 2005; Pozza et al, 2010; Rodrigues et al., 2013; Pozza, 2021). Os sintomas são lesões irregulares, com aparência encharcada nas bordas, de coloração marrom-escura a negra, e em seguida aumentam de tamanho e podem ou não desenvolver halos amarelos ao redor das lesões (Figura 3). Essas podem coalescer, formando grandes áreas necrosadas e ocasionar deformação e/ou rompimento do limbo foliar. Em folhas novas, o halo amarelo nas lesões pode não ser notado. As lesões ocorrem com maior frequência nas bordas das folhas, onde a bactéria pode penetrar por ferimentos causados devido a danos mecânicos ou aberturas naturais (Belan et al. 2014, Pozza et al. 2010, Rodrigues et al., 2013).

2.2 Epidemiologia da mancha aureolada

A temperatura e o fotoperíodo, bem como outras variáveis ambientais, influenciam tanto o patógeno quanto o hospedeiro, favorecendo ou não a ocorrência e o progresso da doença (Silva, 2014). Os fatores do ambiente podem determinar a distribuição geográfica, a incidência e a severidade da doença, sendo em muitos casos específicos para o patógeno em questão. Dentre esses fatores, a temperatura é a mais frequentemente correlacionada com a epidemiologia da doença, em seguida a umidade e a luz (Colhoun, 1973). Apesar da importância das doenças causadas por bactérias, há pouca informação disponível na literatura referentes às amplitudes térmicas e aos regimes de luz favoráveis ao seu desenvolvimento. Sob condições naturais, a temperatura e a luminosidade oscilam diuturnamente, mas quando constantes, sob condições artificiais, permitem avaliar o efeito sobre a biologia do patógeno (Andrade et al. 2007). A determinação da influência dos fatores do ambiente no cultivo *in vitro* e a infecção de plantas é fundamental para embasar inoculações artificiais (Mueller; Buck, 2003).

A temperatura é essencial para multiplicar a bactéria e regular a velocidade de reações de diversas enzimas de *Pseudomonas syringae* (Hockett; Burch; Lindow, 2013), contudo, há variações da atividade de cada gene ou enzima dentro dessa mesma espécie. Para *P. syringae* os genes e enzimas controladores da síntese de fitotoxinas e da motilidade do flagelo são reprimidos a 30°C, enquanto as enzimas do metabolismo, do transporte de substâncias e da tradução de proteínas são estimuladas na mesma temperatura (Hockett; Burch; Lindow, 2013). De fato, a multiplicação da *Psg in vitro* foi realizada a 28°C em diversos trabalhos (Ithiru et al., 2013; Pérez, 2015; Rodrigues et al., 2017; Zoccoli; Takatsu; Uesugi, 2011) porém, não se sabe ao certo qual temperatura proporciona maior crescimento *in vitro* e qual o intervalo possível para multiplicar essa bactéria.

Freitas (2017) relatou no ensaio *in vitro* o maior crescimento populacional de *Psg* no intervalo entre 23 e 31°C, com pico em 28,1°C. Foi relatado no ensaio *in vivo* a inoculação por ferimento proporcionando maior Área abaixo da curva de progresso da severidade (AACPS) da mancha aureolada em relação à inoculação sem ferimento. Sendo a máxima AACPS em temperaturas de 20,9 e 19,3°C, quando inoculadas com e sem ferimento respectivamente.

Em relação à doença, ainda são discutidas as condições climáticas e ambientais favoráveis ao seu progresso (Sera et al., 2004). Cardoso e Mohan (1980) relataram no estado do Paraná maior intensidade da doença quando a temperatura variou entre 13,1 e 20,5ºC, umidade relativa de 57 a 73% e precipitação de 111,3 mm mensais. No entanto, Zoccoli et al. (2011) realizaram estudos na região do Triângulo Mineiro e no Alto Paranaíba e demonstraram ser as análises climatológicas mensais e anuais e precipitações pluviais insuficientes para diferenciar as variáveis do clima e áreas favoráveis ou desfavoráveis para a ocorrência, persistência e severidade da doença. De acordo com esses autores, a determinação dessas variáveis é essencial para determinar a forma de manejo adequada da mancha aureolada, sobretudo em relação à faixa de temperatura na qual as plantas são mais suscetíveis, do tempo de duração do molhamento foliar favorável à infecção e também da velocidade e da duração do vento.

O vento constante nas lavouras situadas em altitudes elevadas, a chuva e as práticas culturais colaboram para a disseminação da bactéria dentro da planta e de planta para planta (Godoy et al, 1997; Pozza et al, 2010; Carvalho e Chalfoun 1998). As ocorrências de chuvas de granizo podem favorecer a disseminação da bactéria, além do molhamento foliar e da elevação da umidade, os quais podem causar ferimentos ou portas de entrada para o patógeno. Conforme relatado por Rodrigues et al. (2013), a incidência da doença é reduzida nos cafezais em períodos com menor ocorrência de chuvas. No entanto, ainda não havia sido estudada a influência da interação da temperatura e do fotoperíodo no desenvolvimento *in vitro* da bactéria, bem como na severidade da mancha aureolada, para assim prever as condições favoráveis à doença no campo.

2.3 Inoculação artificial de *Pseudomonas syringae* pv. *garcae*

Estudos de etiologia, epidemiologia e controle, entre outros, requerem métodos de inoculação eficientes, práticos, de baixo custo e reproduzíveis em casa de vegetação e campo (Henz et al., 1988). Na literatura foram descritos vários métodos de inoculação de bactérias fitopatogênicas em plantas, como atomização com pressão, infiltração, inoculação com o uso

de abrasivos, aspersão com ou sem ferimentos, enxertia, infestação do solo, imersão de sementes, infiltração a vácuo em sementes, técnica do palito, micropipeta e almofada de alfinetes (Henz et al., 1988; Mariano et al., 2000; Romeiro et al., 2001; Araújo et al., 2005; Kauffman et al. 1973; Klement, 1963).

Os métodos para *Psg* foram descritos sem e com ferimentos. Oliveira e Romeiro (1990), Ithuri et al. (2013) e Rodrigues et al. (2017) utilizaram como um dos métodos de inoculação sem ferimento a pulverização da suspensão bacteriana nas concentrações $1{,}1x10^9$ UFC/ml, 10^8 UFC/ml e $3x10^8$ UFC/ml, respectivamente. Já, entre os métodos de inoculação com ferimento, a injeção da suspensão bacteriana de *Psg* com seringa e agulha é um dos mais ultilizados. Ithuri et al. (1970), Oliveira e Romeiro, (1990), Ithuri et al. (2013) e Rodrigues et al. (2017) utilizaram esse método nas concentrações de 10^9UFC/ml, $1{,}1x10^9$ UFC/ml, 10^8 UFC/ml e $3x10^8$ UFC/ml respectivamente. Além do método de injeção da suspensão bacteriana, outros métodos com ferimento já foram descritos, entre eles, o de abrasão com carborundum (Costa et al. 1957; Robbs 1979), corte com lâmina esterilizada com posterior pulverização da suspensão bacteriana (Ithiru et al. 2013), abrasão com lixa e ferimento com multiagulhas (Rodrigues et al. 2017).

Como pode ser observado, ainda não se chegou a um consenso sobre o método mais eficiente para inocular *P. syringae* pv. *garcae* em plantas de cafeeiro, sendo relatados vários métodos com e sem ferimento. Além disso, também não foi determinada qual concentração de inóculo proporciona maior intensidade da doença em determinado método de inoculação, com ou sem possibilidade de realizar ferimentos nas plantas, de forma a criar porta de entrada para a bactéria.

2.4 Distribuição espaço temporal de doenças de plantas

Para entender o progresso de epidemias é fundamental conhecer a origem e a quantidade do inóculo inicial, as formas de disseminação do patógeno e a influência das variáveis climáticas e ambientais. Esses fatores podem influenciar na dispersão de patógenos e na infecção, assim como as práticas de manejo da doença. Também é necessário conhecer a distribuição espacial das plantas doentes (Campbell e Madden 1990). Para elaborar protocolos de amostragem de uma doença, é essencial conhecer o padrão de distribuição espacial da doença (Jeger 1990). De tal modo, Madden et al. (2007) descrevem três classificações para o padrão espacial de plantas doentes: o aleatório, o agregado e o regular. De acordo com esses autores, há relação entre o padrão espacial agregado e as oportunidades de infecção, e isso ocorre quando, em condições naturais, o patógeno é disperso a curtas distâncias na interação planta a

planta, como as bacterioses em viveiro de mudas (Romeiro 2005), devido ao adensamento de plantas. Assim, a probabilidade de uma planta localizada mais próxima à fonte de inóculo ser infectada é maior em relação às mais distantes (Bergamin Filho et al. 2002; Gottwald et al. 1989; Gottwald 2010), ou seja, pode haver dependência espacial entre os indivíduos doentes, variando de acordo com as características do hospedeiro (suscetibilidade), do patógeno e do ambiente.

Gottwald et al. (1989), Gottwald et al. (1989), Gottwald et al. (1990) e Carmo et al. (1996) estudaram a distribuição espacial de bactérias em viveiros. Gottwald et al. (1989), ao analisarem a distribuição espacial da mancha bacteriana em citros (*Citrus Bacterial Spot* - CBS) (*Xanthomonas campestris* pv. *citrumelo*) em viveiros de citros na Flórida, observaram padrão espacial não agregado ou ao acaso, porém foi identificado um epicentro potencial da epidemia por mapeamento de contorno isopático identificando a direção leste como predominante no progresso da doença. Além disso, antes do surto da doença ocorreram chuvas com vento. Segundo os autores, a disseminação do CBS está diretamente associada com essas chuvas. Entretanto, o mesmo autor, analisando outros quatro viveiros na Flórida, detectou padrão espacial agregado na dispersão do CBS (Gottwald et al., 1990). Esses autores também constataram várias formas de introdução do patógeno, pois foram detectados dois focos distintos da doença. Em viveiros de Laranja doce (*Citrus sinensis* (L.) Osb.), Toranja Duncan (*Citrus paradisi* Macfad.), e Citrumelo Swingle (*Poncirus trifoliata* (L.) Raf. X *C. paradisi*) na Argentina, Gottwald et al. (1989) analisaram o progresso do Cancro Cítrico (CBC) causado por *Xanthomonas campestris* pv. *citri.* Nos três viveiros as proporções de plantas doentes mantiveram baixas e regulares por um longo tempo (média de 350 dias), porém, após ocorrerem tempestades com ventos fortes, a taxa de progresso da doença aumentou. Também ocorreu distribuição espacial agregada e focos secundários foram identificados em datas posteriores de avaliação da doença nos viveiros. Entretanto, em todos esses artigos, embora os autores tenham avaliado o progresso da doença, não foi avaliado quantitativamente o efeito das covariáveis ambientais e seu efeito no progresso espaço-temporal da doença.

Em outro estudo, Carmo et al. (1996) inocularam a bactéria *Xanthomonas campestris* pv. *vesicatoria* em plântulas de pimentão (*Capsicum annuum* L.) com cerca de 15 dias após a emergência, sendo inoculado uma planta por bandeja. Carmo et al. (1996) constataram o aumento da incidência e da severidade da pústula bacteriana do pimentão variando em cada ensaio de acordo com as variáveis climáticas. O intervalo de 19°C a 35°C, umidade relativa de 70,7 a 84,1% e elevada precipitação (195,6 mm), em geral acompanhada por ventos fortes no terceiro ensaio, foram as condições mais favoráveis para o desenvolvimento da doença. Nesse

estudo os autores afirmaram a importância da chuva como um fator determinante no progresso da epidemia (CARMO et al., 1996).

Em outro estudo, Carmo et al. (2001) constataram a resposta rápida das bactérias em relação às variações climáticas, principalmente a temperatura. Os autores também verificaram o aumento linear da intensidade da mancha bacteriana do pimentão (*Xanthomonas campestris* pv. *vesicatoria*) com o incremento da quantidade de inóculo inicial.

Em patossistemas de etiologia fúngica também foi reportada a relação entre a epidemia de doenças e a aleatoriedade do foco inicial. Scott et al. (2014) verificaram a associação da presença e quantidade de inóculo inicial do patógeno *Sclerotinia sclerotiorum* e *S. Menor* na cultura de piretro (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.) favorecendo a epidemia.

Em relação as variáveis ambientais, Freitas et al. (2016) constataram correlação negativa significativa entre a ocorrência da doença Sigatoka Amarela em bananeiras e a precipitação durante as avaliações. A alta taxa de produção foliar foi influenciada por essa elevada precipitação, compensando as perdas causadas pela doença não ocorrida nos meses de menor precipitações. Ou seja, as características do ambiente têm relação com a dependência espacial.

Sendo assim, há a necessidade de ferramentas capazes de medir a influência de covariáveis como as variáveis climáticas no progresso espaço-temporal da doença, devido à natureza incerta, ambígua, não-linear em muitos dos sistemas agrícolas (Pozza et al. 2012). Segundo Alves et al. (2006), utilizando a ferramenta geoestatística há a possibilidade de identificar a magnitude e o grau de dependência espacial, mapear a variabilidade espacial das epidemias, além de observar o progresso ao longo do tempo ou sua distribuição temporal. A geoestatística permite modelar o progresso espacial e assim possibilita estudar hipóteses sobre aspectos epidemiológicos de doenças de plantas (Alves et al. 2009; Alves e Pozza 2010; Alves et al. 2006; Jaime-Garcia et al. 2001; Leal et al. 2010; Mouen Bedimo et al. 2007; Nelson et al. 1999; Ortiz et al. 2010; Roham et al. 2014; Wallace and Hawkins 1994; Zucoloto et al. 2009).

Assim, Mouen Bedimo et al. (2007) utilizaram semivariogramas e mapas de dispersão obtidos por krigagem para avaliar focos de infecção primária de *Coffee berry disease* (*Colletotrichum kahawae* Waller & Bridge) em Camarões. Os autores analisaram a distribuição espacial ao longo do tempo e verificaram plantas contaminadas por etapas a partir do primeiro cafeeiro infectado. Também para a cultura do cafeeiro (*Coffea arabica L.*), Alves et al. (2009) verificaram padrão agregado para a distribuição da ferrugem (*Hemileia vastatrix* Berkeley & Broome) e da cercosporiose (*Cercospora coffeicola* Berk. & Cooke), ou seja, na forma de focos ao longo da lavoura. Dessa forma, a atual estratégia de controle da doença aplicada em área total pode ser substituída por aplicação de fungicidas específicos em locais de ocorrência com

maior intensidade da doença. Para outras espécies de hospedeiros, Blank et al. (2016), utilizando a geoestatística, verificaram que a severidade do cancro bacteriano (*Clavibacter michiganensis subsp. michiganensis*) em tomate (*S. lycopersicum*) tem autocorrelação espacial de padrão agrupado. Além disso, a análise geoestatística também foi eficiente no trabalho de Roumagnac et al. (2004), no qual o modelo exponencial esférico melhor descreveu os semivariogramas da distribuição espaço-temporal da doença *Bacterial blight of onion* (BBO) em cebola (*Allium spp.*), cujo agente etiológico é *Xanthomonas axonopodis pv. allii*. Ainda nesse mesmo trabalho, a origem do inóculo inicial e, consequentemente, os estágios iniciais da epidemia foram associados às sementes possivelmente infectadas com a doença, e também houve focos secundários após chuvas acompanhadas de ventos fortes. Alves e Pozza (2010), por exemplo, verificaram, via análise geoestatística, a formação de gradiente de intensidade da antracnose do feijoeiro-comum (*Colletotrichum lindemuthianum* Sacc. & Magnus, Briosi & Cavara) em torno da fonte de inóculo primário, com padrão agregado de distribuição e dependência espacial com a fonte de inóculo.

REFERÊNCIAS

AGRIOS, G.N. **Plant pathology.** 5. ed. Londres: Elsevier Academic Press, 2005.

ALMEIDA, I.M.G.; RODRIGUES, L.M.R.; PATRÍCIO, F.R.A. Crestamentobacteriano das folhas causado por *Pseudomonas cichorii* em café no estadode São Paulo **Summa Phytopathologica**, Botucatu, v.38, supl., 2012. 1CD-ROM.

ALVES MC, DA SILVA FM, POZZA EA, DE OLIVEIRA MS (2009) Modeling spatial variability and pattern of rust and brown eye spot in coffee agroecosystem. **Journal of pest science** 82:137-148.

ALVES MC, POZZA EA, MACHADO JC, ARAÚJO DV, TALAMINI V, OLIVEIRA MS (2006) Geoestatística como metodologia para estudar a dinâmica espaço-temporal de doenças associadas a *Colletotrichum spp.* transmitidos por sementes. **Fitopatologia Brasileira** 31:557-563.

AMARAL, J.F.; TEIXEIRA, C.; PINHEIRO, E.D. A bacterium causing halo blight of coffee. **Arquivos do Instituto Biológico**, v.23, p.151-155, 1956.

ANDRADE, G.C.G. et al. Características culturais e severidade da mancha foliar de *Quambalaria eucalypti* sob diferentes regimes de temperatura, luz e período de molhamento foliar. **Fitopatologia Brasileira** 32:329-334. 2007.

ARAÚJO, D.V.; MARIANO, R.L.R.; MICHEREFF, S.J. Inoculation methods of *Acidovorax avenae subsp. citrulli* in melon. **Summa Phytopathologica**, v.31, p.69-73, 2005.

BARTA, T.M.; WILLIS, D.K. Biological and molecular evidence that *Pseudomonas syringae* patovars *coronafaciens*, *striafaciens* and *garcae* are likely the same pathovar. **Journal of Phytopathology**, v.153, p.492- 499, 2005.

BELAN, L.L.; POZZA, E.A.; FREITAS, M.L.O.; SOUZA, R.M.; JESUS JUNIOR, W.C.; OLIVEIRA, J.M. Diagrammatic Scale for Assessment of Bacterial Blight in Coffee Leaves. **Journal of Phytopathology** (1986), v.162, p.801-810, 2014.

BELAN, L.L.; POZZA, E.A.; SOUZA, R.M.; RAIMUNDI, M.K.; SILVA, J.L.; FREITAS, M.L.O.; SOUZA, P.E. Influência da luz e período de molhamento foliar em diferentes métodos de inoculação artificial de *Pseudomonas syringae* pv. *garcae*. In: 46º Congresso Brasileiro de Fitopatologia, 2013, Ouro Preto - MG. CBFito Sustentável - **Anais do 46º Congresso Brasileiro de Fitopatologia**, 2013. p.255-255.

BELAN, L.L.; POZZA, E. A.; SOUZA, R.M. A Mancha Aureolada do Cafeeiro. **Revisão Anual de Patologia de Plantas**, v.22, p.227-256, 2014

BERGAMIN FILHO A, HAU B, AMORIM L, LARANJEIRA FF (2002**) Análise espacial de epidemias**. Rev A Pat Plantas 10:155-218

BLANK, L., COHEN, Y., BORENSTEIN, M., SHULHANI, R., LOFTHOUSE, M., SOFER, M., AND SHTIENBERG, D. 2016. **Variables associated with severity of bacterial canker and wilt caused by *Clavibacter michiganensis subsp. michiganensis* in tomato greenhouses**. Phytopathology 106:254-261.

CAMPBELL CL, MADDEN LV (1990) Introduction to plant disease epidemiology.

CARDOSO, R.L; MOHAN, S.K. Ocorrência e intensidade do crestamento bacteriano causado por *Pseudomonas garcae* em relação a alguns fatores climáticos, no Estado do Paraná. In: CONGRESSO BRASILEIRO DE PESQUISAS CAFEEIRAS 7, 1979, Araxá, MG. **Resumos**. Rio de Janeiro: IBC/GERCA. p. 65-66, 1980.

CARMO MGF, KIMURA O, MAFFIA LA, CARVALHO AOC (1996) **Progresso da pústula bacteriana do pimentão, causada por** *Xanthomonas campestris pv. vesicatoria* **em condições de viveiro**. Fitopat Bras 20:66-70

CARMO MGF, MACAGNAN D, CARVALHO AO (2001) Progresso da mancha-bacteriana do pimentão a partir de diferentes níveis iniciais de inóculo e do emprego ou não do controle com oxicloreto de cobre. Hortic Bras 19:210-215

CARVALHO, V.L.; CHALFOUN, S.M. Manejo integrado das principais doenças do cafeeiro. **Informe Agropecuário**, v.19, p.27-35, 1998.

CHEN, Z. Morphocultural and pathogenic comparisons between *Colletotrichum kahawae* and *C. gloeosporioides* isolated from coffee berries. **PhD Thesis**. Universidade Técnica de Lisboa. Lisboa. 2002.

COLHOUN, J. Effects of environmental factors on plant disease. **Annual Review of Phytopathology** 11:343-364. 1973.

CONAB – Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira Café Safra 2021. Disponível em: http://www.conab.gov.br/. Acessado em 28/09/21.

COSTA, A.S.; AMARAL, J.F.; VIEGAS, A.P.; SILVA, D.M.; TEIXEIRA, C.G.; PINHEIRO, E.D. Bacterial halo blight of coffee in Brazil. **Phytopathologische Zeitschrift**, v.28, p.427-444, 1957.

COSTA, A.S.; SILVA, D.M.A Mancha aureolada do cafeeiro. **Bragantia**, v.19, p.LXII-LXVIII, 1960.

DESTÉFANO, S.A.L.; RODRIGUES, L.M.R.; BERIAM, L.O.S.; PATRÍCIO, F.R.A.; THOMAZIELLO, R.A.; RODRIGUES NETO, J. Bacterial leaf spot caused by *Pseudomonas syringae* pv. *tabaci* in Brazil. **New Disease Reports**, v.22, n.5, 2010.

FREITAS, A. S.; POZZA, E. A. ; ALVES, M. C. ; COELHO, G. ; ROCHA, H. S. ; POZZA, A. A. A. . Spatial distribution of Yellow Sigatoka Leaf Spot correlated with soil fertility and plant nutrition. PRECISION AGRICULTURE JCR, p. 93-107, rint. 2016

FREITAS, M.L.O. Epidemiologia e controle da mancha aureolada do cafeeiro. **Tese de Doutorado.** 2017.

GODOY, C.V.; BERGAMIN FILHO, A.; SALGADO C.L. Doenças do cafeeiro (Coffea arabica L.). In: Kimati H, Amorim L, Bergamin-Filho, A, Camargo LEA and Rezende JAM (eds) **Manual de Fitopatologia**. 3rd edition, 2nd volume. Agronômica Ceres, São Paulo, Brazil, p.184-200, 1997.

GOTTWALD T, TIMMER L, MCGUIRE R (1989) Analysis of disease progress of Citrus canker in nurseries in Argentina. **Phytopathology** 79:1276-1283.

GOTTWALD T. R. and GRAHAM J. H. Spatial pattern analysis of epidemics of citrus bacterial spot in Florida citrus nurseries. Phytophathology 80:181-190. 1990.

GOTTWALD T. R., MILLER C., BRLANSKY R. H., GABRIEL D. W., and CIVEROLO E. L. Analysis of spatial distribution of citrus bacterial spot in a Florida citrus nursery. Plant Disease 73:207-303. 1989.

GOTTWALD TR (2010) Current Epidemiological Understanding of Citrus Huanglongbing*. **Annual review of phytopathology** 48:119-139.

HENZ, G.P.; TAKATSU, A.; REIFSCHNEIDER, F.J.B. Avaliação de métodos de inoculação de *Xanthomonas campestris* pv. *campestres* para detecção de fontes de resistência em brássicas. **Fitopatologia Brasileira**, Brasília, v.13, n.3, p.207-210, 1988.

HOCKETT, K. L.; BURCH, A. Y.; LINDOW, S. E. Thermo-regulation of genes mediating motility and plant interactions in *Pseudomonas syringae*. **PLoS One**, San Francisco, v. 8, n. 3, p. e59850, 2013.

ITHURI, J.M.; GICHURU, E.K.; GITONGA, P.N.; CHESEREK, J.J.; GICHIMU, B.M. Methods for early evaluation for resistance to bacterial blight of coffee. **African Journal of Agricultural Research**. v.8, n.21, p.2450-2454, 2013.

JAIME-GARCIA R, ORUM T, FELIX-GASTELUM R, TRINIDAD-CORREA R, VANETTEN H, NELSON M (2001) Spatial analysis of *Phytophthora infestans* genotypes and late blight severity on tomato and potato in the Del Fuerte Valley using geostatistics and geographic information systems. **Phytopathology** 91:1156-1165.

JEGER M (1990) Mathematical analysis and modeling of spatial aspects of plant disease epidemics. **Epidemics of Plant Diseases.** Springer, pp 53-95.

JESUS JUNIOR, W.C.J.; ZAMBOLIM, L.; SILVA, H.S.; VALE, F.X.R.; SILVA, C.L. Controle químico da mancha aureolada do cafeeiro. **Fitopatologia Brasileira**, v.27, p.61, 2002. (Suplemento)

KADO, C.I.; HESKETT, M.G. Selective media for isolation of *Agrobacterium, Corynebacterium Erwinia, Pseudomonas* and *Xanthomonas*. **Phytopathology**, v.60, p.969-976, 1970.

KAIRU, G.M. Biochemical and pathogenic differences between Kenyan and Brazilian isolates of *Pseudomonas syringae* pv *garcae*. **Plant Pathology**, v.46, p.239-246, 1997.

KAUFFMAN, H.E.; REDDY, A.P.K.; HSIEH, S.P.Y.; MERCA, S.D. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. **Plant Disease Reporter**, v.57, n.6, p.537-541, 1973.

KIMURA, O.; ROBBS, C.F.; FERRARI, J.A.R. Algumas observações relacionadas com as bacterioses do cafeeiro. In: CONGRESSO BRASILEIRO DE PESQUISAS CAFEEIRAS, 4, 1976, Caxambu. **Resumos**. Rio de Janeiro: IBC/GERCA, 1976, p.104.

KIMURA, O.; ROBBS, C.F.; RIBEIRO, R.L.D. Estudos sobre o agente da "Mancha Aureolada do cafeeiro" (Pseudomonas garcae Amaral et al.) **Arquivos da Universidade Federal Rural**, v.3, n.2, p.15-18, 1973.

KING, E.O.; WARD, M.K.; RANEY, D.E. Two simple media for the demonstration of pyocianin and fluorescin. **Journal of Laboratory and Clinical Medicine**, v.44, n.1, p.301-307, 1954.

KLEMENT, Z. Rapid detection of the pathogenicity of phytopathogenic pseudomonas. **Nature**, v.199, p.299-300, 1963.

KOROBKO, A. WONDINAGEGNE, E. Bacterial blight of coffee (*Psedomonas syringae* pv. *garcae*) in Ethiopia. In Rudoldh, K.; Burr, T.J.;Mansfield, J.W.; Stead, D.; Vivian, A.; Von Kietzele, J. **Pseudomonas syringae and related pathogens**. Netherlands Springer, 1997. p.538-541.

LEAL RM, BARBOSA JC, COSTA MG, JUNIOR JB, YAMAMOTO PT, DRAGONE J (2010) Distribuição espacial de huanglongbing (greening) em citros utilizando a geoestatística. **Revista Brasileira de Fruticultura**, Jaboticabal 32:808-818.

MADDEN LV, HUGHES G, VAN DEN BOSCH F (2007) The study of plant disease epidemics. **American Phytopathological Society** St Paul, MN.

MARIANO, R.L.R.; ASSIS, S.M.P. Inoculação de bactérias fitopatogênicas. In: Mariano, R.L.R. (Coord.) **Manual de práticas em fitobacteriologia**. Recife: Imprensa Universitária, Universidade Federal de Pernambuco, 2000. p.53–63.

MOHAN, S.K. Investigações sobre Pseudomonas garcae Amaral et al. Em cafeeiros. In: CONGRESSO BRASILEIRO DE PESQUISAS CAFEEIRAS, 4, 1976, Caxambu. **Resumos**. Rio de Janeiro: IBC/GERCA, 1976, p.56.

MOHAN, S.K.; CARDOSO, R.L.; PAIVA, M.A. Resistência em germoplasma de coffea ao crestamento bacteriano incitado por *Pseudomonas garcae* AMARAL et al. **Pesquisa Agropecuária Brasileira**, v.13, n.1, p.53-64, 1978

MOUEN BEDIMO J, BIEYSSE D, CILAS C, NOTTÉGHEM J (2007) Spatio-temporal dynamics of arabica coffee berry disease caused by *Colletotrichum kahawae* on a plot scale. **Plant Disease** 91:1229-1236.

MUELLER, D.S. & BUCK, J.W. Effects of light, temperature, and leaf wetness duration on daylily rust. **Plant Disease** 87:442-445. 2003.

NELSON MR, ORUM TV, JAIME-GARCIA R, NADEEM A (1999) Applications of geographic information systems and geostatistics in plant disease epidemiology and management. **Plant Disease** 83:308-319.

OLIVEIRA, J.R.; ROMEIRO, R.S. Reação de folhas novas e velhas de cafeeiro a infecção por *Pseudomonas cichorii* e *P. syringae* pv. *garcae*. **Fitopatologia Brasileira**, v.15, n.4, p.355-356. 1990.

ORTIZ B, PERRY C, GOOVAERTS P, VELLIDIS G, SULLIVAN D (2010) Geostatistical modeling of the spatial variability and risk areas of southern root-knot nematodes in relation to soil properties. **Geoderma** 156:243-252.

PATRÍCIO, F.R.A.; BERIAM, L.O.S.; ROSSI, A.; MORAES, A.; ALMEIDA, I.M.G. Controle químico da mancha aureolada em uma região montanhosa. **Tropical Plant Pathology**, v.35, supl., p.S119, 2010.

PÉREZ, C. D. P. Interação do nitrogênio com o potássio na intensidade da ferrugem e da mancha aureolada do cafeeiro em solução nutritiva. 2015. 57 f. **Dissertação (Mestrado em Fitopatologia)** -Universidade Federal de Lavras, Lavras, 2015.

POZZA, E. A. (2021) Cafeicultura do cerrado. Belo Horizonte; **EPAMIG**, 2021. p.349-429.

POZZA, E. A.; BELAN, LEÔNIDAS LEONI ; SANTOS, L. A. ; ALVES, M. C. . **Geoestatística e sistemas de apoio a decisão aplicados ao manejo de doenças de plantas.** Inovação, Difusão e Integração: Bases para a Sustentabilidade da Cafeicultura. 1ed.Alegre-ES: CAUFES, 2012, v , p. 155-178.

POZZA, E.A.; CARVALHO, V.L.; CHALFOUN, S.M. Sintomas de injúrias causadas por doenças do cafeeiro. In: Guimarães, R.J.; Mendes, A.N.G.; Baliza, D.P. (Org.). **Semiologia do cafeeiro**. 01 ed. Lavras: Editora UFLA, 2010, p.67-106.

RAIMUNDI, M.K. Caracterização e identificação de isolados bacterianos patogênicos ao cafeeiro. 2014. **Dissertação de Mestrado**. Universidade Federal de Lavras.

RAMALHO, T.O.; FIGUEIRA, A.R.; SOTERO, A.J.; WANG, R.; GERALDINO DUARTE, P.S.; FARMAN, M.; GOODIN, M.M. Characterization of Coffee ringspot virus-Lavras: A model for an emerging threat to coffee production and quality. **Virology** (New York, N.Y. Print), v.464-465, p.385-396, 2014.

RAMOS, A.H.; SHAVDIA, L.D. A dieback of coffee in Kenya. **Plant Disease Reporter**, v.60, n.10, p.831-835, 1976.

ROBBS, C.F.; KIMURA, O.; RIBEIRO, R.L.D.; OYADOMARI, L.C. "Crestamento bacteriano das folhas": nova enfermidade do cafeeiro (*Coffea arábica L.*) incitada por *Pseudomonas cichorii* (Swingle) Stapp. **Arquivos da Universidade Federal Rural**, v.4, n.2, p.1-5, 1974.

RODRIGUES NETO, J.; FIGUEIREDO, P.; MARIOTTO, P.R.; ROBBS, C.F. Pseudomonas andropogonis (Smith, 1911) Stapp, 1928, agente causal da "mancha escura bacteriana" em folhas de cafeeiro (*Coffea arabica L.*). **Arquivos do Instituto Biológico**, v.48, p.31-36, 1981.

RODRIGUES NETO, J.; SILVA, C.H.D.; BERIAM, L.O.S.; PATRÍCIO, F.R.A.; RODRIGUES, L.M.R.; THOMAZIELO, R.A. Mancha bacteriana do cafeeiro causada por *Pseudomonas syringae* pv. *tabaci*. **Summa Phytopathologica**, v.32, supl., p.85, 2006.

RODRIGUES, L. M. et al. Aggressiveness of strains and inoculation methods for resistance assessment to bacterial halo blight on coffee seedlings. **Journal of Phytopathology**, Berlin, v. 165, n. 2, p. 105-114, 2017.

RODRIGUES, L.M.R.; ALMEIDA, I.M.G.; PATRÍCIO, F.R.A.; BERIAM, L.O.S.; MACIEL, K.W.; BRAGHINI, M.T.; GUERREIRO FILHO, O. **Mancha aureolada do cafeeiro causada por *Pseudomonas syringae* pv. *garcae***. Campinas: Instituto Agronômico, 2013. 24 p.

RODRIGUES, L.M.R.; ALMEIRA, I.M.G.; PATRÍCIO, F.R.A.; BERIAM, L.O.S.; MACIEL, K.W.; BRAGHINI, M.T.; GUERREIRO FILHO, O. **Mancha aureolada do cafeeiro causada por *Pseudomonas syringae* pv. *garcae***. Boletim técnico IAC, Campinas, n. 212, 2013.

ROHAM R, PIRDASHTI H, YAGHUBI M, NEMATZADEH G (2014) Spatial distribution of nutsedge (*Cyperus spp. L.)* seed bank in rice growth cycle using geostatistics. **Crop Protection** 55:133-141.

ROMEIRO, R.S. **Bactérias Fitopatogênicas**. Editora UFV. 2 ed. atual. e ampl. Viçosa, 417p. 2005.

ROMEIRO, R.S. **Bactérias Fitopatogênicas**. Editora UFV. 2 ed. atual. e ampl. Viçosa, 417p. 2005.

ROMEIRO, R.S. Inoculação de bactérias fitopatogênicas em plantas. In: Romeiro, R.S. **Métodos em bacteriologia de plantas**. Viçosa: Universidade Federal de Viçosa, 2001, cap.8, p.65-80.

ROUMAGNAC, P., PRUVOST, O., CHIROLEU, F., AND HUGHES, G. 2004. Spatial and temporal analyses of bacterial blight of onion caused by *Xanthomonas axonopodis pv. allii*. Phytopathology 94:138-146.

SERA, G.H.; SERA, T.; ALTÉIA, M.Z.; ANDROCIOLI FILHO, A.; AZEVEDO, J.A.; PETEK, M.R.; ITO, D.S. Associação de *Pseudomonas syringae* pv. *garcae* com algumas características agronômicas em cafeeiros F2 segregantes para o gene erecta. **Ciência e Agrotecnologia**, v.28, n.5, p.974- 977, 2004.

SILVA, M. G. D. Interação da luz, da temperatura e do molhamento foliar *em Cercospora coffeicola* e na cercosporiose em cafeeiro. **Tese de Doutorado**. 2014.

UNITED STATES DEPARTMENT OF AGRICULTURE. Production, Supply and Distribution Online. Disponível em:<http:// https://www.fas.usda.gov/data/brazil-coffee-annual-6/>. Acesso em: 28 set. 2021.

VALE, F.X.R. ; ZAMBOLIM, L. **Controle de doenças de plantas: grandes culturas**. 1.ed. Visconde do Rio Branco, MG: Suprema, 1997. v.2, 1132p.

WALLACE M, HAWKINS D (1994) Applications of geostatistics in plant nematology. **Journal of nematology** 26:626.

YAMADA, J.K. Resistência de isolados de *Pseudomonas syringae* pv. *garcea* a cobre. 2014. **Dissertação de Mestrado**. Universidade Federal de Lavras.

ZAMBOLIM, L.; VALE, F.X.R.; ZAMBOLIM, E.M. Doenças do Cafeeiro. In: KIMATI, A.; AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A.; CAMARGO, L. E. A. **Manual de Fitopatologia**. 4.ed. São Paulo: Agronômica Ceres, 2005. v.2, p.165-180.

ZOCCOLI, D.M.; TAKATSU, A.; UESUGI, C.H. Ocorrência de mancha aureolada em cafeeiros na Região do Triângulo Mineiro e Alto Paranaíba. **Bragantia**, Campinas, v. 70, n. 4, p.843-849, 2011.

ZUCOLOTO M, LIMA JSS, COELHO RI (2009) Uso da 467 geoestatística na probabilidade de ocorrência do Mal-do-Panamá em bananeira prata anã. Rev Fac Nal Agr Medellín 62:4793-4796

SEGUNDA PARTE - ARTIGOS

(Formatação baseada nas exigências do *Journal of Phytopathology*, versão **Publicada** 28 de Maio de 2024*)*

ARTIGO 1

Efficiency of inoculation methods for the assessment of bacterial halo blight in coffee seedlings

Eficiência de métodos de inoculação de mancha aureolada em mudas de café

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Efficiency of inoculation methods for the assessment of bacterial halo blight in coffee seedlings

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ABSTRACT

Bacterial halo blight (BHB), caused by *Pseudomonas coronafaciens* pv. *garcae* (Pcg), is one of the most significant diseases affecting coffee trees worldwide, resulting in considerable damage and notable losses within coffee production systems. Further research into the epidemiology and etiology of BHB in coffee plants is essential for developing effective management strategies. Understanding the efficiency of each inoculation methodology in inducing symptoms is crucial for advancing this research. Therefore, this study aimed to assess the efficiency of seven Pcg inoculation methods using five concentrations to enhance the highest BHB intensity. The experimental design was a complete randomized block design in a factorial (7 x 5) variance analysis with four replications. The first three pairs of leaves of coffee seedlings of the cultivar Catuaí Vermelho IAC-99 were inoculated and evaluated for the incidence and severity of BHB. The inoculation methods of Pcg by injury provided higher BHB AUDPCI and AUDPCS values. Among these, the inoculum injection and multineedle

wounding at inoculum concentrations of 1.6 x 10^9 CFU mL^{-1} demonstrated higher disease intensity.

Keywords: bacterial halo blight; *Coffea arabica* diseases; epidemiology; incidence; inoculum concentration; severity.

INTRODUCTION

Bacterial halo blight (BHB), caused by *Pseudomonas coronafaciens* pv. *garcae* (Pcg; synonym *Pseudomonas syringae* pv. *garcae*) (Barta and Willis 2005), has become a significant challenge for Brazilian coffee (*Coffea arabica* L.) production in the past decade. This disease was first reported in 1955 in the city of Garça, São Paulo, as a natural occurrence in coffee trees (Amaral et al. 1956). Initially, it was not considered a significant economic threat for several years. However, under favorable climatic conditions, it can now cause losses of up to 70% in seedling nurseries and crops (Belan et al. 2014b).

The symptoms of BHB occur mainly in young plant tissues, especially leaves, branches, and fruits. On the leaves, the lesions appear as irregular dark brown spots surrounded by a yellowish halo, which is not visible on young leaves. These lesions may also coalesce, forming large necrotic areas and deforming or rupturing the leaf blade (Pozza et al. 2022; Belan et al. 2014b).

Despite its importance, further research is needed to develop management strategies for the BHB. Efficient inoculation procedures are essential for studying the epidemiology and etiology of Pcg, as well as host-pathogen interactions and disease resistance in breeding programs (Martins et al. 2021; Rodrigues et al. 2023). The variables such as inoculation technique, inoculum concentration, environmental conditions, and plant growth stage are important to be considered when creating an inoculation procedure (Agrios 2005).

Several inoculation methods of Pcg, with and without injuries, were previously described in the literature. For nonwounding inoculations, Oliveira and Romeiro (1990), Ithiru et al. (2013), and Rodrigues et al. (2017) used bacterial suspension sprays at concentrations of 1.1 x 10^9 CFU mL^{-1}, 10^8 CFU mL^{-1}, and 3 x 10^8 CFU mL^{-1}, respectively.

Among wound inoculation methods, injecting a Pcg bacterial suspension with a syringe is most commonly used. Ithiru et al. (1970), Oliveira and Romeiro (1990), Ithiru et al. (2013), and Rodrigues et al. (2017) used this method, with concentrations of 10^9 CFU mL^{-1}, 1.1 x 10^9 CFU mL^{-1}, 10^8 CFU mL^{-1}, and 3 x 10^8 CFU mL^{-1}, respectively. In addition to the bacterial suspension injection method, other wounding methods have been described, including abrasion with carborundum (Costa et al. 1957; Robbs 1979), cutting with a sterilized blade and subsequent spraying with a bacterial suspension (Ithiru et al. 2013), abrasion with sandpaper, and multineedle wounds (Rodrigues et al. 2017).

The selection of a methodology is contingent upon the specific research objectives. However, it has not yet been determined which of these methodologies is the most efficient in inducing the highest BHB intensity. Hence, it also becomes crucial to identify the optimal inoculum concentration for achieving the maximum BHB intensity when utilizing a specific inoculation method, enabling bacterial penetration. As a result, this study aimed to evaluate the efficiency of inoculation methods with and without injury, examining their interactions with various concentrations of Pcg.

MATERIAL AND METHODS

The experiment was conducted twice to evaluate the efficiency of methods (Table 1) for inoculating coffee leaves (*Coffea arabica* L.) with Pcg and the effect of increasing the concentration of the bacterial inoculum on the manifestation of disease symptoms. Seedlings of the coffee cultivar Catuaí Vermelho IAC-99, which is susceptible to the pathogen, were used, with plants grown in polyethylene bags (0.065 m in diameter by 0.20 m in height) filled with soil substrate, bovine manure, and sand (2:1:1) until they had three to four pairs of true leaves. The experiment was conducted in a controlled environment at 23 ± 2°C, with a photoperiod of 12 hours, a mean luminous intensity of 17 $\mu mol.m^{-2.s-1}$, and a relative humidity maintained above 80% using nebulizers.

The experiment was implemented in a factorial scheme (7 x 5) with seven inoculation methodologies and five inoculum concentrations, totaling 35 treatments. Factor 1 comprised seven inoculation methodologies (Table 1). Factor 2 included inoculum concentrations of 2.5 x 10^7, 1 x 10^8, 4 x 10^8, and 1.6 x 10^9 CFU mL^{-1}. The control group was inoculated with a sterile saline solution using all the inoculation methods. The experimental design was a complete randomized block with four biological replicates, comprised of one technical replicate.

Table 1 Methods used for the coffee leaves (*Coffea arabica* L.) inoculation with *P. coronafaciens* pv. *garcae*.

Inoculation methods	
Without injury	Sprayed
With injury	Multineedle assembly
	Scissor cut
	Sandblasting
	Steel wool friction
	Injection
	Wind

Inoculum preparation

The isolated pathotype Pcg 'souche' (CIRM, plant-associated bacteria; CIRM-CFBP1634) was used for seedling inoculation. Preparation of the inoculum and sowing of the pathogen were performed in 523 media following methods described by Kado and Heskett (1970). After 24 hours, the bacterial cells were suspended in sterile saline (0.85% NaCl), and the respective concentrations were determined in a spectrophotometer (OD_{600}), as described by Oliveira and Romeiro (1990).

***In vivo* assay**

Regardless of the treatment, all the seedlings were conditioned for 24 hours before inoculation in an air-conditioned room under the above-mentioned circumstances. These plants received daily irrigation, as needed, to prevent the water from reaching the aerial part of the seedlings.

Six leaves per seedling were inoculated and evaluated, corresponding to the first, second, and third pair of leaves wholly developed from the apex of the seedling. The controls received treatments containing sterile saline instead of the bacterial suspension. The inoculation without injury was performed by spraying the respective inoculum suspensions onto the abaxial and adaxial surfaces of the leaves using a hand sprayer (5 mL/plant). For wound inoculation, six methods were used (Table 1). All the equipment and the sand used to cause the injury were sterilized by autoclaving at 120°C for 60 minutes. For inoculation with multineedle wounds, a set of 10 needles was fixed on a resistant surface (Figure 1A and Aa). Scissors were used to create a cut 0.5 cm in length from the edges of the leaf blade (Figure 1F). Two random inoculation points were made in the leaf mesophyll for these two methods. With the steel wool wound (Figure 1C), the inoculum was manually rubbed onto a demarcated area of 1 cm^2 on the adaxial leaf surface of the seedlings to create microgrooves. The set of needles, scissors, and steel wool were immersed in the respective concentrations of the bacterial suspensions at the time of inoculation. In the injection method, the bacterial suspension was inserted into the leaves by hypodermic syringes. The injection of the inoculum was performed at two points on the leaf blade in the region near the central vein on the abaxial side of the leaves (Mafia, Alfenas, and Gonçalves 2007).

In the inoculation process using sandblasting, medium-grain sand (0.25 to 0.50 mm) was sprayed onto seedlings from a distance of 50 cm using an air gun within a sand-filled container (Figure 1B). The setup, connected to a compressor with a continuous airflow of 50 m/s for 30 seconds, created injuries to the leaf blades. For the wind wound treatment (Figure 1D), seedlings were placed close together and exposed to a constant airflow of 8 m/s for one hour using a fan. This airflow rate simulates the average wind speed in southern Minas Gerais in April (INMET 2017) when BHB epidemics typically occur. After the plants were exposed

to sandblasting and wind, five different concentrations of bacterial suspensions were sprayed on the abaxial and adaxial leaf surfaces (5 mL/plant).

Figure 1 A: Multineedle assembly; Aa: inoculation of coffee (*Coffea arabica* L.) leaves with *Pseudomonas coronafaciens* pv. *garcae* using the multineedle set immersed in the bacterial suspension; B: the air gun used for inoculation with a sandblast injury; C: inoculation via steel wool friction followed by spraying of the inoculum suspension; D: inoculation by wounds caused by wind; E: injection of the inoculum suspension into the leaf parenchyma; F: inoculation by cuts made with scissors.

Incidence and severity assessment of bacterial halo blight

After inoculation, observations were performed every 24 hours to identify symptom occurrence and quantify the disease incidence (INC). The severity of the disease was also estimated every two days using a diagrammatic scale (Belan et al. 2014a). Assessments were carried out until the first leaf fell, which occurred 16 days after the appearance of the first symptoms. In total, 16 incidence and eight severity assessments were completed.

At the end of the experiments, a standard sample of injured tissue was removed and used for an exudation test and the isolation of the pathogen in 523 media to confirm the causal agent and identity of the bacteria.

The area under the disease progress curve for incidence (AUDPCI) and disease severity (AUDPCS) were calculated based on the incidence and disease severity values over time, as proposed by Shaner and Finney (1977).

Statistical analysis

The experiments were conducted twice, and a joint analysis of the data was performed to evaluate whether there was a difference between the experiments over time. Among the controls and their respective treatments, orthogonal mean contrasts were used to verify the occurrence of differences ($p < 0.05$). If there was a difference between the controls and the treatments, they were not included in the statistical analysis.

The data were subjected to analysis of variance (ANOVA), including normality (Shapiro-Wilk) and homoscedasticity (Bartlett), and the significance was determined using the F test ($p < 0.05$). The inoculation method data were compared using the Scott-Knott test ($p < 0.05$), while the inoculum concentrations data were subjected to linear regression analysis with a 5% probability of error. The inoculum concentrations that resulted in the maximum intensity of the disease were obtained by deriving values from the adjusted regression equation,

which was set equal to zero. Despite the interaction between the two factors, an additional analysis unfolding the main factor was also performed to determine the optimal inoculation method regardless of concentration. These analyses were performed in R software (version 3.1.4).

RESULTS

In the joint data analysis, there were no significant differences ($p < 0.05$) between the trials, so the data presented refers to the mean of the two experiments. There were no symptoms of BHB in the coffee seedlings inoculated with sterilized saline for any inoculation methods evaluated. Thus, these controls differed statistically from the other treatments and were not included among the treatments for the remaining analyses.

Significant interaction ($p < 0.05$) was observed between the inoculation methods for the AUDPCI and AUDPCS. Similarly, significant interaction was observed in the contrast between inoculation methods and concentrations for these variables. Conversely, the significance ($p < 0.05$) between concentrations was observed only for the AUDPCS (Table 2; Table 3).

Table 2 Analysis of variance of the effect of seven different inoculation methods and four *P. coronafaciens* pv. *garcae* inoculum concentrations on the area under the disease progress curve for incidence (AUDPCI) of coffee leaves.

Factor	df[a]	SS[b]	MS[c]	F value	P value
Inoculation methods	6	44892975.723136	7482162.620523	3531.941	0.0000
Concentrations	3	13970.642753	4656.880918	2.198	0.0946
Inoculation methods x concentrations	18	226709.419329	12594.967740	5.945	0.0000
Blocks	3	4823.370396	1607.790132	0.759	0.5203
Error	81	171592.655579	2118.427847		
Total	111	45310071.811192			
CV = 3.98%					

df[a] = degrees of freedom; SS[b] = som of squares; MS[c] = mean of squares.

Table 3 Analysis of variance of the effect of seven different inoculation methods and four *P. coronafaciens* pv. *garcae* inoculum concentrations on the area under the disease progress curve for severity (AUDPCS) of coffee leaves.

Factor	df[a]	SS[b]	MS[c]	F value	P value
Inoculation methods	6	46429.538561	7738.256427	477.852	0.0000
Concentrations	3	1195.083632	398.361211	24.600	0.0000
Inoculation methods x concentrations	18	2872.666518	159.592584	9.855	0.0000
Blocks	3	79.643718	26.547906	1.639	0.1867
Error	81	1311.700082	16.193828		
Total	111	51888.632511			
CV = 20.76%					

df[a] = degrees of freedom; SS[b] = som of squares; MS[c] = mean of squares.

The AUDPCI values were higher in inoculation methods that included injury, except for wounds caused by wind (Figure 2). For the AUDPCS variable, the injection inoculation method provided greater disease severity in the coffee seedling leaves. This method provided a 43.4% increase in AUDPCS relative to that achieved with the multineedle wound inoculation method. Furthermore, the injection method was more efficient than the sandblasting, steel wool, and scissor inoculation methods. Spray inoculation and wind-induced injuries resulted in lower AUDPCI and AUDPCS values (Figure 2).

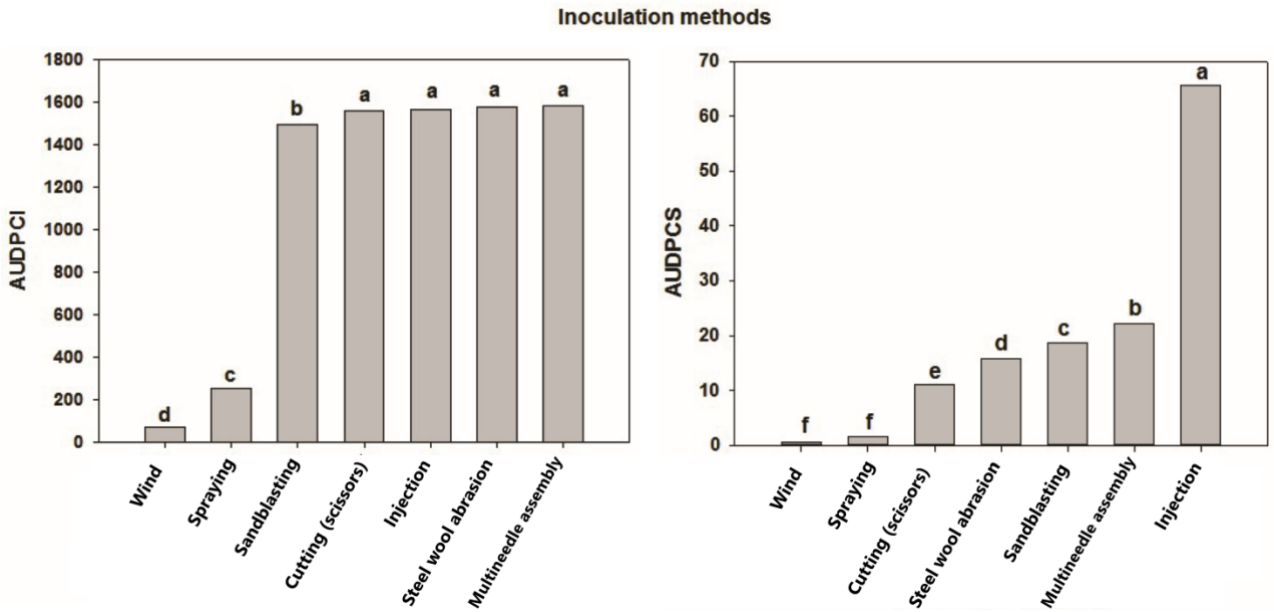

Figure 2 The area under the disease progress curve for incidence (AUDPCI) and severity (AUDPCS) of bacterial halo blight (*Pseudomonas coronafaciens* pv. *garcae*) on coffee (*Coffea arabica* L.) leaves subjected to different methods of inoculation. Bars with the same letter do not differ at the 5% probability level according to a Skott-Knott test.

In the analysis of factor interactions, the use of atomization and sandblasting resulted in a significant difference ($p < 0.05$) in inoculum concentrations for the AUDPCI variable (Figure 3). When these methods were used, the concentrations that provided the highest incidence rates were 2.5 x 10^7 and 4.0 x 10^8 CFU mL^{-1}, respectively (Figure 3). For the method of sandblasting injury, there was a slight decrease in the AUDPCI value after reaching the concentration associated with the greatest disease incidence.

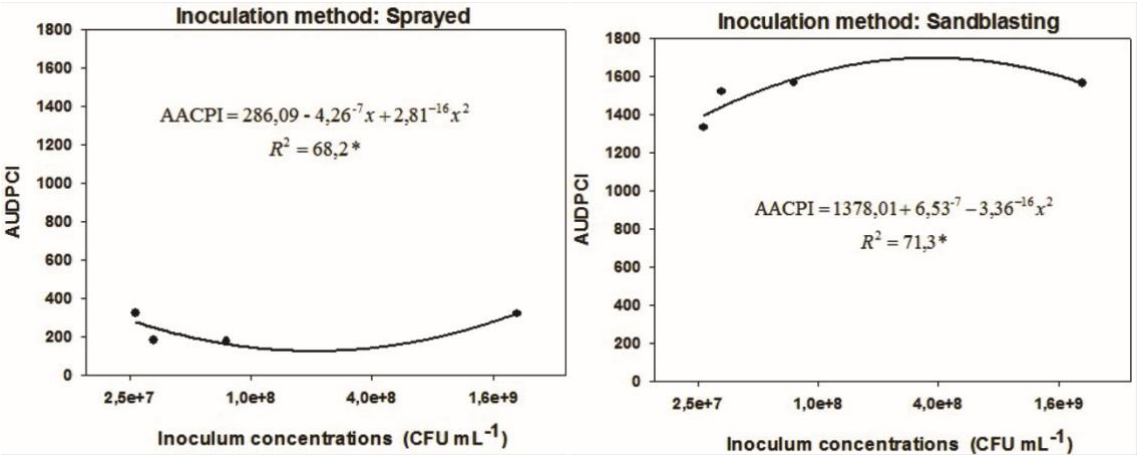

Figure 3 The area under the disease progress curve for incidence (AUDPCI) of bacterial halo blight (*Pseudomonas coronafaciens* pv. *garcae*) on coffee (*Coffea arabica* L.) leaves subjected to spraying with inoculum suspensions and sandblasting injuries.

For the AUDPCS variable, there were differences between the inoculum concentrations when wound inoculation was performed with steel wool abrasion, sandblasting, a multineedle assembly, and injection of the inoculum suspension. In the steel wool abrasion and multineedle wound inoculation methods, disease severity increased linearly with increasing inoculum concentration (Figure 4). In this case, the highest concentration of inoculum used in this study (1.6×10^9 CFU ml^{-1}) provided the maximum disease severity (Figure 4).

For the sandblasting wound inoculation and inoculum suspension injection methods, concentrations of 4×10^8 and 1.6×10^9 CFU ml^{-1}, respectively, resulted in the greatest disease severity. In this case, for the AUDPCI variable, there was a decrease in the AUDPCS value after reaching the greatest disease severity (Figure 4).

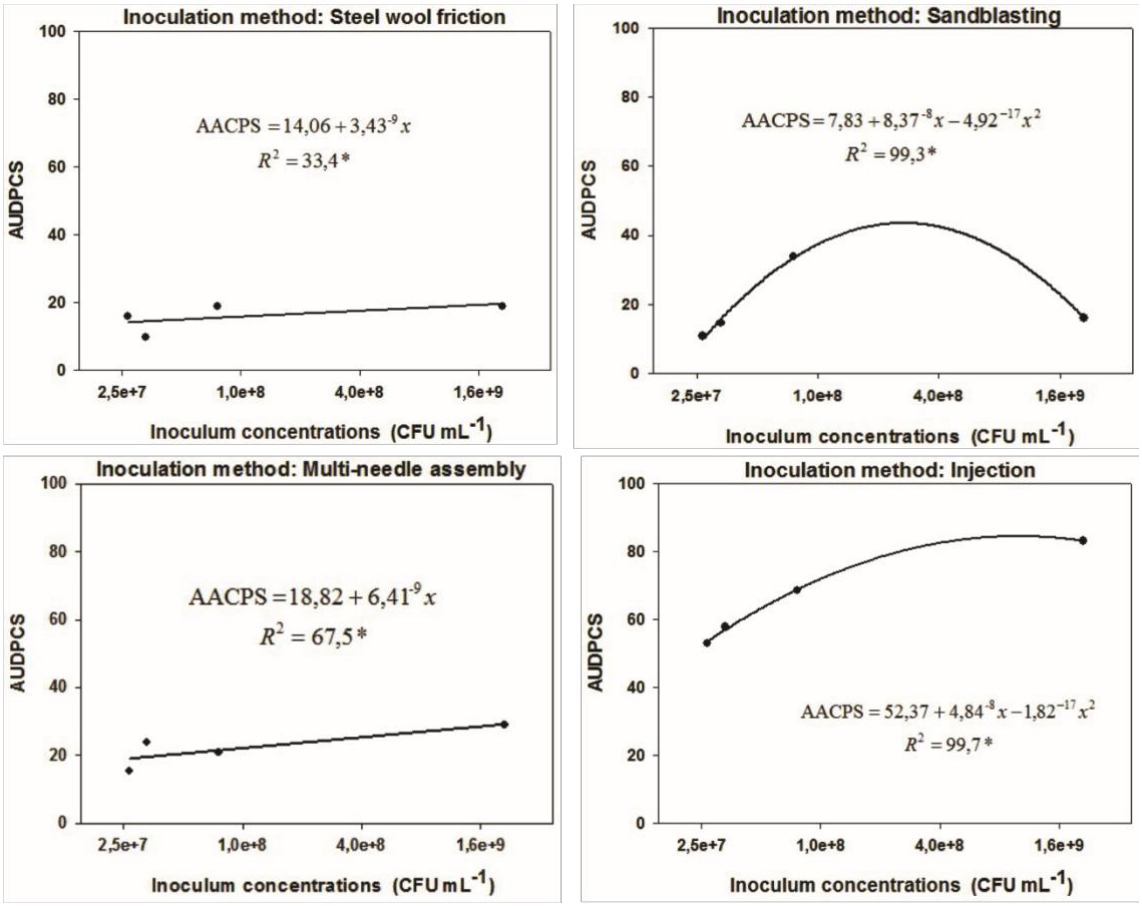

Figure 4 The area under the disease progress curve for the severity (AUDPCS) of bacterial blight (*Pseudomonas coronafaciens* pv. *garcae*) on coffee (*Coffea arabica* L.) leaves inoculated by steel wool abrasion, sandblasting, a multineedle assembly and the injection of the inoculum suspension.

DISCUSSION

Leaf limb injuries may be considered the most likely pathways for the penetration of phytopathogenic bacteria into plant tissues due to natural openings such as stomata and hydathodes (Agrios 2005; Goodman 1982; Schumann and D'Arcy 2010). This fact supports the higher disease incidence and severity observed when inoculation methodologies involving leaf blade injury were used. Phytobacteria can not cross plant cuticles or cell walls and, thus, can not penetrate plant cells (Goodman 1982). Therefore, leaf limb injuries, in addition to facilitating phytobacterial penetration into plant tissues, provoke extravasation of intracellular fluid, attracting bacteria and serving as a culture medium (Melotto et al. 2006; Madigan et al. 2017).

The inoculation method in which the bacterial suspension was injected into the plant tissue provided a higher disease incidence and severity than the other methods. Within the plants inoculated with this method, there was no difference in disease incidence among the inoculum concentrations used. In this case, the bacteria were deposited directly into the leaf mesophyll, providing colonization of the intercellular spaces without the need to overcome plant defense barriers and structural mechanisms. Therefore, the disease severity increased as the concentration of bacteria in the inoculum suspension introduced into the intracellular spaces increased. In fact, Ithiru et al. (2013) did not identify differences between coffee genotypes regarding disease resistance or the reaction to different isolates when using the injection inoculation method (10^8 CFU ml^{-1}). According to these authors, this method caused greater disease severity than wound inoculation with cuts made by blades.

Rodrigues et al. (2017) used a cork stopper and sandpaper embedded in an inoculum suspension at a concentration of 3 x 10^8 CFU mL^{-1} to cause injuries as part of their inoculation methods. Using both methods of inoculation, the symptoms of BHB occurred on plant leaves. In the case of the cork stopper, despite creating wounds, there was no oxidation of tissues at the

wound site since this is a relatively less drastic method of causing injury, avoiding confusion between disease symptoms and mechanical damage to the tissue of the leaves. Compared with using carborundum or multineedle inoculation, this technique reduces the oxidation of injured tissues, since the oxidative compounds released after damaging cells can affect colonization by pathogens (Rodrigues et al. 2017). Costa et al. (1957) reported that leaves inoculated with a carborundum wound developed less severe BHB; therefore, this method was less efficient than inoculation with a multineedle apparatus. In this study, abrasion with steel wool was also not the best method; whereas it was effective in inducing Pcg infection, it resulted in lower intensity BHB infections than the other procedures.

As a standard method for inoculating coffee leaves with Pcg had yet to be defined, the optimal inoculum concentration had also not been standardized for reproducible research. Different concentrations of Pcg suspension, such as 10^9 CFU mL^{-1} (Ithiru et al. 1970), 1.1 x 10^9 CFU mL^{-1} (Belan et al. 2016), 10^8 CFU mL^{-1} (Ithiru et al. 2013), and 3 x 10^8 CFU mL^{-1} (Rodrigues et al. 2017), are described in the literature.

Furthermore, the injection inoculation method resulted in a bigger foliar lesion area as the inoculum concentration increased. The severity was reduced after applying a concentration of 1.6 x 10^9 CFU mL^{-1} due to early defoliation. Similarly, with the sandblasting method, the disease incidence increased proportionally to the inoculum concentration, which reached its peak at 4 x 10^8 CFU mL^{-1}; however, the disease incidence decreased when the highest inoculum concentration evaluated was used.

Although there are reports of injuries occurring due to factors such as mechanical harvesting and hail, rain, and wind (due to rubbing leaves) (Zoccoli et al. 2011), the wind inoculation method had the lowest disease incidence and severity. The wind velocity was 8 m/s, simulating the observed average wind speed intensity in the southern region of Minas Gerais in April 2017 (INMET 2017). It is possible that the wind speed over the exposure time (one hour)

was not sufficient to create wounds on the leaves, or its direct impact may have dehydrated the leaves, causing the stomata to close.

Despite wounding methods have been demonstrated to have more severe symptoms for inoculating Pcg, the inoculation by spraying phytopathogenic bacterial solutions is often utilized in scientific research (Silva Júnior 2007; Malavolta Júnior et al. 2002; Halfeld-Viera 2006; Araújo 2005; Amaral et al. 1956; Belan et al. 2013; Botrel 2013; Yamada 2014; Rodrigues et al. 2017). The similarity of atomization or spraying with nonmechanized field conditions is one of the reasons for this being a frequently used method; furthermore, it does not cause injury to the foliar tissue, so it does not break the natural defense barriers of plants. Without injury, to penetrate a plant, bacteria move to infection sites by chemotaxis, and when they reach a specific population density, detected by quorum sensing, they penetrate the host (Ng and Bassler 2009). For this inoculation method, an inoculum concentration of 1.6×10^9 CFU mL^{-1} provided a higher AACPI of BHB in coffee leaves.

Yamada (2014) used an inoculum suspension concentration of 1.1×10^9 CFU mL^{-1} (higher than the value identified in this study 2.5×10^7 CFU mL^{-1} for spray inoculation) for a resistance test of different isolates of this bacterium. This fact justifies the importance of the present study in developing standardized experimental methods for assessing pathosystems involving BHB and coffee trees.

In this research all the tested methods induced BHB symptoms in coffee leaves. The spraying method, at a concentration of 2.5×10^7 CFU mL^{-1}, resulted in lower BHB levels when compared to the wounding methods. However, it still accurately reproduced the characteristic symptoms in a controlled environment. In contrast, the most effective methods for inducing the highest BHB severity involved plant injuries, specifically inoculum injection and multineedle wounding, both applied at concentrations of 1.6×10^9 CFU mL^{-1}.

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REFERENCES

Agrios GN (2005) Plant pathology, vol 5th. Elsevier Acad. Press., Burlington. Mass, EUA.

Amaral JF, Teixeira C, Pinheiro ED (1956) A bacterium causing halo blight of coffee. Arquivos do Instituto Biológico, 23:151–155. http://dx.doi.org/10.1590/S0006-87052011000400017

Araújo DV, Mariano RLR, Michereff SJ (2005) Inoculation methods of *Acidovorax avenae* subsp. *citrulli* in melon. Summa Phytopathologica, 31(1), 69–73.

Barta TM, Willis, DK (2005) Biological and Molecular Evidence that *Pseudomonas syringae* Pathovars *coronafaciens*, *striafaciens* and *garcae* are Likely the Same Pathovar. Journal of Phytopathology, 153:492–499. https://doi.org/10.1111/j.1439-0434.2005.01008.x

Belan LL, Pozza EA, Freitas MLO, Souza RM, Jesus Junior WC, Oliveira JM (2014a) Diagrammatic Scale for Assessment of Bacterial Blight in Coffee Leaves. Journal of Phytopathology, 162:801–810. https://doi.org/10.1111/jph.12272

Belan LL, Pozza EA, Souza RM, Raimundi MK, Silva JL, Freitas MLO, Souza PE (2013) Influência da luz e período de molhamento foliar em diferentes métodos de inoculação artificial de *Pseudomonas syringae* pv. *garcae*. In: 46º Congresso Brasileiro de Fitopatologia, 2013, Ouro Preto - MG. CBFito Sustentável - Anais do 46º Congresso Brasileiro de Fitopatologia, 255–255.

Belan LL, Pozza EA, Souza RM (2014b) A Mancha Aureolada do Cafeeiro. Revisão Anual de Patologia de Plantas, 22:227–256.

Botrel DA (2013) Fungos sapróbios como agentes de biocontrole da mancha aurelada (*Pseudomonas syringae* pv. *garcae*) no cafeeiro. Dissertation, Federal University of Lavras.

Costa AS, Amaral JF, Viegas AP, Silva DM, Teixeira CG, Pinheiro ED (1957) Bacterial halo blight of coffee in Brazil. Phytopathologische Zeitschrift, 28(4), 427–444.

Goodman RN (1982) The infection process. In: Mount, M.S., Lacy, G.H. Phytopathogenic prokaryotes. New York: Academic, 1:31–62.

Halfeld-Vieira BA, Nechet KL (2006) Ocorrência da mancha bacteriana do maracujazeiro em Roraima. Fitopatologia Brasileira, (2)31. https://doi.org/10.1590/S0100-41582006000200023

INMET Instituto Nacional de Meteorologia. http://www.inmet.gov.br/portal/index.php?r=home/page&page=rede_estacoes_conv_graf Accessed 28 September 2017

Ithiru JM, Gichuru EK, Gitonga PN, et al (2013) Methods for early evaluation for resistance to bacterial blight of coffee. African Journal of Agricultural Research, 8:2450–2454. https://doi.org/10.5897/AJAR2013.6717

Ithiru JM, Gichuru EK, Masanga JO, Alakonya AE (1970) A Comparative analysis associated to virulence of *Pseudomonas syringae* pv. *garcae*, the causative agent of Bacterial Blight of coffee in Kenya. The Journal of Agricultural Science, 2:76. https://doi.org/10.25081/jsa.2018.v2.893

Kado CI, Heskett MG (1970) Selective media for isolation of *Agrobacterium*, *Corynebacterium Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology, 60:969–976.

Madigan MT, Bender KS, Buckley DH, Sattley WM, Stahl DA (2017) Brock Biology of Microorganisms. 15th Ed. Pearson Education, 1064.

Mafia RG, Alfenas AC, Gonçalves RC (2007) Detecção, isolamento e inoculação de bactérias fitopatogênicas. In: Alfenas, A.C.; Mafia, R.G. (Eds.). Métodos em Fitopatologia. Viçosa: UFV, 138–160.

Malavolta Júnior VA, Almeida IMG, Rodrigues Neto J, Beriam LOS, Melo PCT (2002) Caracterização de *Pseudomonas syringae* pv. *syringae* em tomateiro no Brasil e reação de cultivares/genótipos de tomateiro a esse patovar e ao patovar tomato. Arquivos do Instituto Biológico, São Paulo, 69(1), 63–66, jan/mar.

Melotto M, Underwood W, Koczan J, Nomura K, He S Y (2006) Plant stomata function in innate immunity against bacterial invasion. Cell. 126: 969–980. https://doi.org/10.1016/j.cell.2006.06.054

Martins SA, Resende MLV, Monteiro ACA, Santiago WD, Vasconcelos VAM, Reichel T, Pereira MHB, Botelho DMS (2021) Resistance of genotypes and defence compounds against bacterial halo blight of coffee. Journal of Phytopathology, 69:766–776. https://doi.org 10.1111/jph.13050

Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. Annual review of genetics, 43: 197–222. https://doi.org/10.1146/annurev-genet-102108-134304

Oliveira JR, Romeiro RS (1990) Reação de folhas novas e velhas de cafeeiro a infecção por *Pseudomonas cichorii* e *P. syringae pv. garcae.* Fitopatologia Brasileira, 15(4), 355–356.

Pozza EA, Santinato F, Sera GH, Matiello JB, Filho OG, Santinato R, Silva RP, Benvenga SR (2022) Manejo sustentável de doenças em cafeeiros de alta produtividade. In: Santinato F.

(Eds.). A moderna cafeicultura brasileira: tecnologias que afetam a produtividade. 01 ed. Jaboticabal: Funep, 510–511.

Robbs CF, Kimura O, Ribeiro RLD, Oyadomari LC (1974) "Crestamento bacteriano das folhas": nova enfermidade do cafeeiro (*Coffea arabica* L.) incitada por *Pseudomonas cichorii* (Swingle) Stapp. Arquivos da Universidade Federal Rural, 4(2), 1–5.

Rodrigues LMR, Almeida IMG, Patrício FRA, Beriam LOS, Maciel KW, Braghini MT, Filho OG (2017) Aggressiveness of Strains and Inoculation Methods for Resistance Assessment to Bacterial Halo Blight on Coffee Seedlings. Journal of Phytopathology, 165:105–114. https://doi.org/10.1111/jph.12543

Rodrigues LMR, Andrade VT, Destéfano SAL, Braghini MT, Caixeta LB, Guerreiro-Filho O (2023) Inheritance of Resistance to Bacterial Halo Blight and Bacterial Leaf Spot in Arabica Coffee, 107(12). https://doi.org/10.1094/PDIS-09-22-2282-RE

Rodrigues LMR, Almeida IMG, Patrício FRA, Beriam LOS, Maciel KW, Braghini MT, Guerreiro Filho O (2013) Mancha aureolada do cafeeiro causada por *Pseudomonas syringae* pv. *garcae*. Campinas: Instituto Agronômico, 24 p.

Schumann GL, D'arcy CJ, (2010) Essential Plant Pathology. St. Paul, MN, USA, 59(3), 384.

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056. https://doi.org/10.1094/Phyto-67-1051

Silva Júnior TAF (2007) *Pseudomonas cichorii* em tomateiro: ocorrência no estado de São Paulo, gama de hospedeiras e reação de genótipos. Dissertation, Universidade Estadual de São Paulo Júlio de Mesquita Filho.

Zoccoli DM, Takatsu A, Uesugi CH (2011) Ocorrência de mancha aureolada em cafeeiros na Região do Triângulo Mineiro e Alto Paranaíba. Bragantia, Campinas, 70(4), 843–849. http://dx.doi.org/10.1590/S0006-87052011000400017

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ARTIGO 2

Effect of temperature and photoperiod on *Pseudomonas syringae* pv. *garcae* inoculum production

Efeito da temperatura e fotoperíodo na produção de inóculo *Pseudomonas syringae* pv. *garcae*

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Effect of temperature and photoperiod on *Pseudomonas syringae* pv. *garcae* inoculum production

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ABSTRACT

Bacterial halo blight (BHB), whose etiological agent is the bacterium *Pseudomonas syringae pv. garcae* (Psg), is one of the diseases occurring in coffee (*Coffea arabica* L.) nurseries and fields, causing damage to the crop. This study aimed to investigate the influence of five temperatures and three photoperiods on the concentration of *Psg* for the inoculation of coffee leaves. The study was carried out in a factorial design (5 x 3), with four replicates, including the temperatures of 20, 25, 30, 35, and 40 °C, and three photoperiods: continuous light, 12-hour light/12-hour dark, and continuous dark. Petri dishes with inoculum suspensions were incubated for 48 hours, during which bacterial concentrations and cell sizes were measured. The resulting inoculum was then used to inoculate coffee seedlings at 23 ± 2 °C by pricking one leaf pair with a multi-needle device and immersing it in the inoculum. Disease severity was assessed, and the area under the disease progress curve (AUDPC) was calculated to evaluate the progression of the disease. The interaction between temperature and photoperiod was significant for the inoculum concentration of Psg. The highest mean inoculum concentration of 1.22 x 10^9 CFU/ml was obtained under the 12-hour light photoperiod. The effect of temperature on inoculum was adjusted by third-degree regressions, estimating the highest inoculum concentration, which was

2.2 x 10^9 CFU/ml, observed at 25 °C under a 24-hour light photoperiod. There was an interaction ($p < 0.05$) between photoperiod and temperature on the dimensions of Psg cells. The longest mean length and mean width of the bacterial cells were observed under photoperiods of 0 hours and 24 hours of light. At 30 °C, the 12-hour light photoperiod produced the largest bacterial cells. While temperature during inoculum production significantly affected BHB severity, photoperiod did not. The highest disease severity was linked to inoculum produced at 25 °C with a 12-hour light photoperiod.

Keywords: bacterial inoculation; bacterial halo blight; *Coffea arabica* L.; concentration; disease severity.

INTRODUCTION

Coffee is one of the most important commodities in the global market, with *Coffea arabica* and *Coffea canephora* being the predominant species under cultivation. Brazil is the main producer and exporter, accounting for 39% of the global coffee production in 2023 (USDA, 2024). Projections for 2024 estimate Brazil's coffee production at 58.8 million 60 kg bags of green bean equivalent, with the state of Minas Gerais contributing 68% of the national *C. arabica* production (CONAB, 2024).

However, several factors have reduced the productivity of coffee plantations, including climatic adversities, pests, and diseases. Fungi, viruses, bacteria, and nematodes are examples of etiological agents of coffee diseases. Among these, the bacterium *Pseudomonas syringae* pv. *garcae* (Psg) stands out for causing bacterial halo blight (BHB). This disease is common in seedling nurseries and crop fields in the main producing regions. The pathogen spreads rapidly, and the disease causes defoliation and branch desiccation and is difficult to control, causing significant losses from the nursery to the field. Disease effects are particularly evident in regions over 900 m altitude with a temperature close to 20 °C, exposed to the wind, and a leaf wetness duration of more than 4 hours or where the crop is mechanized harvesting (Pozza et al. 2022).

The bacterium mainly infects new tissues, such as seedlings in nurseries, new plants in the field, and expanding leaves near the apical meristem during the production phase, in addition to causing rot in coffee branches and fruits (Rodrigues et al. 2013). In the leaves, the initially observed symptoms are small irregular lesions with a soggy appearance, with subsequent tissue necrosis or blight. This necrotic area may increase in size, and yellow halos

may appear around it, explaining the name of the disease. The lesions can also coalesce and deform or break the leaf blade (Belan et al. 2014b).

The development of lesions, their coalescence, and the extent of the yellow halo are influenced by the coffee plant's resistance and specific environmental conditions. Factors such as temperature, photoperiod, and other environmental variables play crucial roles in affecting both the pathogen and the host, as well as their interaction, either promoting or hindering the onset and progression of the disease (Silva, 2014). Environmental factors can determine the geographic distribution, incidence, and severity of disease and, in many cases, are specific to the pathogen in question. Among these factors, temperature is most frequently correlated with disease epidemiology, followed by humidity and light (Colhoun, 1973; Campbell and Madden, 1990).

In the field, temperature and luminosity oscillate daily, but when these factors remain constant under artificial conditions, it is possible to evaluate their impact on pathogen biology (Andrade et al. 2007). Knowing the effect of environmental factors *in vitro* and the infectivity of pathogens is essential for producing inocula and determining the ideal conditions for artificial inoculation (Mueller and Buck, 2003). Because disease occurrence depends on environmental factors, the morphology, and physiology of the pathogen are also influenced by environmental conditions, which can alter the infectious capacity of the pathogen and consequently the intensity of the disease. Therefore, the temperature and photoperiod during the incubation period can alter the morphological characteristics and therefore the infectivity of Psg, in addition to its inoculum production.

Temperature is essential for Psg to multiply and regulate the reaction rate of its various enzymes, however, the activity of each gene or enzyme can vary within a species. For Psg, genes and enzymes that control toxin synthesis and flagellum motility are repressed at 30 °C, while enzymes associated with substance transport and protein translation are stimulated at this same temperature (Hockett, Burch, and Lindow, 2013). Multiplication of Psg *in vitro* was performed at 28 °C in several studies (Freitas et al. 2022, Raimundi et al. 2021, Pèrez et al. 2017; Rodrigues et al. 2017; Ithiru et al. 2013). Freitas (2017) observed the highest Psg population growth *in vitro* in the 23 to 31 °C range, with a peak at 28.1 °C, close to the temperature mentioned above. According to the same author, in the in vivo assay, wounding inoculation resulted in a higher area under the disease progress curve (AUDPC) values for BHB than inoculation without wounding. The highest AUDPC was obtained at 20.9 and 19.3 °C temperatures, i.e., lower than the optimal temperature *in vitro*, when samples were inoculated

with and without wounding, respectively. However, it is uncertain which temperature range leads to the greatest Psg population growth and what the relationship with luminosity is.

Temperature and photoperiod are two environmental factors that can significantly influence bacterial cell morphology, including alterations in cell size, shape, and structure. These morphological changes can profoundly affect bacterial growth, survival, and pathogenicity (Lam, Wheeler, and Tang, 2014). Consequently, investigating how these factors impact bacterial cell morphology is essential for understanding bacterial ecology, physiology, and pathogenesis. Such knowledge is also crucial for developing effective strategies for BHB management.

Therefore, the objectives of this study were to investigate the effects of temperatures and photoperiods on the production of Psg inoculum and the severity of BHB caused by this inoculum in coffee seedling leaves.

MATERIALS AND METHODS

The experiment was performed in two stages, *in vitro* and *in vivo*, and repeated twice.

Effect of temperature and photoperiod on the inoculum concentration and size of Psg cells *in vitro*

The Psg isolate was obtained from pathotype Psg 'souche' (CIRM, plant-associated bacteria; CFBP1634). Preparation of the inoculum and sowing of the pathogen were performed in 523 media following methods described by Kado and Heskett (1970).

From pure colonies, a suspension with a concentration of 1 x 10^8 CFU/ml was prepared and calibrated in a spectrophotometer according to the method described below to determine the inoculum concentration. A total of 100 µL of this suspension was pipetted into Petri dishes containing MB1 medium, spread evenly with a Drigalski spatula, and closed with plastic film.

Five temperatures, 20, 25, 30, 35, and 40 °C, and three light exposures (photoperiods) were evaluated in a factorial (5 x 3) design, totaling 15 treatments. A randomized block design with four replicates was used, i.e., there were 60 plates in total. The plates were kept in BODs for 48 hours and exposed to continuous light, 12-hour light/12-hour dark, or continuous dark. The absence of light was achieved by wrapping the plates with aluminum foil.

After 48 hours, the inoculum concentration was determined in a spectrophotometer at an optical density of 600 nm. Ten milliliters of 0.85% saline solution were added to each Petri dish. Next, a fraction of this suspension was pipetted into an ELISA plate well. The absorbance value was then measured in each treatment. This value was converted into an inoculum concentration following Oliveira (1988):

$IC = 600/0.028 + [1.516 * (10^{-10})]$

Where:

IC = inoculum concentration (CFU/ml)

To measure the size of Psg cells from the bacterial suspensions described above, the suspensions were scraped onto slides, which were then covered with coverslips for immediate reading. The slides were observed under a Zeiss Axio Observer Z1® microscope at 1000x magnification. Images of the bacterial cells were obtained, and the width and length of 30 cells per treatment were measured using AxioVision® software.

Pathogenicity test in seedlings *in vivo*

The inoculum suspensions obtained for each treatment in the previous step were used to inoculate healthy seedlings of *C. arabica* var. Catuaí Vermelho IAC-99, which is susceptible to the pathogen. These seedlings had three to four pairs of true leaves and were grown in polyethylene bags 0.065 m in diameter and 0.20 m in height. The growth substrate consisted of soil, cattle manure, and sand at a ratio of 2:1:1.

Inoculation was performed with the suspensions and corresponding inoculum concentrations obtained in the same treatments and following the same experimental design as those described in the *in vitro* experiment. Thus, the first pair of fully developed leaves from the apex of the plant was inoculated in each of the 60 seedlings. The pathogen was inoculated via wounds using a multi-needle set immersed in the inoculum suspension (Oliveira et al. 2024). The controls were inoculated with a sterile saline solution. After inoculation, the seedlings were kept in a humid chamber for 24 hours in a controlled environment at 23 ± 2 °C with a photoperiod of 12 hours. They received localized daily irrigation as needed so as not to wet the leaves.

The evaluation of BHB severity began 48 hours after inoculation and was evaluated every two days, only in the two inoculated leaves, totalizing ten evaluations. The severity was estimated with the aid of a diagrammatic scale for BHB proposed by Belan et al. (2014a).

A standard sample of wounded tissue was collected and used for the water drop exudation test. The microorganism was then cultured in Petri dishes with MB1 medium for identification of Psg and confirmation of the bacterial etiology.

The values of severity from the graded scale were integrated over time to obtain AUDPC, according to the equation proposed by Shaner & Finney (1977).

Statistical analysis

Firstly, the assumptions of the analysis of variance were checked. To assess normality and homoscedasticity, the Shapiro-Wilk and Bartlett tests were used, respectively. The data followed the assumptions of the analysis of variance and did not need to be transformed. A joint analysis was performed for the experiments repeated over time.

For both experiments, the analysis of variance followed a 5 x 3 factorial arrangement with a randomized block design, with factor 1 comprised of five temperatures such as 20, 25, 30, 35, and 40 °C, and factor 2 with three photoperiods, namely, continuous light, 12-hour light/12-hour dark and continuous dark, totaling15 treatments.

When the F test ($p < 0.05$) was significant for photoperiod, Tukey's test of means was performed ($p < 0.05$). For the temperature variable, linear regression models were fitted for both inoculum concentrations *in vitro* and AUDPC *in vivo*. Linear regression models were also fitted for the variable bacterial cell size (length and width). The models were selected based on the significance of the t-test of the parameters of the fitted regression models ($p < 0.05$), the highest coefficient of determination (R^2), and the smallest mean square of the deviations obtained in the analysis of variance.

The maximum points of the mean concentration (10^9 CFU/ml), length (µm), and width (µm) variables were obtained by equalizing the fitted regression models to zero, and then their first-order derivative was calculated. These statistical analyses were performed in R software (version 3.4.2).

(a) Pearson correlation analysis ($p < 0.05$) was carried out using R software (version 3.4.2) to verify the correlations among temperatures, AUDPC, inoculum concentrations, and bacterial cell length and width.

(b) RESULTS

There was no difference in the joint analysis of the experiments repeated in time for either the *in vitro* or *in vivo* experiments. Thus, the following data correspond to the means of the two experiments.

Psg inoculum concentration and cell size under different temperatures and photoperiods *in vitro*

The interaction between photoperiod and temperature significantly affected bacterial inoculum concentration (CFU/ml) ($p < 0.05$). The highest mean inoculum concentration of 1.22 x 10^9 CFU/ml was obtained under the 12-hour light photoperiod (Figure 1).

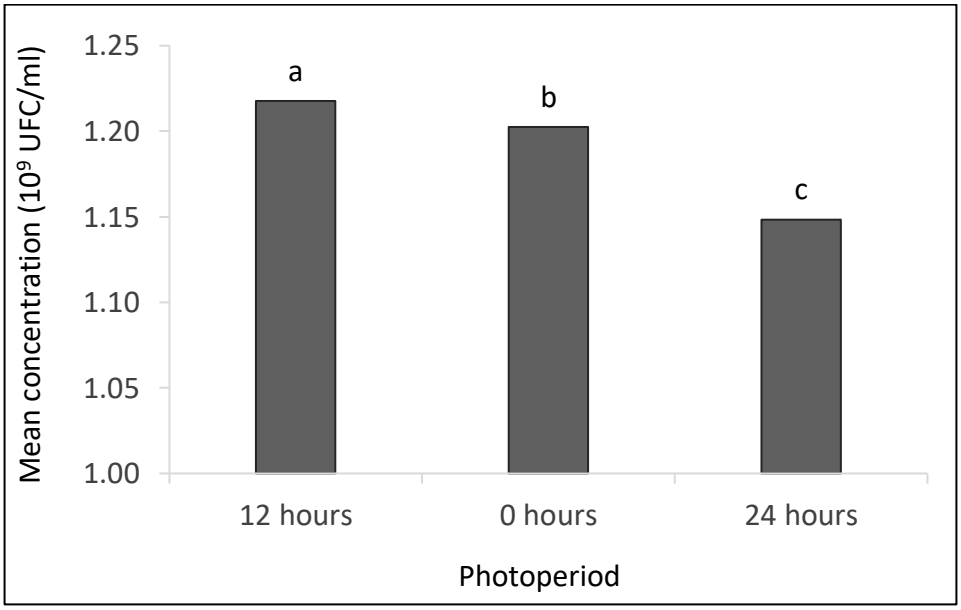

Figure 1. Mean inoculum concentration of *Pseudomonas syringae pv. garcae* (10^9 CFU/ml) under different photoperiods. Bars followed by the same letter do not differ by Tukey's test ($p < 0.05$).

The effect of temperature on inoculum was adjusted by third-degree regressions, estimating the highest inoculum concentration, which was 2.2 x 10^9 CFU/ml, observed at 25 °C under a 24-hour light photoperiod (Figure 2). At temperatures of 35 °C and above, bacterial growth was nearly zero across all three photoperiods.

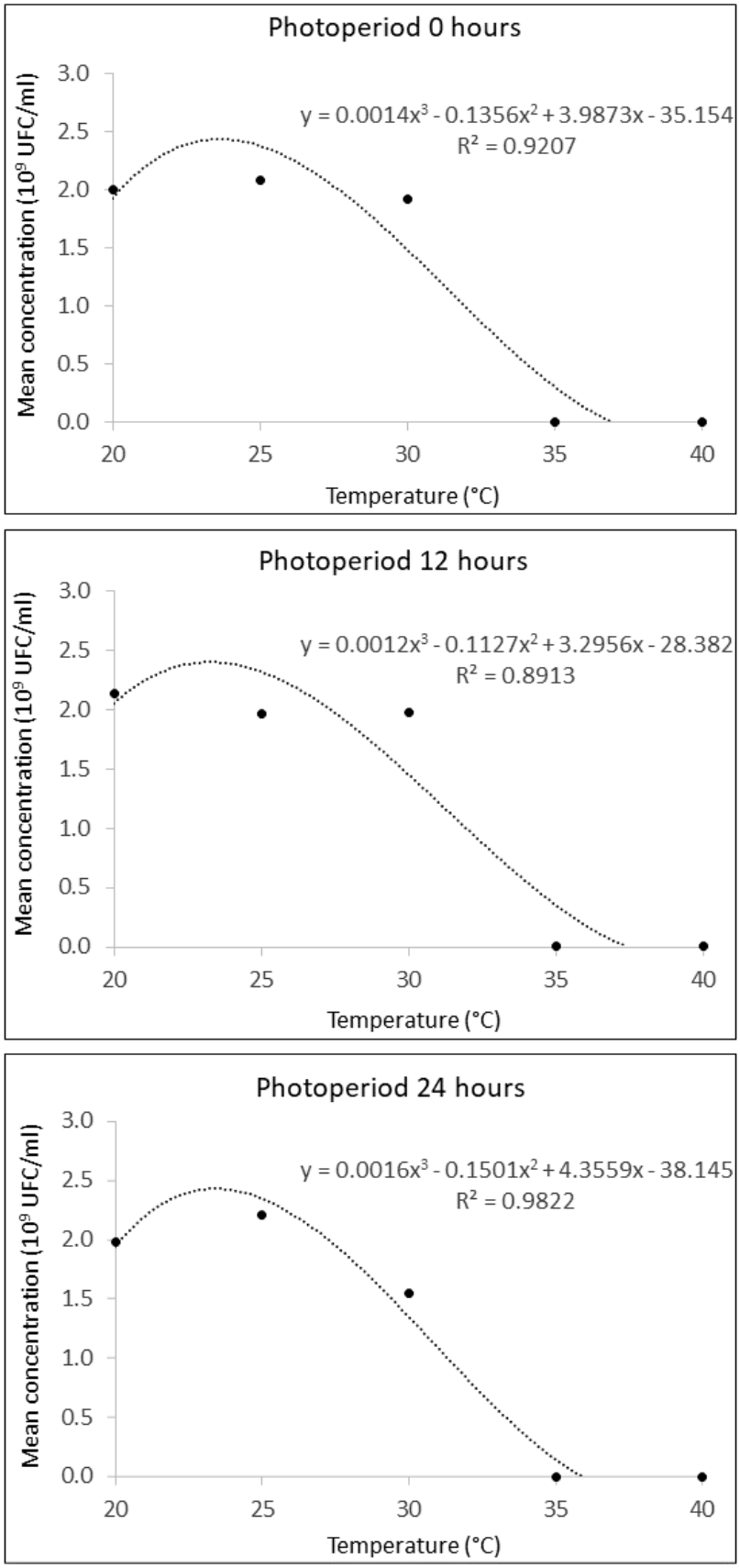

Figure 2. Mean inoculum concentration of *Pseudomonas syringae pv. garcae* (10^9 CFU/ml) as a function of temperature under different photoperiods (0, 12, and 24 hours of light).

There was an interaction ($p < 0.05$) between photoperiod and temperature on the dimensions of Psg cells. The longest mean length (2.41 µm) and mean width (0.38 µm) of the bacterial cells were observed under photoperiods of 0 hours and 24 hours of light (Figure 3).

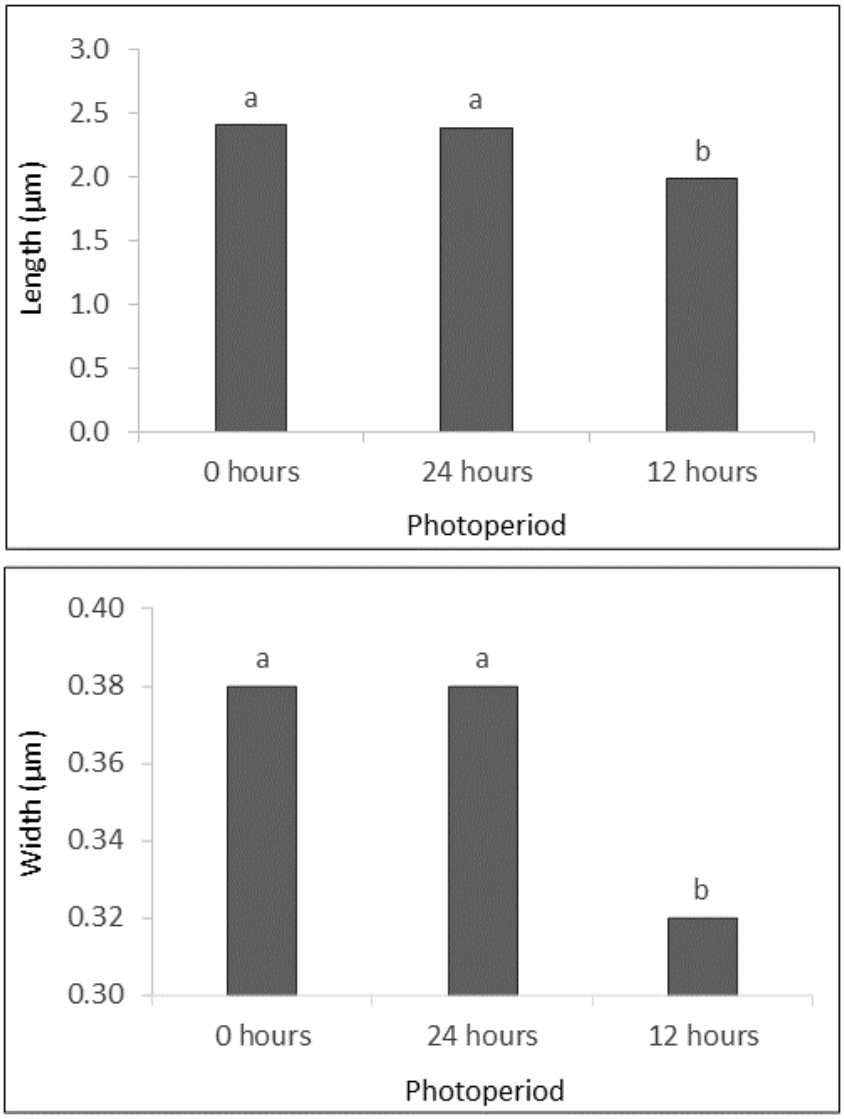

Figure 3. Length and width of *Pseudomonas syringae pv. garcae* cells under different photoperiods. Bars followed by the same letter do not differ by Tukey's test ($p < 0.05$).

At 30 °C, the 12-hours light resulted in the largest bacterial cells, with dimensions of 4.4 µm in length and 0.7 µm in width. No bacterial growth was detected at 40 °C, while at 35 °C, growth occoured only under continuous dark and continuous light conditions (Figure 4).

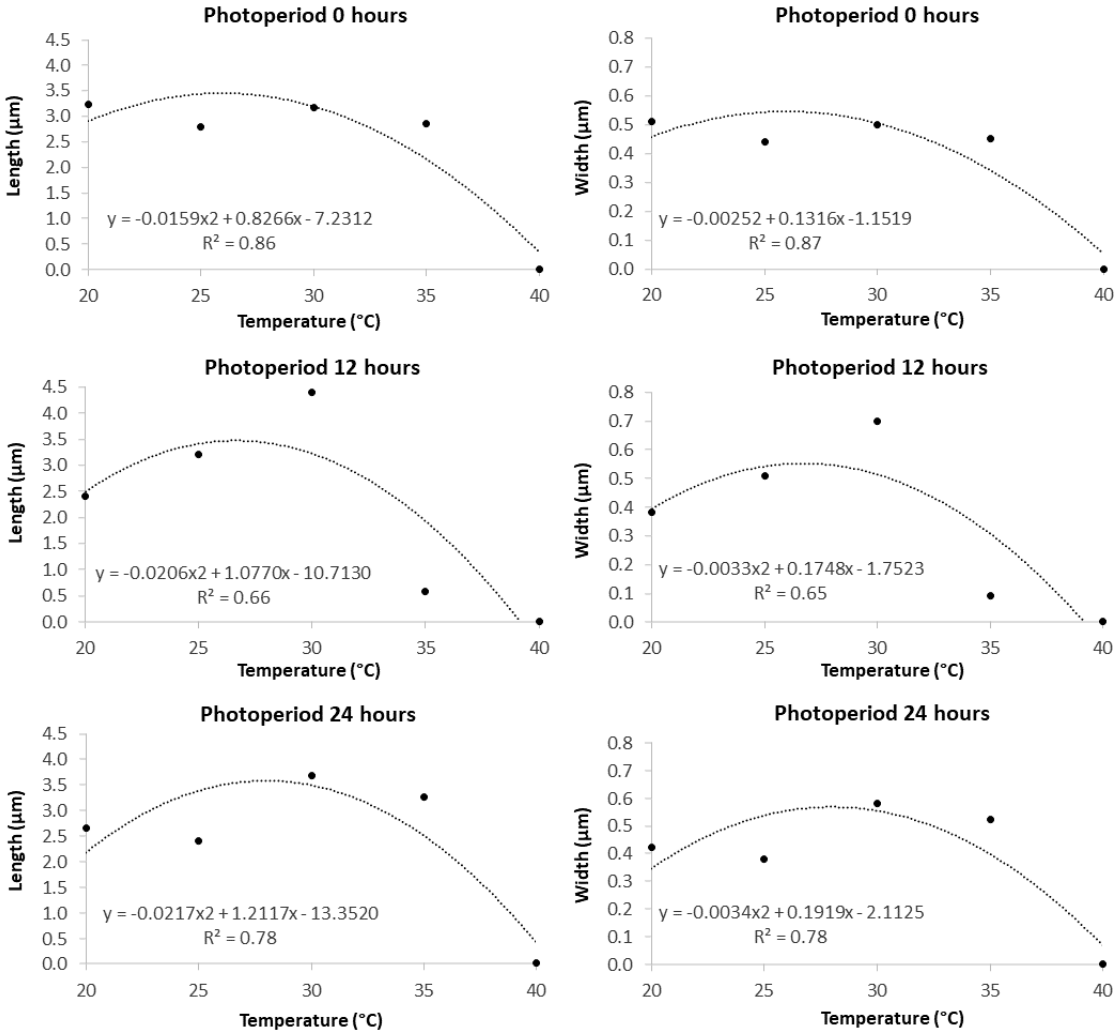

Figure 4. Length and width of *Pseudomonas syringae pv. garcae* bacterial cells as a function of temperature under different photoperiods (0, 12, and 24 hours of light).

The severity of bacterial halo blight in coffee seedlings inoculated with Psg suspensions produced under different temperatures and photoperiods

The highest BHB severity, reaching 4.9 %, was observed 20 days after inoculation (dai) with inoculum produced under a 12-hour light photoperiod at 25 °C (Figure 5). In contrast, the lowest severity (0 %) across all photoperiods occurred two dai when the inoculum was cultivated at 30 °C.

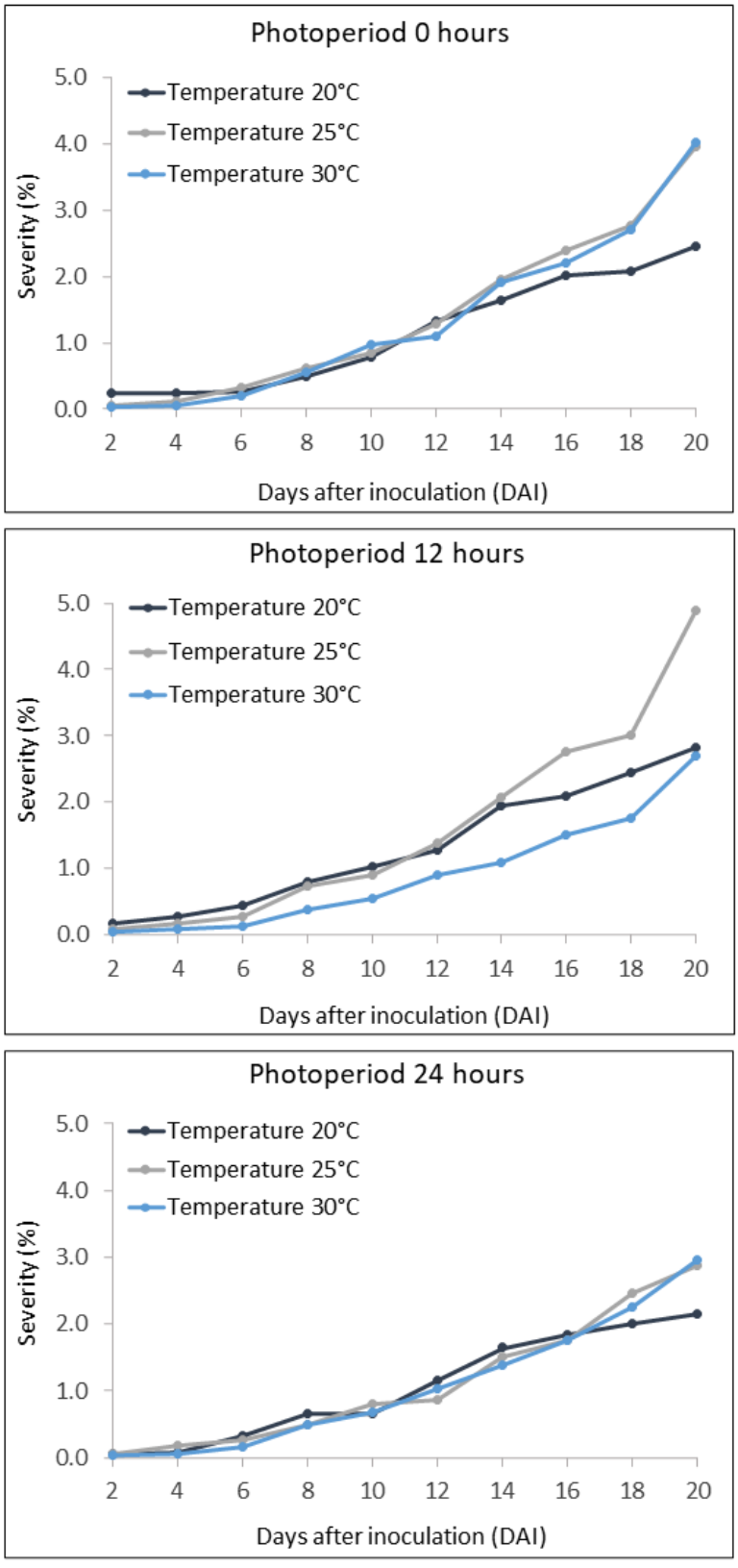

Figure 5. Progress of severity (%) of bacterial halo blight in coffee seedlings (*Coffea arabica* L.) inoculated with *Pseudomonas syringae pv. garcae* suspensions produced under temperatures of 20, 25, and 30°C, and photoperiods of 0, 12, and 24 hours of light.

There was difference ($p < 0.05$) for the AUDPC of BHB when the inoculum was produced at different temperatures. The highest AUDPC was recorded at 25 °C, with a gradual decline observed as the temperature increased. As expected, bacterial cells cultivated at 35 and 40 °C did not result in any symptoms in coffee seedlings, as bacterial multiplication did not occur at this temperature (Figure 6).

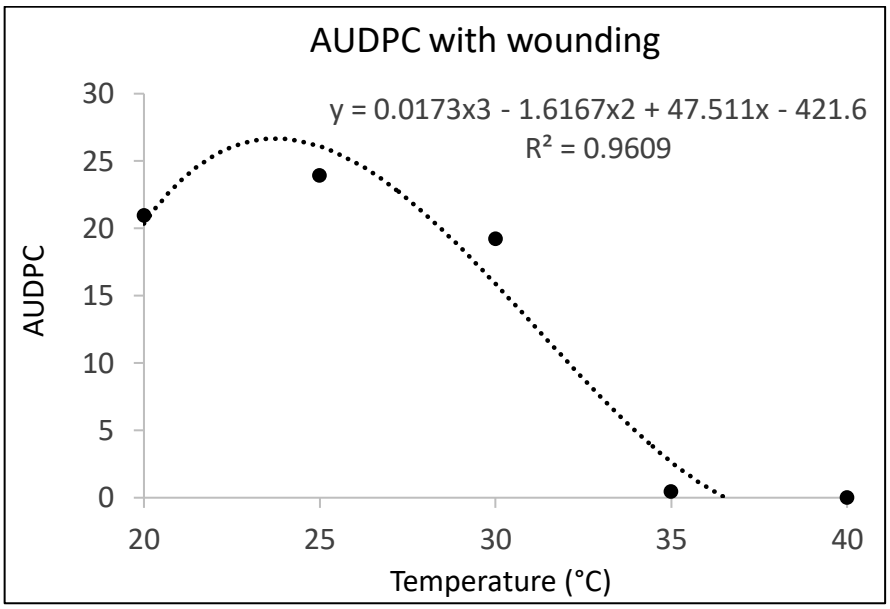

Figure 6. The area under the disease progress curve (AUDPC) of bacterial halo blight (*Pseudomonas syringae pv. garcae*) in coffee seedlings (*Coffea arabica* L.) with wounding inoculation.

There was a significant ($p < 0.05$) negative correlation between the *in vitro* incubation temperature (°C) and AUDPC (-0.73) and between temperature (°C) and inoculum concentration (-0.89) (Table 1). Conversely, there was a significant ($p < 0.05$) positive correlation between the variables AUDPC and inoculum concentration (CFU/ml). In this case, the coefficient was also high (0.82); that is, the higher the inoculum concentration, the higher the AUDPC.

Table 1. Correlation coefficients between the variables temperature (°C), AUDPC, inoculum concentration (CFU/ml), length, and width of bacterial cells.

	Temperature	**AUDPC**	**Concentration**	**Length**	**Width**
Temperature	ns	-0.73*	-0.89*	-0.62*	-0.62*
AUDPC	-0.73*	ns	0.82*	0.55*	0.55*
Concentration	-0.89*	0.82*	ns	0.67*	0.66*
Length	-0.62*	0.55*	0.67*	ns	1.00*
Width	-0.62*	0.55*	0.66*	1.00*	ns

* Pearson correlation ($p < 0.05$); ns = not significant

DISCUSSION

Despite the importance of bacterial diseases, there is limited information in the existing literature concerning the thermal ranges and light regimes conducive to their reproduction. Determining the environmental factors essential for *in vitro* cultivation and plant infection is crucial to supporting artificial inoculation (Mueller and Buck, 2003). In studies involving Psg, Belan et al. (2016) prepared the inoculum at 28 °C with a 24-hour light photoperiod, while Freitas et al. (2022) utilized a 12-hour photoperiod at the same temperature. In contrast, Beriam et al. (2017), Domingues et al. (2015), and Rodrigues et al. (2016), conducted their incubations at 28 °C without photoperiod. Therefore, it is essential to study the optimal temperatures and photoperiods for inoculum production to effectively induce BHB symptoms.

In this study, the highest values of inoculum concentration of Psg were 2.2 x 10^9 CFU/mL obtained at 25 °C under a 24-hour photoperiod. This result is diferred from those found by Freitas (2017), in which the highest Psg *in vitro* growth was obtained in the range of 23 to 31 °C, with a peak at 28.1 °C, with 12-hour light photoperiod. The use of different photoperiods for other *Pseudomonas* genera has been described in the literature. Bodelier et al. (1997) studied the effect of 14- and 20-hour photoperiods in the *Pseudomonas chlororaphis* growth. According to the authors, the 20-hour light caused an increase in *P. chlororaphis* growth. Although often used in some scientific research, this amount of time is unnecessary to obtain a high concentration for Psg inoculum.

The influence of temperature on *in vitro* growth has also been observed in various *Pseudomonas syringae* pathovars. For example, Young et al. (1977) reported a stable growth rate for *P. syringae* within the temperature range of 23 to 33 °C, indicating no significant

alteration in bacterial growth within this range. Similarly, *P. syringae* pv. *phaseolicola* exhibited an optimal growth rate at 27 °C under the same incubation conditions (Nüske and Ritsche, 1989).

In previous studies, various concentrations of Psg suspension have been successfully employed to induce symptoms post-inoculation. Notable concentrations include 10^9 CFU/mL (Ithiru et al., 1970; Belan et al., 2016), 10^8 CFU/mL (Ithiru et al., 2013), and 3 x 10^8 CFU/mL (Rodrigues et al., 2017). The Psg concentration of 1.22 x 10^9 CFU/mL obtained in this study aligns closely with the 1.6 x 10^9 CFU/mL reported by Oliveira et al. (2024).

The photoperiod during inoculum production did not significantly affect the AUDPC, as the concentration was sufficient to cause infection across all three photoperiods studied. In contrast, the temperature during inoculum production had a notable impact on AUDPC, primarily due to its influence on inoculum concentration. The highest disease severity was observed with inoculum produced at 25 °C, while the lowest severity occurred with inoculum produced at 30 °C. This finding is consistent with Freitas et al. (2017), who observed the highest BHB severity in seedlings maintained at 20.9 °C when exposed to temperatures ranging from 15 to 31 °C.

Bacteria of the genus *Pseudomonas* typically range from 1.5 to 5.0 µm in length and 0.5 to 1.0 µm in width (Krieg, 1984). In this study, a temperature of 30 °C combined with a 12-hour light photoperiod produced the largest Psg cells, with dimensions of 4.4 µm in length and 0.7 µm in width, fitting within the expected size range.

Temperature is well-known to influence bacterial cell size and pathogenicity. In this study, the inoculum produced at 25 °C proved to be the most effective in inducing BHB symptoms in coffee seedlings than those produced at 30 °C. While larger bacterial cells might be more effective at colonizing large wounds or damaged tissues, their size could limit their ability to navigate through narrower spaces, potentially restricting their spread within the plant (Yang, Blair, and Salama, 2016).

Bacteria can detect changes in local temperature through multiple mechanisms, including altering DNA topology, RNA structuring and metabolism, and protein activity and processing. The histone-like nucleoid structuring protein, H-NS, present in several Gram-negative pathogens, can repress the expression of virulence genes. At low temperatures, the DNA in the promoter region of certain virulence genes adopts a bent conformation, stabilized by H-NS, which binds to curved DNA at A: T sites, blocking RNA polymerase access to the promoter. At higher temperatures, this homotypic H-NS binding is reduced as the DNA bend relaxes, allowing RNA polymerase access to the promoter. This reduction in RNA polymerase

binding at the promoter leads to decreased transcription of genes encoding virulence factors when the bacterium is outside the host (Lam, Wheeler, and Tang, 2014).

The negative correlation between temperature (°C) and AUDPC, and between temperature and inoculum concentration (CFU/ml), indicated that the lower the temperature, the greater the inoculum concentration, and the highest disease severity. In the study carried out by Young et al. (1977), the growth rate of *P. syringae* fell dramatically at temperatures above 33 °C. Nevertheless, in the same study, all species of bacteria of the *Pseudomonas* genus were tolerant to cold, i.e., psychrotrophic, an important factor explaining the survival of the pathogen and the occurrence of the disease at mild temperatures.

There is little information available in the literature regarding correlations among these factors concerning bacterial diseases. In pathosystems involving certain fungi, such as the genus *Botrytis*, a negative correlation is common; i.e., an increased inoculum concentration and disease severity are observed as a function of decreased temperature (Latorre et al. 2002; Nair and Allen, 1993; Sirjusingh and Sutton, 1996). Conversely, for the variables AUDPC and inoculum concentration (CFU/ml), there was a significant positive correlation with an R^2 of 0.82; that is, the higher the inoculum concentration was, the greater the disease severity. According to Silveira et al. (2003), the severity of melon fruit blotch increased with increasing inoculum concentration of *Acidovorax avenae* subsp. *citrulli*.

CONCLUSIONS

The highest inoculum concentration occoured under a 24-hour photoperiod at the optimal growth temperature of 25 °C for *Pseudomonas syringae* pv. *garcae*, while the higher BHB severity occoured under a 12-hour light photoperiod at same temperature. Notably, larger bacterial cell sizes were observed when incubation occurred at 30 °C under the 12-hour light photoperiod. These findings underscore the importance of carefully controlling temperature and photoperiod to optimize bacterial growth and disease severity, providing crucial insights for the effective management of bacterial halo blight in coffee seedlings.

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REFERENCES

AMARAL, J. F.; TEIXEIRA, C.; PINHEIRO, E. D. A bacterium causing halo blight of coffee. **Arquivos do Instituto Biológico**, São Paulo, 23:151–155, 1956.

ANDRADE, G. C. G. et al. Características culturais e severidade da mancha foliar de Quambalaria eucalypti sob diferentes regimes de temperatura, luz e período de molhamento foliar. **Fitopatologia Brasileira**, 32:329–334. 2007.

COMPANHIA NACIONAL DE ABACTECIMENTO – CONAB, 2024. **Safra brasileira de café**. Available in: <https://www.conab.gov.br/info-agro/safras/cafe>. Access in: July 11, 2024.

BELAN, L. L. et al. Diagrammatic scale for assessment of bacterial blight in coffee leaves. **Journal of Phytopathology**, 162:801–819, 2014a.

BELAN, L. L. et al. Occurrence of *Pseudomonas syringae* pv. *garcae* in coffee seeds. **Australian Journal of Crop Science**, Lavras, 10(7):1015–1021, 2016.

BELAN, L. L.; SOUZA, R. M. A mancha aureolada do cafeeiro. **Revisão Anual de Patologia de Plantas**, 22:227–256, 2014b.

BERIAM, L. O. S.; PATRÍCIO, F. R. A.; MACIEL, K. W. Diferenciação de bactérias do gênero *Pseudomonas* patogênicas ao cafeeiro por técnicas serológicas. **Arquivos do Instituto Biológico**, São Paulo, 84:1–7, 2017.

BODELIER, P. L. E. et al. Effects of photoperiod on growth of and denitrification by *Pseudomonas chlororaphis* in the root zone of *Glyceria maxima*, studied in a gnotobiotic microcosm. **Plant soil**, 190:91–103, 1997.

CAMPBELL, C. L.; MADDEN, L. V. **Introduction to plant disease epidemiology**. New York: John Wiley, 1990. 523p.

COLHOUN, J. Effects of environmental factors on plant disease. **Annual Review of Phytopathology**, 11:343–364. 1973.

DOMINGUES, M. V. P. F. et al. Controle químico e biológico da mancha aureolada em mudas de cafeeiro. In: IX SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, Curitiba–PR, SBICafé, 2015.

FREITAS, M. L. O. et al. Copper formulations in bacterial blight control and toxic effects on coffee seedlings. **Bioscience Journal**, 38, e38043, 2022.

HOCKETT, K. L.; BURCH, A. Y.; LINDOW, S. E. Thermo-regulation of genes mediating motility and plant interactions in *Pseudomonas syringae*. **PLoS One**, San Francisco, 8(3):e59850, 2013.

ITHIRU, J. M. et al. A comparative analysis associated to virulence of *Pseudomonas syringae* pv. *garcae*, the causative agent of bacterial blight of coffee in Kenya. **The Journal of Agricultural Science**, 2:76, 1970.

ITHIRU, J. M. et al. Methods for early evaluation for resistance to bacterial blight of coffee. **African Journal of Agriculture Research**, Lesotho, 8(21):2450–2454, 2013.

KADO, C. I.; HESKETT, M. G. Selective media for isolation of *Agrobacterium*, *Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*. **Phytopathology**, 60:969–976, 1970.

KRIEG, N. R. Gram-negative aerobic rods and cocci (Family I. *Pseudomonadaceae*). In: KRIEG, N. R; HOLT, J. G. **Bergey's Manual of Systematic Bacteriology**, vol. 1. Williams & Wilkins, Baltimore, 1984. p. 140–219.

LAM, O.; WHEELER, J.; TANG, C. M. Thermal control of virulence factors in bacteria: A hot topic. **Virulence**, 5(8):852–862, 2014.

LATORRE, G.; RIOJA, M. E.; LILLO, C. Efecto de la temperatura en el desarrollo de la infección producida por *Botrytis cinerea* en flores y bayas de uva de mesa. **Ciência e Investigacion Agrária**, 29(3):145–152, 2002.

MACIEL, K. W. et al. Bacterial halo blight of coffee crop: Aggressiveness and genetic diversity of strains. **Bragantia**, 77(1):96–106, 1 jan. 2018.

MELOTTO, M. et al. Plant stomata function in innate immunity against bacterial invasion. **Cell**, London, 126(5):969–980, 2006.

MUELLER, D. S.; BUCK, J. W. Effects of light, temperature, and leaf wetness duration on daylily rust. **Plant Disease**, 87:442–445, 2003.

NAIR, N. G.; ALLEN, R. N. Infection of grape flowers and berries by *Botrytis cinerea* as a function of time and temperature. **Mycological Research**, Cambridge, 97(8):1012–1014, 1993.

NÜSKE, J.; FRITSCHE, W. Phaseolotoxin production by *Pseudomonas syringae* pv. *phaseolicola*: the influence of temperature. **Journal of Basic Microbiology**, 29:441–447, 1989.

OLIVEIRA, J. M. et al. Efficiency of inoculation methods for the assessment of bacterial halo blight in coffee seedlings. **Journal of Phytopathology**, 172(3), 2024.

OLIVEIRA, J. R. Idade da folha e suscetibilidade do cafeeiro a *Pseudomonas cichorii* e a *Pseudomonas syringae* pv. *garcae*. 1988. 79p. Dissertação (Mestrado em Fitopatologia) – Universidade Federal de Viçosa, Viçosa, MG, 1988.

PATRÍCIO, F. R. A. et al. Controle químico da mancha aureolada em uma região montanhosa. **Tropical Plant Pathology**, 35:S119, 2010.

PAUL L. E. et al. Effects of photoperiod on growth of and denitrification by *Pseudomonas chlororaphis* in the root zone of *Glyceria maxima*, studied in a gnotobiotic microcosm. **Plant and Soil**, Netherlands, 190:91–103, 1997.

PÉREZ, C. D. P. et al. Nitrogênio e potássio na intensidade da mancha aureolada do cafeeiro em solução nutritiva. **Coffee Science**, 12:60–68, 2017.

POZZA, E. A. et al. Manejo sustentável de doenças em cafeeiros de alta produtividade. In: Santinato F. (Eds.). **A moderna cafeicultura brasileira: tecnologias que afetam a produtividade**. 01 ed. Jaboticabal: Funep, 2022. p. 510–511.

RAIMUNDI, M. K. et al. Diagnosis of leaf bacterial diseases of coffee reveals the prevalence of halo blight. **Ciência e Agrotecnologia**, v. 45, 2021.

RODRIGUES, L. M. et al. Aggressiveness of strains and inoculation methods for resistance assessment to bacterial halo blight on coffee seedlings. **Journal of Phytopathology**, Berlin, 165(2):105–114, 2017.

RODRIGUES, L. M. R. et al. **Mancha aureolada do cafeeiro causada por *Pseudomonas syringae* pv. *garcae***. Campinas: Instituto Agronômico de Campinas, 2013. 24p.

SHANER, G.; FINNEY, R. E. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. **Phytopathology,** 70:1183–1186, 1977.

SILVA, M. G. D. Interação da luz, da temperatura e do molhamento foliar em Cercospora coffeicola e na cercosporiose em cafeeiro. 2014. Tese (Defesa de Doutorado).

SILVEIRA, E. B. et al. Influência da temperatura, umidade, concentração de inóculo de *Acidovorax avenae* subsp. *citrulli* e idade do fruto no desenvolvimento da mancha-aquosa em melão. **Fitopatologia Brasileira**, 29(1):36, 2004.

SIRJUSINGH, C.; SUTTON, J.C. Effects of wetness duration and temperature on infection of geranium by *Botrytis cinerea*. **Plant Disease**, St. Paul, 80(2):160–165, 1996.

UNITED STATES DEPARTMENT OF AGRICULTURE – USDA. 2024. **Production – coffee**. Available in: <https://fas.usda.gov/data/production/commodity/0711100>. Access in: August 9, 2024.

YANG, D. C.; BLAIR, K. M.; SALAMA, N. R. Staying in Shape: The Impact of Cell Shape on Bacterial Survival in Diverse Environments. **Microbiology and Molecular Biology Reviews**, 80(1):187–203, 2016.

YOUNG, J. M.; LUKETINA, R. C.; MARSHALL, A. M. The effects on temperature on growth in vitro of *Pseudomonas syringae* and *Xanthomonas pruni*. **Journal of Applied Bacteriology**, Oxford, 42(3):345–354, 1977.

(Formatação baseada nas exigências da *Plant Pathology)*

ARTIGO 3

Geostatistical analysis of the spatiotemporal distribution of bacterial halo blight on coffee seedlings

Análise geoestatística da distribuição espaço temporal da mancha aureolada em mudas de cafeeiro

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Geostatistical analysis of the spatiotemporal distribution of bacterial halo blight on coffee seedlings

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SUMMARY

Bacterial halo blight (BHB), caused by *Pseudomonas syringae* pv. *garcae*, is a major bacterial threat to coffee nurseries and can significantly reduce crop yields. Understanding its spatiotemporal distribution is critical for improving disease management. This study aimed to analyze the spatial and temporal progression of BHB in coffee seedling nurseries using a predefined initial inoculum. The experiment was conducted in a coffee nursery with *Coffea arabica* Catuaí Vermelho IAC 99 seedlings. Four seedbeds were delimited, 1.05 m wide by 2 m long, containing 496 seedlings each, arranged in rows and columns. Four central seedlings in each seedbed were inoculated with *P. syringae pv. garcae* at 10^8 CFU/mL using a multi-needle technique and reintroduced once symptoms appeared. Disease progression was monitored over eight weeks. Climatic data, including rainfall, temperature, and relative humidity, were recorded, and geostatistical methods were applied to evaluate the spatial

distribution of infected plants. Symptoms first appeared in non-inoculated seedlings 16 days after the reintroduction of infected plants, with the highest infection rate, reaching 9%, observed on day 49. Spatial analysis revealed that the disease spread up to 47 cm from the initial inoculum within 37 days, with a strong spatial dependence, fitting a spherical semivariogram model. Kriging maps demonstrated the formation of secondary foci over time, and coalescence was observed as the epidemic progressed. A positive correlation between rainfall and disease incidence was found, indicating that favorable environmental conditions accelerated disease spread. These findings offer valuable insights to the control of BHB in nurseries.

Keywords: *Coffea arabica.* epidemiology. epidemic. geostatistics. *Pseudomonas syringae* pv. *garcae.* spatial distribution.

INTRODUCTION

Coffee (*Coffea* spp.) is one of the most important commodities traded globally. Brazil is the largest producer and exporter, playing a crucial role in the country's economy and supporting millions of livelihoods. In 2023, Brazil's coffee production reached 55.1 million 60-kg bags of green bean equivalent, reflecting an 8.2% increase compared to the previous year (Conab 2024). However, production is often threatened by several diseases, including bacterial halo blight (BHB), caused by *Pseudomonas syringae* pv. *garcae* (Amaral et al. 1956). This bacterium can cause losses of up to 70% in seedling nurseries and mature crops, particularly in regions above 900 meters in altitude with temperatures around 20°C, where crops are exposed to wind, have over 4 hours of leaf wetness, or are harvested mechanically (Pozza et al. 2022).

This disease mainly affects new tissues in seedlings and newly planted crops. It causes dark irregular spots and yellowish halo in developed leaves, and dryness in branches, and fruits, reducing coffee productivity in Brazil's major coffee-producing regions such as Minas

Gerais, São Paulo, and Paraná. However, with the advent of mechanized harvesting, these symptoms have become more severe in lignified branches in mature crops due to injuries caused by the machinery, allowing the pathogen to penetrate both plagiotropic and orthotropic branches (Belan and Souza 2014; Pozza et al. 2021).

Based on this current scenario, disease control is essential, involving the use of antibiotics and copper-based bacteriostatic products in sprays. However, when the disease is present in the field alongside other species or pathovars, such as bacterial spot (*Pseudomonas syringae* pv. *tabaci*), chemical control becomes less effective, and there are few resistant cultivars available (Fernandes et al. 2020; Marin et al. 2018; Pozza et al. 2021). The principle of exclusion can be applied to reduce the use of these chemicals and promote environmental and financial sustainability for producers. This involves reducing the initial inoculum by using healthy seedlings and allowing only clean, disinfected machinery into the field to prevent the pathogen's spread to disease-free areas (Belan et al. 2016).

The favorable environment for BHB epidemics in nurseries is due to the dense planting of seedlings, constant irrigation, and frequent contact between leaves. Under these conditions, it is common to observe clumps of infected seedlings (Belan and Souza, 2014) forming a disease gradient that spreads from a central point and can intensify over time (Belan et al. 2018). This variation in BHB incidence, originating from an initial inoculum source, can be studied using geostatistics, a tool used to analyze the epidemiological aspects of plant diseases and model spatial progression (Alves et al. 2006; Alves et al. 2009; Alves et al. 2010; Belan et al. 2018; Leal et al. 2010; Mouen Bedimo et al. 2007; Roham et al. 2014).

Geostatistics has previously been employed by Belan et al. (2018) to model the spatial progression of BHB in a commercial coffee nursery with agrochemical applications, focusing on naturally randomly occurring initial foci. However, viable inoculum of *P. syringae pv. garcae* has been detected in coffee seeds from infected plants. Consequently, seeds can also

contribute to the spread of the bacterium in seedling nurseries, leading to random foci of diseased seedlings (Belan et al. 2016). When these seedlings are transplanted into the field, they may serve as an initial inoculum for future disease epidemics, provided environmental conditions are favorable and the cultivar is susceptible. This can facilitate the long-distance dissemination of the bacterium.

Monitoring an epidemic from its onset until symptom stabilization allows the acquisition of more accurate data on the spatiotemporal dynamics of the disease, which will significantly inform and guide decision-making regarding management strategies. Understanding the rate and pattern of disease progression over time and space may inform management practices, such as increasing seedling spacing, applying copper-based fungicides, eliminating initial foci, and avoiding the early deployment of seedlings from affected areas. Therefore, the aim with this study was to evaluate the spatial and temporal progression of a BHB epidemic in a nursery, using a predefined initial inoculum of *P. syringae pv. garcae* in a susceptible coffee cultivar, and to examine the correlation between disease dynamics and environmental variables.

MATERIALS AND METHODS

Experimental area

The study was conducted in a coffee seedling nursery (*C. arabica* L.), using the Catuaí Vermelho IAC 99 cultivar, susceptible to *P. syringae* pv. *garcae*. The nursery is located in Lavras, Minas Gerais, Brazil, at geographical coordinates 21°13'35.36"S and 44°58'31.04"W, at an altitude of 918 m. A CR10X meteorological station from Campbell Scientific Inc.® was installed in the nursery to monitor weather variables, including temperature, relative humidity,

leaf wetness, and wind speed and direction during the experiment. The nursery was shaded with a 5-mm polyethylene screen that allowed 50% of sunlight to pass through.

The seedlings were arranged side by side, without spacing (248 seedlings/m^2), totaling 1,984 seedlings distributed in 16 rows and 31 columns across four seedbeds (1.05 × 2 meters), each containing 496 seedlings. The seedlings were grown in polyethylene bags with a diameter of 0.065 m and a height of 0.20 m, and the distance between the seedling stems was 6.5 cm. These containers were filled with a substrate composed of soil, cattle manure, and sand in a 2:1:1 ratio. Irrigation was performed twice daily by sprinkling for 15 minutes, providing approximately 6.5 mm of water a day. Other cultural practices, such as fertilization, were carried out following the recommendations for the use of soil conditioners and fertilizers for seedling nurseries in Minas Gerais (Ribeiro et al. 1999). Fungal disease management was conducted with two applications of the fungicides Nativo® (Trifloxystrobin and Tebuconazole) and Cercobin 875 WG (Thiophanate-methyl) at the recommended doses for coffee crops, in December 2016 and January 2017.

The inoculum was prepared from *P. syringae pv. garcae* pathotype 'souche' (CIRM, plant-associated bacteria; CFBP1634) on plates containing Kado & Heskett (1970) 523 medium using parallel streaks. The plates were stored in a BOD incubator for 24 hours at 25°C with a 12-hour photoperiod, using fluorescent lights with an intensity of 3707.41 lux. Pure bacterial colonies were suspended in a sterile saline solution (0.85% NaCl) at a bacterial concentration of 10^8 CFU/mL, determined using a spectrophotometer (OD_{600}), as described by Oliveira and Romeiro (1990). Inoculation was performed by wounding the leaf lamina with a multi-needle tool on four seedlings taken from the center of each seedbed, once the first pair of leaves had fully developed. These inoculated seedlings were kept at the same environmental conditions, but separate from the others until the first symptoms appeared and the identity of the *P. syringae*

126 *pv. garcae* pathogen was confirmed. Then, the seedlings were placed back in the center of each
127 seedbed (columns 14 and 15; rows 7 and 8) on January 17, 2017.
128
129 **Assessment of bacterial halo blight**
130
131 Starting on January 17, 2017, after the seedlings with symptoms were placed in the
132 seedbeds, the healthy seedlings were visually inspected daily. Assessments of disease severity
133 (percentage of leaf area affected) and incidence began on February 2, 2017 (16 days later) when
134 the first symptoms were detected in previously healthy seedlings. The assessments occurred
135 every 7 days, in four leaves per seedling. When the lower canopy of the seedlings was difficult
136 to view, the leaves were gently opened with a 10 cm wooden stick to check for symptoms. The
137 stick was immediately discarded after use to prevent cross-contamination of other leaves.
138 The evaluation period was 8 weeks until the disease progression stabilized. The severity
139 assessments were based on the diagrammatic scale proposed by Belan et al. (2014). Weekly
140 data on the average severity percentage for the four leaves per seedling, for each position in the
141 grid of the four seedbeds, were used for geostatistical analysis.
142
143 **Temporal analysis and correlation with climatic variables**
144
145 Progress curves of the incidence of BHB were plotted using the incidence values; these
146 values were calculated as the percentage of seedlings with symptoms, average among the four
147 seedbeds over time (days after the first evaluation - DAFE) (Campbell and Madden 1990). The
148 averages of the meteorological variables (rainfall, wind speed, temperature, relative humidity,
149 hours of leaf wetness, and sun exposure) of the 1st, 2nd, 3rd, 4th, 7th, and 10th days before the
150 weekly evaluations were plotted. Pearson's correlation analysis was performed between

meteorological variables and the incidence of diseased seedlings at 1, 2, 3, 4, 7, and 10 days before the weekly evaluations. The “SigmaPlot version 14.0” program was used. The weather station used was a Campbell Scientific model CR1000. The software “SigmaPlot version 14.0” was used to plot the graphs.

Three-dimensional spatial analysis of disease progression

Three-dimensional graphs were generated to show the incidence and mean severity of haloed spot disease in the four seedbeds over time (Campbell and Madden 1990). The graphs were made in the software “SigmaPlot version 14.0”.

Georeferenced sampling mesh

A regular grid was established for each site with 496 georeferenced points; these points were distributed at a spacing of 0.065 m×0.065 m, occupying the entire experimental area of each site (2.1 m^2). Considering the average diameter of the polyethylene bags (0.065 m) used to produce the seedlings, the seedlings were georeferenced in the seedbeds in the coordinate system (x;y). For this purpose, one of the site vertices was considered as the coordinate (0;0). Thus, for the seedlings located in the first row and first column, the coordinate of (0.033; 0.033) was given. The location of the healthy and diseased plants in each evaluation enabled the elaboration of maps representing the progress of the disease over time.

Model fit

The semivariograms are represented by graphs of the estimated semivariance ($\hat{\gamma}(h)$) versus distance (h). The data were fitted to the theoretical model of the spherical semivariogram (Burrough and McDonnell 1986). The fit of the model was obtained using the ordinary least squares (OLS) method.

$$\gamma(h) = \begin{cases} 0, h = 0 \\ (C_0)^2 + \frac{a}{h} \cdot sen\left(\frac{h}{a}\right), h > 0 \end{cases}$$

From the fit of the mathematical model to the calculated values of $\hat{\gamma}(h)$, the parameters of the theoretical model for the semivariogram, were estimated; these parameters included the nugget effect (C_0), threshold (C_0+C), and reach (a).

Kriging mapping

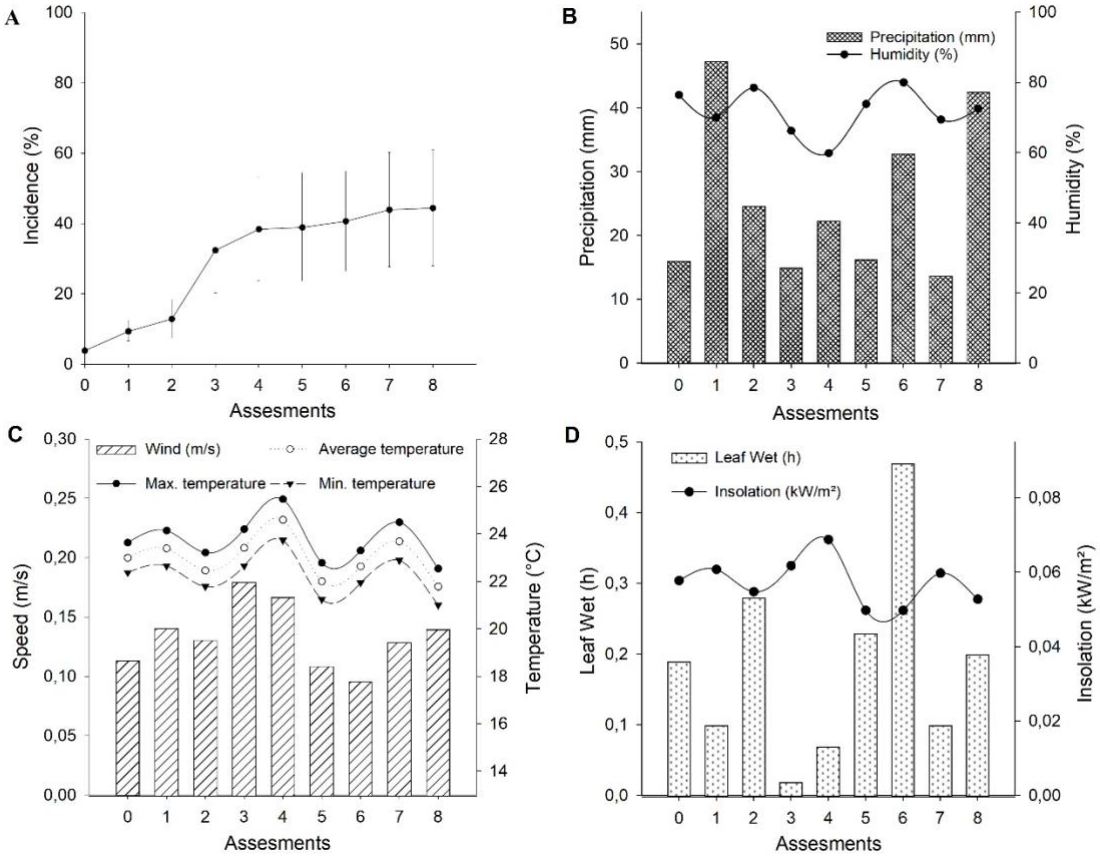
After the adjustment of the semivariograms, data interpolation was performed by ordinary kriging to visualize the spatial distribution patterns of the disease over time.

For the statistical and geostatistical analyses and the plotting of the maps, the computer system "R" was used with the geoR package (Diggle and Ribeiro Junior 2007).

RESULTS

During the epidemic, a disease gradient from the four diseased seedlings was introduced to the center of the seedbed. Specifically, dissemination of *P. syringae pv. garcae* and progression of the BHB occurred over time. The interval was 16 days between the introduction of seedlings with symptoms until the detection of symptoms of BHB. Thereafter,

197 temporal progression of the disease occurred in the 4 beds (Figure 1A), with a consequent
198 increase in the number of seedlings with symptoms of the disease over time around these central
199 seedlings (Figure 1A). An increase in the incidence of the disease over time was observed, and
200 the maximum value of 9% or 44 diseased plants was found 49 days after the beginning of the
201 epidemic. Then, the disease epidemic stabilized, and no further increases in the number of
202 plants with symptoms occurred.
203



204

205 **Fig 1** Progress curve of the incidence of bacterial halo blight *(P. syringae* pv. *garcae*) (A),
206 average precipitation and average relative humidity (RH) (B), maximum, average, and
207 minimum temperatures and average wind speed (C), and hours with average leaf wetness and
208 average sun exposure (D) collected weekly in the coffee seedling nursery between the
209 experimental time.
210

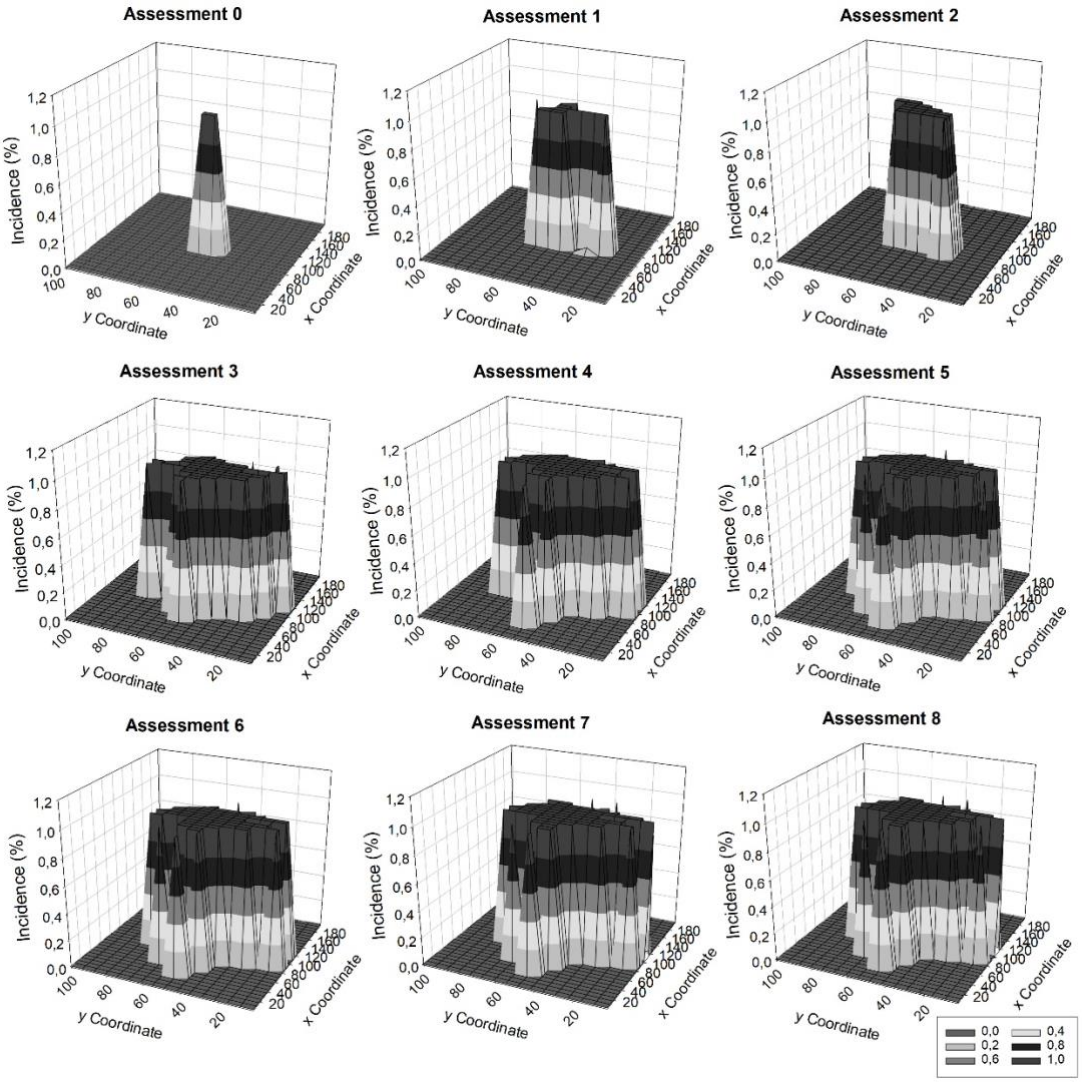
During the disease epidemic, an increase in precipitation was observed (Figure 1B), but no correlation existed between the variables relative to air humidity, temperature, wind speed, or insolation. The number of hours of leaf wetness and incidence of BHB were measured in the seedling nursery (Table 1). A significant positive correlation was observed between the rainfall variable and the disease incidence ($p < 0.01$, Table 1).

Table 1 - Correlation coefficients between the incidence of bacterial halo blight of coffee tree in the seedling nursery and the variables of rainfall, relative air humidity, temperature, mean wind speed, insolation, and number of hours with leaf wetness at 1, 2, 3, 4, 7 and 10 days before the evaluation of the disease. Lavras – MG.

	1 day	**2 days**	**3 days**	**4 days**	**7 days**	**10 days**
Precipitation (mm)	0.404^{ns}	**0.659***	0.558^{ns}	0.563^{ns}	0.542^{ns}	0.589^{ns}
Relative air humidity	-0.338^{ns}	-0.129^{ns}	-0.059^{ns}	0.001^{ns}	-0.098^{ns}	-0.116^{ns}
Wind Speed (m/s)	0.532^{ns}	0.304^{ns}	0.066^{ns}	-0.171^{ns}	-0.186^{ns}	-0.236^{ns}
Average Temperature (°C)	-0.108^{ns}	-0.120^{ns}	-0.107^{ns}	-0.096^{ns}	-0.075^{ns}	-0.029^{ns}
Maximum Temperature (°C)	-0.091^{ns}	-0.098^{ns}	-0.104^{ns}	-0.009^{ns}	-0.060^{ns}	-0.011^{ns}
Minimum Temperature (°C)	-0.117^{ns}	-0.140^{ns}	-0.125^{ns}	-0.105^{ns}	-0.110^{ns}	-0.069^{ns}
Leaf wetting (h)	0.219^{ns}	0.225^{ns}	0.151^{ns}	0.174^{ns}	0.136^{ns}	0.175^{ns}
Insolation (kW/m^2)	0.283^{ns}	0.383^{ns}	-0.017^{ns}	-0.108^{ns}	-0.107^{ns}	-0.220^{ns}

*Pearson correlation (p <0.01); ns=not significant

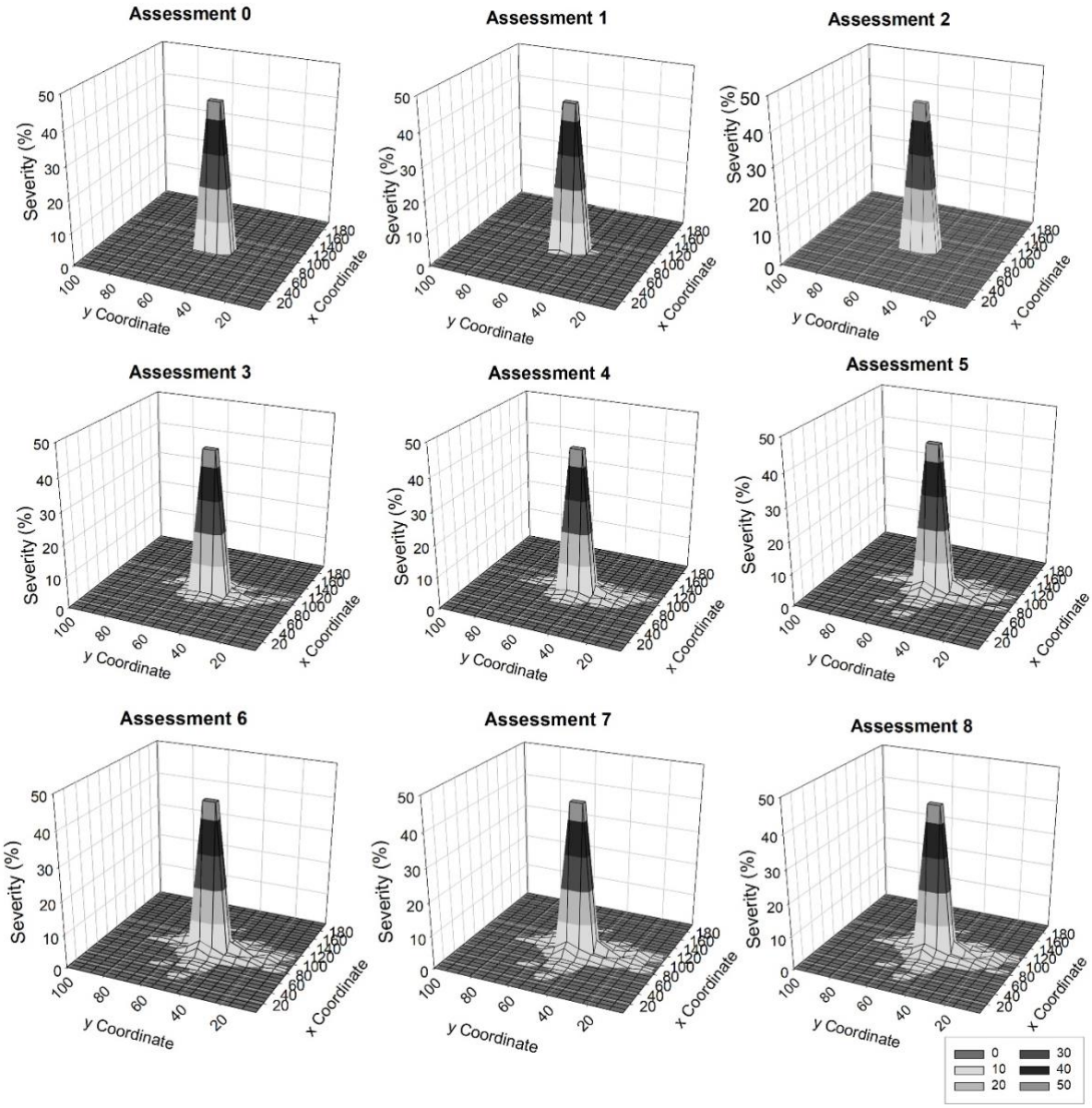
During the epidemic, spatial dispersion of the inoculum from the four diseased seedlings (initial inoculum) introduced in the center of the bed was observed, with consequent spatiotemporal progression of the incidence and severity of BHB (Figures 2 and 3).



226

227 **Fig 2** Three-dimensional graphs of the average incidence of bacterial halo blight (*Pseudomonas*
228 *syringae* pv. *garcae*) on coffee seedlings (*Coffea arabica* L.) in the nursery during all
229 assessments.

230



231

232 **Fig 3** Three-dimensional graphs of the means of the four beds with bacterial halo blight
233 (*Pseudomonas syringae* pv. *garcae*) on coffee seedlings (*Coffea arabica* L.) in the nursery
234 during all assessments.

235

236 Spatial dependence of BHB in the coffee seedling nursery was observed (Table 2). The
237 spherical isotropic model was adequate for representing the phenomenon under study, and the
238 parameters of this model were used to prepare the maps by kriging (Table 2, Figure 5).

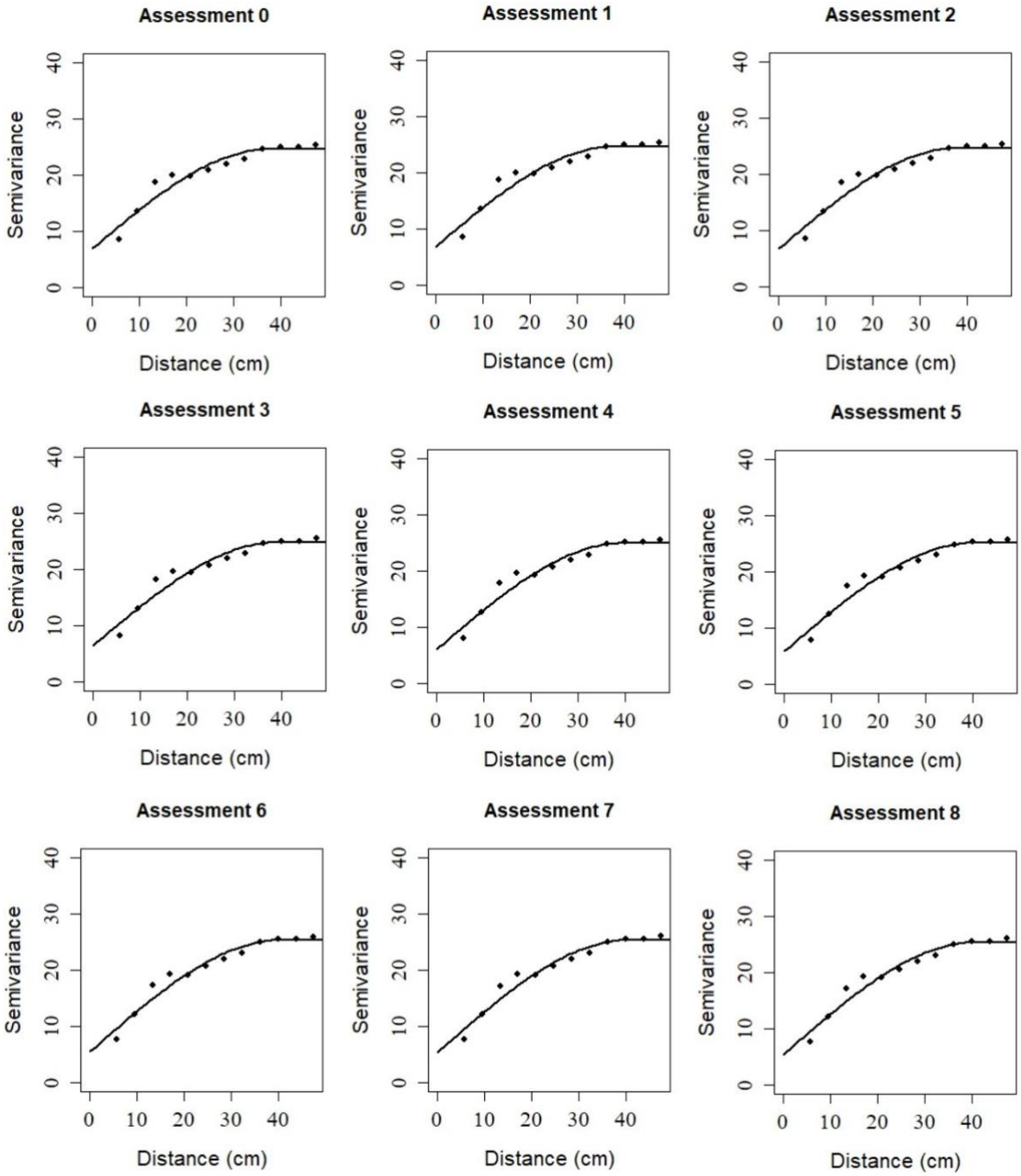
239

240 Table 2 - Parameters and estimated coefficients of the spherical semivariogram obtained by the
241 ordinary least squares (OLS) method on all evaluation times.

Evaluation	Model	A_o	C_o	$C+C_o$	$Co/C+C_o$	DEG
0	**Spherical**	42.45	4.12	21.26	0.20	**Strong**
1	**Spherical**	43.74	4.12	20.58	0.20	**Strong**
2	**Spherical**	46.31	4.12	21.95	0.19	**Strong**
3	**Spherical**	45.04	3.60	21.50	0.16	**Strong**
4	**Spherical**	47.60	3.46	22.11	0.16	**Strong**
5	**Spherical**	46.31	3.48	22.29	0.16	**Strong**
6	**Spherical**	46.31	2.81	23.16	0.12	**Strong**
7	**Spherical**	47.60	3.52	22.50	0.16	**Strong**
8	**Spherical**	47.60	3.51	22.50	0.16	**Strong**

242 A_o = reach, C_o = nugget effect, $C+C_o$ = plateau, $Co/C+C_o$ = ratio indicative of the degree of
243 spatial dependence (DGE, where 0 to 0.25 = strong, 0.25 to 0.75 = moderate and 0.75 to 1 =
244 weak) (Cambardella et al., 1994).
245

246 The degree of dependence was strong, with the number of DEGs ranging from 0.12 to
247 0.20 (Table 2). An increase in the plateau value over time was observed (Table 2, Figure 4).
248 The lowest reach value was approximately 43 cm in the second evaluation after the introduction
249 of the initial inoculum in the seedbed, corresponding to an increase in the number of
250 symptomatic seedlings and/or the appearance of secondary foci of the disease until reaching
251 approximately 47 cm.
252



253

254 **Fig 4** Graphs of the semivariograms fitted to the spherical model, using the ordinary least
255 squares (OLS) method, of the severity of bacterial halo blight (*Pseudomonas syringae* pv.
256 *garcae*) in coffee (*Coffea arabica* L.) seedlings in the nursery.

257

258 After confirming spatial dependence, kriging maps of the disease progression over time
259 were generated (Figure 5). At the beginning of the epidemic, only the seedlings introduced into

the seedbed showed symptoms of the disease (Figure 5). However, from the second evaluation onward, secondary disease foci were identified around the inoculum/initial focus. Over time, an increase in the size of these foci, lateral expansion, and coalescence was observed (Figure 5). Based on the disease distribution maps (Figure 5), an aggregate pattern of the spatial distribution of symptomatic seedlings was observed, and this pattern was maintained over time.

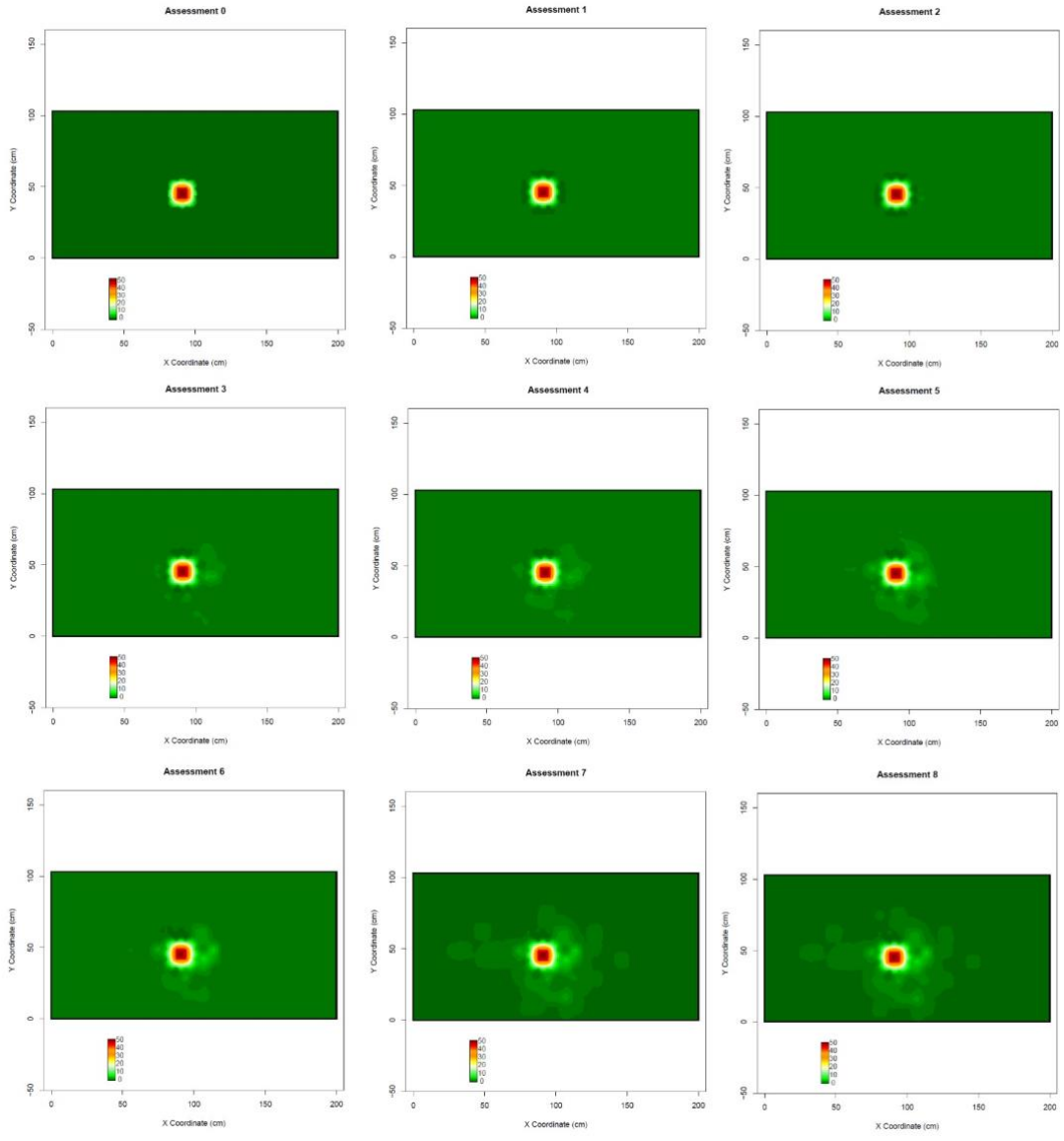


Fig 5 Kriging maps of bacterial halo blight (*Pseudomonas syringae* pv. *garcae*) on coffee (*Coffea arabica* L.) seedlings in a nursery.

DISCUSSION

This study analyzed the spatiotemporal distribution of BHB in coffee seedling nurseries. Controlled infection sites were established by introducing four infected seedlings at the center of each seedbed, enabling close monitoring from the moment of inoculation until disease progression stabilized. As expected, the number of seedlings exhibiting symptoms increased progressively from the central infected seedlings over time. Interestingly, our findings revealed a 9% infection rate 49 days after the onset of the epidemic, which contrasts with the higher 56% incidence reported by Belan et al. (2015) in commercial nurseries over a similar time (61 days). This discrepancy can be attributed to the differences in experimental setup. While our study employed a single-point inoculation, in their study, disease outbreaks arose from naturally occurring, with random foci that likely accelerated disease spread through multiple foci, contributing to higher infection rates.

The environmental conditions in coffee nurseries, including high plant density and the use of sprinkler irrigation, create a conducive environment for BHB epidemics. Previous studies (Carmo et al. 1996; Romeiro 2005; Marcuzzo et al. 2008) have highlighted the importance of climatic variables, such as high humidity and temperature, in promoting the spread of bacterial diseases. Similar to our findings, Carmo et al. (2001) demonstrated a strong positive correlation between disease severity and favorable environmental conditions in pepper plants infected with *Xanthomonas campestris* pv. *vesicatoria*. In nurseries, higher temperatures and excess moisture further facilitate pathogen spread, which underscores the role of microclimatic factors in bacterial disease dynamics (Belan and Souza 2014; Romeiro 2005). According to Romeiro (1976), environmental and microclimatic conditions are among the key factors determining the success or failure of bacterial infections. In his experiments, meteorological data such as relative humidity, precipitation, leaf wetness, air temperature, wind speed, and direction were

295 monitored and recorded. A positive correlation was observed between rainfall and disease
296 incidence two days before assessments, indicating that increased precipitation at the onset of
297 the epidemic favored the dissemination of the disease.

298 Marcuzzo and Denardim (2008) proposed a model to represent the infectious process of
299 phytobacteria in plants. According to this model, favorable environmental conditions
300 (temperature, relative humidity, and leaf wetness) are necessary for reproduction and infection
301 once bacteria reach the leaf surface. This explains the correlation with precipitation; the higher
302 the amount of rainfall, the greater the likelihood of favorable microclimatic conditions for
303 bacterial multiplication (Romeiro 2005) and the formation of aggregates for infection (Souza
304 et al. 2019).

305 The spatial dynamics of BHB in our study also demonstrated a clear spatial dependence on
306 the distance between seedlings and the initial inoculum. The spherical model fitted to the data
307 showed a maximum range of approximately 47 cm at 37 days post-inoculation, consistent with
308 the findings of Belan et al. (2015), who reported a range of 45.6 cm at 36 days in commercial
309 nurseries. The spherical model has also been fitted for diseases with an aggregated distribution
310 pattern (Alves et al. 2006; Alves et al. 2011; Moraes 2014; Mouen Bedimo et al. 2007).

311 According to McBratney and Webster (1986), the nugget effect is an important
312 parameter for the semivariogram as it indicates the unexplained sample variability, given the
313 sampling distance used, highlighting the quality of the model fit. The nugget effect values
314 obtained in this study were considered high (< 25% of the sill value) (Cambardella et al. 1994).

315 The kriging maps generated in our study visually illustrated the increase in symptomatic
316 seedlings over time, highlighting the formation of both primary and secondary foci as the
317 disease spread outward from the initial inoculum. The formation of secondary foci, observed
318 as early as the second assessment at 26 cm from the initial focus, aligns with previous research
319 on the spread of bacterial and fungal pathogens in different crops (Gottwald et al. 1989; Alves

et al. 2006). Similar to the dynamics reported in the *X. campestris* pv. *citri* pathosystem in citrus and *Colletotrichum lindemuthianum* in common bean, we observed rapid disease progression in secondary foci, particularly when climatic conditions favored the epidemic and the host is susceptible to infection (Carmo et al. 1996; Carmo et al. 2001; Gottwald et al. 1989; Gottwald 2010; Rodrigues et al. 2013; Romeiro 2005), especially in bacterial pathosystems. The coalescence of these foci over time contributed to the overall spread of the disease within the nursery.

Therefore, the BHB epidemic in the coffee seedling nursery began 16 days after the initial inoculation and lasted eight weeks. The spatiotemporal progression of the disease was strongly influenced by the initial inoculum, with the pathogen spreading more than 40 cm from the source within 40 days. This study underscores the importance of spatial factors, including plant density, environmental conditions, and proximity to the initial focus, in determining the spread of BHB. The findings also emphasize the role of both primary and secondary infection foci in accelerating disease epidemics, highlighting the need for effective management strategies to limit the spread of BHB in nurseries.

Early detection of BHB in nurseries is essential for implementing effective phytosanitary measures to control the disease. Immediate and proper removal of infected seedlings is crucial to prevent the emergence of new foci and the spread of the bacteria, both within the nursery and in the field, as these seedlings will later be transplanted. If they are infected with *P. syringae pv. garcae* they could serve as a source of inoculum for a potential epidemic. Once the infection becomes established in the plants, controlling the disease becomes more challenging, as it primarily relies on preventive measures using contact products, such as copper-based compounds (Freitas et al. 2022). These insights into BHB epidemiology provide coffee growers with a practical and reliable framework for implementing effective management strategies, helping to reduce crop losses and prevent the spread of *P. syringae pv. garcae*.

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Ethical approval

The author confirms no ethical issues in the article's publication.

Competing interests

The author has no competing interests to declare relevant to this article's content.

Data availability

Data sharing does not apply to this article as no new data were created or analyzed in this study.

REFERENCES

Alves MC, da Silva FM, Moraes JC, Pozza EA, de Oliveira MS. et al. (2011) Geostatistical analysis of the spatial variation of the berry borer and leaf miner in a coffee agroecosystem. *Precision agriculture*, 12:18–31.

368 Alves MC, da Silva FM, Pozza EA, de Oliveira MS (2009) Modeling spatial variability and
369 pattern of rust and brown eye spot in coffee agroecosystem. *Journal of Pest Science*, 82:137–
370 148.
371
372 Alves MC, Pozza EA (2010) Indicator kriging modeling epidemiology of common bean
373 anthracnose. *Applied Geomatics*, 2:65–72.
374
375 Alves MC, Pozza EA, Machado JC, Araújo DV, Talamini V, Oliveira MS (2006) Geoestatística
376 como metodologia para estudar a dinâmica espaço-temporal de doenças associadas a
377 *Colletotrichum spp.* transmitidos por sementes. *Fitopatologia Brasileira*, 31:557–563.
378
379 Amaral JF, Teixeira C, Pinheiro ED (1956) A bacterium causing halo blight of coffee. *Arquivos*
380 *do Instituto Biológico*, 23:151–155.
381
382 Belan LL, Pozza EA, Freitas MLO, Raimundi MK, Souza RM, Machado JC (2016) Occurrence
383 of *Pseudomonas syringae* pv. *garcae* in coffee seeds. *Australian Journal of Crop Science*,
384 10:1015–1021.
385
386 Belan LL, Pozza EA, Alves MC, Freitas MLO (2018) Geostatistical analysis of bacterial blight
387 in coffee tree seedlings in the nursery. *Summa Phytopathologica*, 44:317–325.
388
389 Belan LL; Pozza EA; Freitas MLO.; Souza RM, Jesus Junior WC; Oliveira JM (2014)
390 Diagrammatic Scale for Assessment of Bacterial Blight in Coffee Leaves. *Journal of*
391 *Phytopathology*, 162:801–810.
392

393 Belan LL, Souza RM (2014) A Mancha Aureolada do Cafeeiro. *Revisão Anual de Patologia de*
394 *Plantas*, 22:227–256.
395
396 Belan LL, Pozza EA; Freitas MLO, Pozza AAA, De Abreu MS, Alves E (2015) Nutrients
397 distribution in diseased coffee leaf tissue. *Australasian Plant Pathology*, 44:105–111.
398
399 Burrough PA, Mcdonnell RA (1986) *Principles of geographical information systems.* Ed.
400 London: Oxford University Press, 330p.
401
402 Cambardella CA, Moorman TB, Novak JM, Parkin TB, Karlen DL, Turco RF et al. (1994)
403 Field-scale variability of soil properties in Central Iowa soils. *Soil Science of America Journal*,
404 Madison, 58, 4:1501–1511.
405
406 Campbell CL, Madden, LV (1990) *Introduction to Plant Disease Epidemiology.* New York,
407 NY: John Wiley & Sons.
408
409 Carmo MGF, Kimura O, Maffia LA, Carvalho AOC (1996) Progresso da pústula bacteriana do
410 pimentão, causada por *Xanthomonas campestris* pv. *vesicatoria* em condições de viveiro.
411 *Fitopatologia Brasileira*, 20:66–70.
412
413 Carmo MGF, Macagnan D, Carvalho AO (2001) Progresso da mancha-bacteriana do pimentão
414 a partir de diferentes níveis iniciais de inóculo e do emprego ou não do controle com oxicloreto
415 de cobre. *Horticultura Brasileira*, 19:210–215.
416

417 Companhia Nacional de Abactecimento, CONAB (2024) Produção de café cresce 8,2% em
418 2023 e chega a 55,1 milhões de sacas. https://www.conab.gov.br/ultimas-noticias/5323-
419 producao-de-cafe-cresce-8-2-em-2023-e-chega-a-55-1-milhoes-de-sacas. Accessed 11
420 september 2024.
421
422 Diggle PJ, Ribeiro Junior PJ (2007) *Model-based geostatistics*. Springer, New York. 238p.
423
424 Fernandes LE, dos Santos WG, Carducci FC, Fonseca ICB, Rodrigues LMR, Beriam LOS et al
425 (2020) Resistance of Arabica coffee cultivars to leaf wounds and *Pseudomonas syringae* under
426 field conditions. *Australian Journal of Crop Science*, 14:46–50.
427
428 Freitas MLO, Pozza EA, Neto HS, Pozza AAA, Belan LL, Da Silva HR et al. (2022) Copper
429 formulations in bacterial blight control and toxic effects on coffee seedlings. *Bioscience*
430 *Journal*, 38, pp. e38043.
431
432 Gottwald TR, Miller C, Brlansky RH, Gabrie LDW, Civerolo EL (1989) Analysis of spatial
433 distribution of citrus bacterial spot in a Florida citrus nursery. *Plant Disease*, 73:207–303.
434
435 Gottwald, TR (2010) Current Epidemiological Understanding of Citrus Huanglongbing. *Annual*
436 *Review of Phytopathology*, 48:119–139.
437
438 Kado CI, Heskett MG (1970) Selective media for isolation of *Agrobacterium*,
439 *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. *Phytopathology*, 60:969–976.
440

441 Leal RM, Barbosa JC, Costa MG, Junior JB, Yamamoto PT, Dragone, J (2010) Distribuição
442 espacial de huanglongbing (greening) em citros utilizando a geoestatística. *Revista Brasileira*
443 *de Fruticultura*, 32:808–818.
444
445 Marcuzzo LL, Denardim ND (2008). Colonização de bactérias causadoras de doenças foliares.
446 *Revista de Ciências Agroveterinárias*, 7(2):169–176.
447
448 Marin DB, Alves MC, Pozza EA, Belan LL, Freitas MLO (2019) Multispectral radiometric
449 monitoring of bacterial blight of coffee. *Precision Agriculture*, 20:959–982.
450
451 McBratney AB, Webster R (1986). Choosing functions for semi-variograms of soil properties
452 and fitting them to sampling estimates. *Journal Soil Science*, 37(4):617–639.
453
454 Moraes WB (2014) Dinâmica espaço-temporal da murcha de Ceratocystis em *Mangifera indica*
455 e associação da doença a potenciais vetores. Dissertação de Mestrado. Viçosa, Universidade
456 Federal de Viçosa.
457
458 Mouen Bedimo J, Bieysse D, Cilas C, Nottéghem J (2007) Spatio-temporal dynamics of arabica
459 coffee berry disease caused by *Colletotrichum kahawae* on a plot scale. *Plant Disease*,
460 91:1229–1236.
461
462 Oliveira JR, Romeiro RS (1990) Reação de folhas novas e velhas de cafeeiro a infecção por
463 *Pseudomonas cichorii* e *P. syringae* pv. *garcae. Fitopatologia Brasileira*, 15(4):355–356.
464 1990.
465

Pozza EA (2021) Diagnose e controle de doenças. In: CARVALHO, G. R. *et al*. *Cafeicultura do Cerrado.* Belo Horizonte: EPAMIG, 564p.

Pozza EA, Santinato F, Sera GH, Matiello JB, Filho OG, Santinato R, Silva RP, Benvenga SR (2022) Manejo sustentável de doenças em cafeeiros de alta produtividade. In: Santinato F (Eds.). *A moderna cafeicultura brasileira: tecnologias que afetam a produtividade*. 01 ed. Jaboticabal: Funep, pp. 510–511.

Ribeiro AC, Guimaraes PTG, Alvarez VVH (1999) *Recomendação para o uso de corretivos e fertilizantes em Minas Gerais.* Viçosa: Comissão de Fertilidade do Solo do Estado de Minas Gerais, 359p.

Roham R, Pirdashti H, Yaghubi M, Nematzadeh G (2014) Spatial distribution of nutsedge (*Cyperus spp.* L.*)* seed bank in rice growth cycle using geostatistics. *Crop Protection*, 55:133–141.

Romeiro RS (1976) *Identificação de bactérias fitopatogênicas*. Viçosa, Imprensa universitária. 58p.

Romeiro RS (2005) *Bactérias Fitopatogênicas.* Editora UFV. 2 ed. Viçosa, 417p.

Roumagnac P, Pruvost O, Chiroleu F, Hughes G (2004) Spatial and temporal analyses of bacterial blight of onion caused by *Xanthomonas axonopodis* pv. *allii*. *Phytopathology*, 94:138–146.

491 Souza JOG, Pozza EA, Belan LL, Freitas, MLO, Oliveira JM (2019) Initial stages of infectious
492 process of *Pseudomonas syringae* pv. *garcae* in coffee leaves. *Coffee Science,* 14:338–348.