Key factors to inoculate *Botrytis cinerea* in tomato plants

Álefe Vitorino Borges[1], Rodrigo Moreira Saraiva[1], Luiz Antonio Maffia[1]

[1]Universidade Federal de Viçosa (UFV), Departamento de Fitopatologia 36570-000 Viçosa, MG, Brazil.
Autor para correspondência: Luiz Antonio Maffia (lamaffia@ufv.br)
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ABSTRACT

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Studies addressing the biological control of *Botrytis cinerea* have been unsuccessful because of fails in inoculating tomato plants with the pathogen. With the aim of establishing a methodology for inoculation into stems, experiments were designed to assess: i. the aggressiveness of pathogen isolates; ii. the age at which tomato plants should be inoculated; iii. the susceptibility of tissues at different stem heights; iv. the need for a moist chamber after inoculation; and v. the effectiveness of gelatin regarding inoculum adhesion. Infection with an isolate from tomato plants that was previously inoculated into petioles and then re-isolated was successful. An isolate from strawberry plants was also aggressive, although less than that from tomato plants. Tomato plants close to flowering, at 65 days after sowing, and younger, middle and apical stem portions were more susceptible. There was positive correlation between lesion length and sporulation and between lesion length and broken stems. Lesion length and the percentage of sporulation sites were reduced by using a moist chamber and were not affected by adding gelatin to the inoculum suspension. This methodology has been adopted in studies of *B. cinerea* in tomato plants showing reproducible results. The obtained results may assist researchers who study the gray mold.

Additional keywords: *Solanum lycopersicum*, gray mold, plant age, inoculation site, methodology.

RESUMO

Borges, A.V.; Saraiva, R.M.; Maffia, L.A. **Fatores-chave para a inoculação de *Botrytis cinerea* em tomateiros.** ***Summa Phytopathologica,*** v.40, n.3, p.221-225, 2014.

Estudos abordando o controle biológico de *Botrytis cinerea* vêm tendo insucesso por causa de falhas na inoculação de plantas de tomate com o patógeno. Com o objetivo de estabelecer uma metodologia de inoculação em hastes, experimentos foram delineados para avaliar: i. a agressividade de isolados do patógeno; ii. a idade em que as plantas de tomate devem ser inoculadas; iii. a suscetibilidade de tecidos em diferentes alturas do caule; iv. a necessidade de uma câmara úmida após inoculação; e v. a efetividade da gelatina com relação à adesão do inóculo. A infecção com um isolado de plantas de tomate que foi previamente inoculado em pecíolos e então reisolado foi bem sucedida. Um isolado de plantas de morango também foi agressivo, embora menos que o de plantas de tomate. Tomateiros próximos ao florescimento, aos 65 dias após a semeadura, e porções mais novas de caule, meio e ápice, foram mais suscetíveis. Houve correlação positiva entre comprimento de lesão e esporulação e entre comprimento de lesão e caules quebrados. O comprimento de lesão e a percentagem de lesões com esporulação foram reduzidos pelo uso de câmara úmida e não afetados pela adição de gelatina à suspensão de inóculo. Esta metodologia tem sido adotada em estudos sobre *B. cinerea* em plantas de tomate com resultados reproduzíveis. Os resultados obtidos podem auxiliar pesquisadores que estudam o mofo cinzento.

Palavras-chave adicionais: *Solanum lycopersicum*, mofo cinzento, idade da planta, local de inoculação, metodologia.

The protected cultivation of tomato (*Solanum lycopersicum* L.) is common in a large number of countries and constitutes an interesting alternative for production in off-season periods and in areas where outdoor cultivation is difficult. In this type of cultivation, which has been increasing in Brazil, the crop is protected from rain excess and the temperature is more stable. In addition, yields and production cycles are increased under protected cultivation. However, environmental conditions favorable to pathogens, including *Botrytis cinerea* Pers.: Fr, which causes gray mold or stem canker, may occur in tomato protected cultivation (10).

Over 200 plant species, including tomato plant, are hosts of *B. cinerea*, which infects the flowers, fruits, leaves and stems (12). The infections can lead to severe defoliation, flower death (24), reduction in the fruit market value, stem lesions and, consequently, plant death (9). When the severity of gray mold is high, stem breakage may occur. Tomato stem lesions can result from infections in wounds caused by the defoliation or pruning of plants and/or fungal colonization from the petiole (9, 21, 24). The disease can cause severe losses in greenhouses, leading to the early death of more than 70% of tomato plants (23) and, thus, reducing yields (21).

High relative humidity (>90%) combined with mild temperatures (<20ºC) favors the development of gray mold (16). The effect of temperature on stem infection is similar to what has been reported for other plant organs, which have shown optimum range for infection between 10 and 20°C, although infection may occur from 5 to 26°C (21). Moreover, other factors such as the plant age and the infection

site along with the stem may affect the host susceptibility (10), which decreases with the time following injury (21). These data were obtained from studies conducted in temperate regions. In Brazil, gray mold has been found in tomato plants grown in protected environments near Viçosa, MG (L. A. Maffia, personal communication), although most reports of disease occurrence are from Rio Grande do Sul (4, 18): the disease was found in two of three counties in surveys performed in commercial greenhouses (4). According to those authors, the highest incidence of diseased plants in one of the study regions, from August to October, resulted from gray mold: in October, the incidence was 55%, even following fungicide application.

The significance of gray mold will increase with an increasing area of tomato plants grown under protected cultivation. Therefore, the adoption of disease management measures is vital to ensure successful cultivation. At the Biological Control Unit of "Universidade Federal de Viçosa - UFV", we have been working on gray mold biocontrol since the 1990s in strawberry, roses, eucalyptus and, more recently, tomatoes. Studies aimed at biocontrol in tomato plants have been unsuccessful due to problems with *B. cinerea* inoculation. Understanding the factors related to the successful infection of plants with *B. cinerea* is essential for disease management studies. Thus, the aim of the present study was to define a working methodology to optimize the occurrence of disease from injuries on tomato stems. Specifically, we evaluated: i. the aggressiveness of *B. cinerea* isolates; ii. the optimal plant age for inoculation; iii. the susceptibility of tissues along the stem; iv. the need for a moist chamber to ensure infection; and v. the effectiveness of gelatin regarding adhesion of the pathogen inoculum. Thus, we aimed to define a reproducible methodology for inoculating *B. cinerea* into tomato plants under Brazilian conditions.

MATERIAL AND METHODS

Experiments were performed at the Biological Control Unit and greenhouses of the Department of Plant Pathology of the UFV.

Two *B. cinerea* isolates and a sub-isolate were used: ToV_1b, from tomato plants, preserved on mycelial discs using Castellani's method (5) since 2004 (hereafter referred to as T0); sub-isolate T1, from re-isolation of T0 after its inoculation in tomato tissue; and M1, from strawberry plants. Discs of T0 were placed on plates with potato dextrose agar (PDA) medium, and samples from mycelial growth were picked into tubes with PDA and incubated at 20ºC. To obtain the sub-isolate T1, mycelial discs of 1 cm in diameter were cut from the edges of actively growing colonies of T0 and transferred to wounds done with a knife in fragments of tomato petioles of approximately 5 cm length. The inoculated fragments were kept on nylon screens and moist foam in square plastic boxes and kept at 20 ± 2ºC under a 12 h photoperiod. After 5 days, there was abundant sporulation on the tissue, and conidia were directly transferred with a needle to tubes with PDA, originating the sub-isolate T1. Isolate M1 was directly isolated from greenhouse strawberry plants, from fruits with intense sporulation, to PDA in tubes, by using a needle.

All isolates were grown in tubes with PDA and kept at 20 ± 2ºC under a 12 h photoperiod. After 12 days, conidia were harvested with a glass rod, suspended in sterile distilled water and filtered through two layers of cheese cloth to prepare the inoculum. The suspension was adjusted to $1x10^5$ conidia.mL^{-1} by using a hemocytometer.

'Santa Clara' tomato cultivar was sown on expanded 128-cell polystyrene trays (Styrofoam) containing Tropstrato® commercial organic substrate in a greenhouse at 25 ± 2 ºC. After 25-30 days, the seedlings were transplanted to 10cm-diameter (1L) plastic pots containing the same substrate, then irrigated daily and fertilized according to the plants needs.

Two experiments were performed. In the first one, the effects of the pathogen isolates, the tomato plant age, the height of inoculation on the stem and the use of a moist chamber on the intensity of gray mold were evaluated. Therefore, T0, T1 or M1 was inoculated into tomato plants at 45, 55 or 65 days after sowing on the basal, middle or apical third of the plants, either using a moist chamber or not (moistened cotton wrapped in plastic tape at the inoculation site, left for 24 h following inoculation). Wounds were inflicted via leaf excision on basal, middle and apical third (for all ages) and also sprout excision on middle third (for 65-day-old plants) using pruning shears, and 30 µL of a conidial suspension were applied to each wound.

In the second experiment, the effects of gelatin, as employed by Nobre et al. (20) in leaves, and the use of a moist chamber on the success of inoculation were assessed. Based on the results of the first experiment, T1 was inoculated into wounds at different stem heights of 65-day-old tomato after sowing. A 30µL *B. cinerea* conidial suspension was applied for inoculation, either by adding colorless gelatin (0.1% w/v) or not and with or without a moist chamber.

Each experiment was performed twice, each one under a completely randomized design in a factorial scheme. The first experiment included the factors isolate, plant age, height of inoculation on the stem and moist chamber, with four replicates (one experimental unit = one tomato plant). The second experiment included the factors inoculation height, gelatin, and moist chamber, with three replicates. In all trials, after inoculation the plants were kept at 18 ± 2°C under a 12 h photoperiod in a growth chamber. The gray mold severity, assessed based on the length of lesions, the occurrence of pathogen sporulation on lesions and the broken stem were evaluated at eight days after inoculation. The lesion lengths were expressed as cm, while sporulation sites and broken stem were expressed as percentage (%). As there was no difference in homoscedasticity between the two runs for each experiment, both were analyzed together. The lesion length values were subjected to analysis of variance (ANOVA) based on Marini (19) and, when appropriate, means were compared by using Tukey's test ($\alpha = 0.05$). A descriptive analysis was performed based on the results regarding the occurrence of pathogen sporulation and broken stem. Spearman correlation analysis between lesion lengths, sporulation sites and broken stems was performed. SAS v. 9.1 software was used in the statistical analyses.

RESULTS AND DISCUSSION

After several attempts at the Biological Control Unit, it was not possible to assess the biocontrol of gray mold in tomato plants because of the unsuccessful inoculation of *B. cinerea* into stems. Previously, the pathogen was inoculated into leaves (20), which has been performed by a number of authors (2, 3, 17). A reproducible method for pathogen inoculation is needed because the disease is predominantly found in stems under commercial cultivation, and leaf tests may not mimic field conditions (25). In the present study, key aspects defining stem inoculation methodology, which is the most commonly used technique experimentally (7, 21, 22), were assessed in wounded plant stems. It is worthy to point that the methodology above has been used in temperate countries. In Brazil, where the climate is different from that of temperate countries, both plant and pathogen may behave differently. Therefore, the following study was needed.

The lesion lengths were positively and significantly ($P = 0.0001$)

correlated with sporulation site percentage (r = 0.74) and broken stem percentage (r = 0.51) in the first experiment. Weeker correlation occurred between sporulating site percentage and broken stem percentage (r = 0.30).

Isolate T0 caused no lesions in any of the inoculated plants in either run in the first experiment. Therefore, it was not included in the ANOVA regarding lesion length. There was no significant effect of quadruple (P = 0.8573) or triple (P always above 0.4687) interactions. Regarding the double interactions, there was a significant effect of isolate x inoculation site (P = 0.0016) and plant age x inoculation site (P = 0.0092). Thus, unfolding and analysis were performed within each factor with 14 replicates (the effect of moist chamber was disregarded for the obtained means).

The isolates differed in aggressiveness: T0, originally from tomato plants and preserved, was not aggressive in stems. Higher lesion length values were associated with T1 (Figure 1A), which also sporulated more in lesions, mainly at sprouting wounds (Figure 1B). For both isolates, higher percentage of broken stems occurred when the sprouting wound was inoculated (Figure 1C).

B. cinerea isolates have been reported to maintain their pathogenicity after preservation for four years in glycerol, sand or silica gel, although various isolates have lost the resistance to procymidone; sclerotia sizes and sporulation decreased for some isolates, regardless of the applied preservation method (8). Considering that: i. *B. cinerea* is highly variable, and several factors may affect its aggressiveness (21); ii. the statistical and biological variability of studies may be affected by the selected isolate (6); iii. in the study conducted by Eden et al. (11), tomatoes were inoculated with isolates prior to cultivation for spore production to avoid virulence loss; and iv. we found important results, it is recommended the inoculation of preserved isolates into living tissue prior to their use. The isolate originating from strawberry plants was, unsurprisingly, aggressive given the low specificity of this pathogen. For example, O'Neill et al. (21) used an isolate from cucumber plants in studies on tomato plants. As previously suggested, the fungus should preferably be inoculated into tissues of solanaceous plants (preferably tomato) prior to using it in tomato plants.

In a previous study conducted in Brazil, disease was not observed in 30-day-old tomato seedlings inoculated with *B. cinerea*. In the present study, in general, the older the tomato plant and the younger the involved plant tissue (middle and apical stem), the greater the lesion length (Figure 2A). Similar trends were found regarding occurrence of sporulation, particularly when the inoculation was conducted in the middle third of the plant (Figure 2B). Moreover, the stems broke primarily in the sprouting and apical wound (Figure 2C).

The highest values for lesion length were found in plants at early flowering, approximately 65 days after sowing. It is usually assumed that young plants are not very susceptible to this pathogen and that older plants are more commonly infected (10), although plants aged between 28 and 120 days have been successfully inoculated (21, 25). However, several authors have inoculated plants at approximately 60 days after sowing (1, 22), and upon natural inoculation, the first symptoms appeared at day 64 (18). Thus, given these findings and those of the present study, it is recommended to inoculate tomato plants that are at least 65 days old, preferably close to flowering.

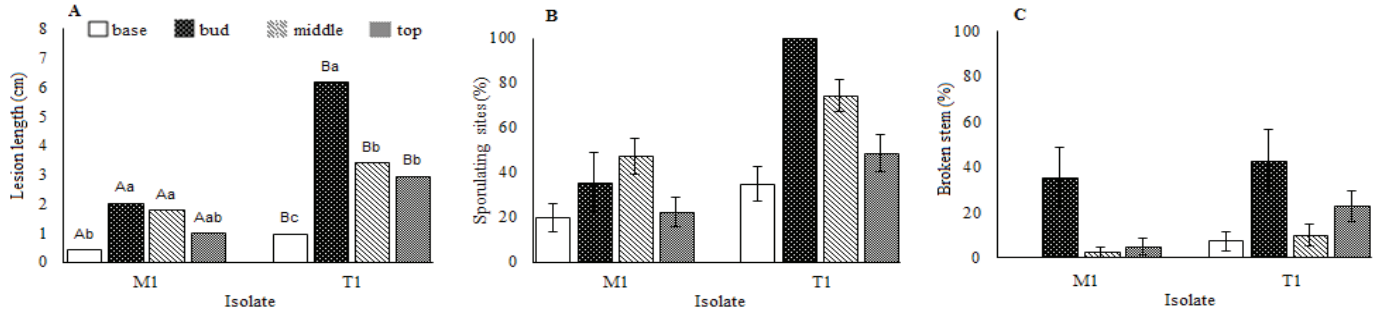

Figure 1. Lesion length (A), sporulating site percentage (B) and broken stem percentage (C) in tomato plants inoculated with *Botrytis cinerea* isolate (M1 or T1) at different stem heights. Means of two experimental runs were pooled (n = 14). In A, means followed by the same uppercase letter regarding different isolate in each stem heights and lowercase letter regarding different stem heights in each isolate do not differ significantly (Tukey, α = 0.05). In B and C, the vertical bars represent the mean standard errors.

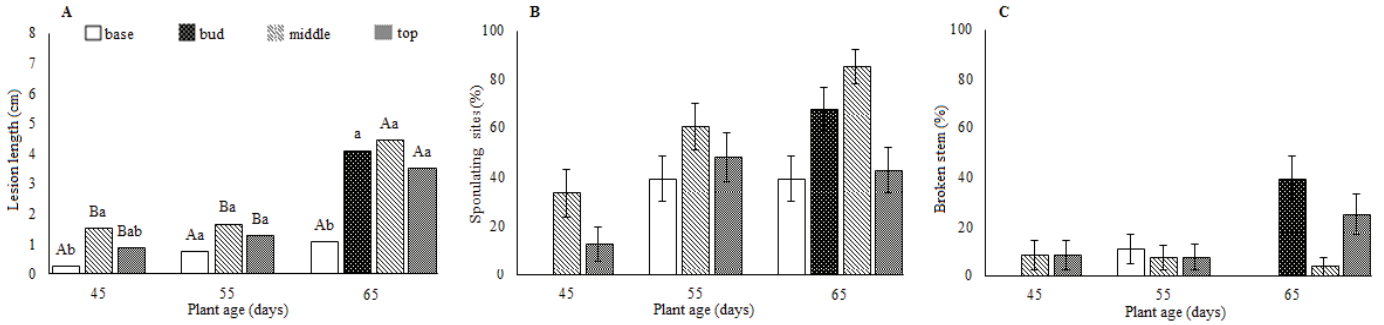

Figure 2. Lesion length (A), sporulating site percentage (B) and broken stem percentage (C) in tomato plants inoculated with *Botrytis cinerea* on different ages (days after sowing) and at different stem heights. Means of two experimental runs were pooled (n = 14). In A, means followed by the same uppercase letter regarding different ages in each stem height and lowercase letter regarding different stem heights in each age do not differ significantly (Tukey, α = 0.05). In B and C, the vertical bars represent the mean standard errors.

The height of inoculation in the stem also affected the results obtained in this study, since the lesion length, pathogen sporulation and broken stem intensity were higher in the middle and apical thirds of the tomato plants. The higher lesion length may be explained by the differences in the physiological state of the inoculated tissues, as the degree of tissue lignification and, consequently, resistance to the pathogen are greatest at the basal sections of stems (15). Studies conducted by using stem segments have shown less progress of infections at the basal and apical segments than at the middle segment (25) or no effect of the site on the infection incidence or lesion size (21). Despite this last contradictory result, considering previous data and those obtained in the present study, it is recommended that *B. cinerea* be mainly inoculated in the middle third and higher heights of tomato plants.

The use of a moist chamber significantly reduced the lesion length ($P = 0.0086$) and resulted in a lesion length of 0.82 cm for M1 and 1.96 cm for T1; without the moist chamber the values were 1.33 cm for M1 and 2.91 cm for T1. Lower sporulation values were also found with the use of the moist chamber: 15% for M1 and 39% for T1; and 45% for M1 and 66% for T1 without the moist chamber. At first, this previously reported finding (9, 11, 21) appears contradictory because spore germination and infection are water-dependent. However, the wounded tissue apparently provides moisture and nutrients for germination and infection (9, 21). It is also likely that the condition of the damp tissue hinders mycelial growth in wounded tissues (24) and that excess moisture may flush out nutrients that facilitate the germination of conidia in the wound (21). The subsequent expansion of the lesion is dependent on the water content of the colonized tissue. In any case, droplet inoculation with fungal conidia into newly wounded tissue is sufficient to enable infection, without the need for a moist chamber.

In tomato leaves, droplet inoculation of a conidial suspension containing gelatin increased the success of infection with *B. cinerea* (20). In the present study, this procedure did not increase the inoculation efficiency; the applied suspension droplet was small (30 µL) and was introduced immediately after wounding in the stem, which facilitated inoculum adhesion.

This is a pioneer study for Brazilian conditions. It was decided to inoculate wounded stems given the significance of infections in this organ (21), which mainly occur through wounds resulting from the excision of leaves and lateral shoots (1, 7, 21). Other authors have assessed the results of inoculation with this pathogen between 4-5 days (13, 22) and 8-12 days (9, 14) after inoculation. In the present study, lesion length, sporulation and stem breakage were assessed at 8 days after inoculation, when fungal sporulation covered the lesions. These procedures have been adopted in biocontrol assays involving *B. cinerea*, inoculating the middle third of plants at 65 days after sowing, without using a moist chamber. The results have been reproducible, enabling the treatment efficiency to be compared. Therefore, the aim is to make a contribution to other researchers who may study gray mold in tomato plants, mainly to Brazilian researchers.

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REFERENCES

1. Aissat, K.; Nicot, P. C.; Guechi, A.; Bardin, M.; Chibane, M. Grey mould development in greenhouse tomatoes under drip and furrow irrigation. Agronomy for Sustainable Development, Paris, v. 28, n. 3, p.403-409, 2008.
2. Akram, A.; Ongena, M.; Duby, F.; Dommes, J.; Thonart, P. Systemic resistance and lipoxygenase-related defence response induced in tomato by Pseudomonas putida strain BTP1. **BMC Plant Biology**, London, v. 8, p.113, 2008.
3. Audenaert, K.; Meyer, G. B.; Höfte, M. M. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. **Plant Physiology**, Bethesda, v. 128, n. 2, p.491-501, 2002.
4. Blume, E.; Jara, A. S. A. Moléstias em tomateiro cultivado em estufas plásticas em quatro municípios da região central do Rio Grande do Sul, Brasil. **Ciência Rural**, Santa Maria, v. 34, n. 3, p.661-666, 2004.
5. Castellani, A. Maintenance and Cultivation of the common pathogenic fungi of man in sterile distilled water. Further researches. **Journal of Tropical Medicine and Hygiene**, Baltimore, v. 70, p.181-184, 1967.
6. Chardonnet, C. O.; Sams, C. E.; Trigiano, R. N.; Conway, W. S. Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. **Phytopatology**, Saint Paul, v. 90, n. 7, p.769-774, 2000.
7. Decognet, V.; Ravetti, F.; Martin, C.; Nicot, P. C. Improved leaf pruning reduces development of stem cankers caused by grey mould in greenhouse tomatoes. Agronomy for Sustainable Development, Paris, v. 30, n. 2, p.465–472, 2010.
8. Delcán, J.; Moyano, C.; Raposo, R.; Melgarejo, P. Storage of *Botrytis cinerea* using different methods. **Journal of Plant Pathology**, Bari, v. 84, n. 1, p.3-9, 2002.
9. Dik, A. J.; Koning, G.; Kohl, J. Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. **European Journal of Plant Pathology**, Wageningen, v. 105, n. 2, p.115–122, 1999.
10. Dik, A. J.; Wubben, J. P. Epidemiology of *Botrytis cinerea* diseases in greenhouses. In: Elad, Y.; Williamson, B.; Tudzinski, P.; Delen, N. (Ed) ***Botrytis*: biology, pathology and control. The Netherlands, Dordrecht: Kluwer Academic Publishers, 2004. p.319-331.
11. Eden, M. A.; Hill, R. A.; Beresford, R.; Stewart, A. The influence of inoculum concentration, relative humidity, and temperature on infection of greenhouse tomatoes by *Botrytis cinerea*. **Plant Pathology**, New York, v. 45, n. 4, p.795-806, 1996.
12. Elad, Y.; Williamson, B.; Tudzinski, P.; Delen, N. *Botrytis* spp. and diseases they cause in agricultural systems – an introduction. In: Elad, Y.; Williamson, B.; Tudzinski, P.; Delen, N. (Ed) ***Botrytis*: biology, pathology and control. The Netherlands, Dordrecht: Kluwer Academic Publishers, 2004. p.1-6.
13. Finkers, R.; van den Berg, P.; van Berloo, R.; ten Have, A.; van Heusden, A. W.; van Kan, J. A. L.; Lindhout, P. Three QTLs for *Botrytis cinerea* resistance in tomato. **Theoretical and Applied Genetics**, Berlin, v. 114, n. 4, p.585-593, 2007a.
14. Finkers, R.; van Heusden, A. W.; Meijer-Dekens, F.; van Kan, J. A. L.; Maris, P.; Lindhout, P. The construction of a *Solanum habrochaites* LYC4 introgression line population and the identification of QTLs for resistance to *Botrytis cinerea.* Theoretical and Applied Genetics, Berlin, v. 114, n. 6, p.1071-1080, 2007b.
15. Glazener, J. A. Accumulation of phenolic compounds in cells and formation of lignin-like polymers in cell walls of young tomato fruits after inoculation with *Botrytis cinerea*. **Physiological Plant Pathology**, Orlando, v. 20, n. 1, p.11-25, 1982.
16. Jarvis, W. R. Managing diseases in greenhouse crops. **Plant Disease**, Saint Paul, v. 73, n. 3, p.190–194, 1989.
17. Li, L.; Li, T.; Xu, T.; Qi, M.; Yu, Z.; Zhang, K. Role of calcium in tomato (Lycopersicon esculentum Mill.) resistance to *Botrytis cinerea*. **African Journal of Biotechnology**, Pretoria, v. 11, n. 37, p.9013-9022, 2012.
18. Lisboa, B. B.; Bochese, C. C.; Vargas, L. K.; Siveira, J. R. P.; Radin, B.; Oliveira, A. M. R. Eficiência de *Trichoderma harzianum* e *Gliocladium viride* na redução da incidência de *Botrytis cinerea* em tomateiro cultivado sob ambiente protegido. **Ciência Rural**, Santa Maria, v. 37, n. 5, p.1255-1260, 2007.
19. Marini, R. P. Approaches to analyzing experiments with factorial arrange-

ments of treatments plus other treatments. **HortScience**, Alexandria, v. 38, n. 1, p.117-120, 2003.

20. Nobre, S. A. M.; Maffia, L. A.; Mizubuti, E. S. G.; Cota, L. V.; Dias, A. P. S. Selection of *Clonostachys rosea* isolates from Brazilian ecosystems effective in controlling *Botrytis cinerea*. ***Biological Control**, Orlando, v. 34, n. 2, p.132-143,* 2005.
21. O'Neill, T.M.; Shtienberg, D.; Elad, Y. Effect of some host and microclimate factors on infection of tomato stems by *Botrytis cinerea*. **Plant Disease**, Saint Paul, v. 81, n. 1, p.36-40, 1997.
22. Sadfi-Zouaoui, N.; Hannachi, I.; Andurand, D.; Essghaier, B.; Boudabous, A.; Nicot, P. Biological control of *Botrytis cinerea* on stem wounds with moderately halophilic bacteria. **World Journal of Microbiology and Biotechnology**, Dordrecht, *v. 24, n. 12, p.2871-2877,* 2008.
23. Shtienberg, D.; Elad, Y. Incorporation of weather forecasting to integrated, chemical-biological management of *Botrytis cinerea.* **Phytopathology**, Saint Paul, v. 87, n. 3, p.332–340, 1997.
24. Shtienberg, D.; Elad, Y.; Ariela, N.; Nitzani, Y.; Kirshner, B. Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses. **European Journal of Plant Pathology**, Dordrecht, v. 104, n. 8, p.753-763, 1998.
25. ten Have, A.; van Berloo, R.; Lindhout, P.; van Kan, J. A. L. Partial stem and leaf resistance against the fungal pathogen *Botrytis cinerea* in wild relatives of tomato. **European Journal of Plant Pathology**, Dordrecht, v. 117, n. 2, p.153-166, 2007.