Anthers colonized by fungi as the nearest source to initiate strawberries postharvest rot

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Abstract

Strawberry cultivation is expanding in Brazil, but few tactics are employed to extent the shelf-life of the strawberries fruits, which are affected by postharvest fungi that cause rot. To clarify how this rot progress, the objective of this work was to study the relationship between fungi that colonize anthers and rot of strawberry fruits during postharvest. The incidence of fungi in the strawberry anthers, the disease progress in strawberry fruits with infested anthers and in vitro fungicides efficiency against Cladosporium sp. were performed. Cladosporium sp. was found in 100 % of the plants and in approximately 65 % of the flowers during strawberry cultivation in a semi-hydroponic system. From 148 anthers without fungal signs placed into Petri dishes containing PDA, 94 were contaminated with Cladosporium and two of them had Penicillium sp. All 40 strawberry fruits which had Cladosporium sp. infesting their anthers were rotten by this fungus after nine days. As the most recurrent fungus was Cladosporium, the most efficient fungicides to control this fungus were azoxystrobin + diphenoxanazole, boscalide + cresoxim-methyl, metconazole, methyl thiophanate, pyrimenthanil, tebuconazole and trifloxystrobin + tebuconazole at the recommended doses for Botrytis cinerea and Mycosphaerella fragariae. Anther colonization may serve as the nearest source of inoculum to incite postharvest disease, as anthers remain attached to the fruits.

Keywords – Blossom rot – Cladosporium – Fragaria x ananassa Duch. – Fusarium

Introduction

Strawberries are appreciated in the fresh market and as an ingredient that composes various dishes in gastronomy. The world's top five strawberry producers according to the latest sense made in 2017 by the United Nations Food and Agriculture Organization in descending order are China (3,724,647 t), United States of America (1,449,280 t), Mexico (658,436 t), Egypt (407,240) and Turkey (400,167 t) (FAO 2017). Brazil ranks 54th (3,390 t) among strawberry producing countries in the world (FAO 2017).
Pollen produced by the male part of the flower is a necessary structure for seed formation and propagation of plant species (Kaczmarska et al. 2008). This flower structure is rich in proteins, lipids, carbohydrates, vitamins, amino acids and minerals (Campos et al. 2008), which may be consumed by contaminating fungi. Deterioration of anthers is a naturally occurring process after pollination (Woodson 1991), but most often, the presence of fungi is not evident.

The objective of this work was to study the relationship between anthers fungal colonization and postharvest rot of strawberries.

Materials & Methods

Study location and fungal incidence evaluation

The study was performed in a greenhouse located at 26°49'08.1"S and 50°59'14.6"W at the Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) at Caçador, Brazil during the years of 2018-2019. Strawberries plants from the cultivar “San Andreas” have been cultivated in double row for two years. During this period, fungus was observed colonizing flower’s anthers. To quantify the fungus incidence, 40 plants were evaluated once by observing their anthers to estimate how many anthers were colonized by fungus. After quantifying, fungi were isolated and identified at the phytopathology laboratory based on morphology following the key proposed by Barnett et al. (1972).

Incidence of fungi in anthers without fungal signs

To account for the fungus in anthers that do not show any signs of the fungus, 148 anthers were collected randomly. Each 10 anthers were inserted into Petri dishes containing PDA (Potato – Dextrose – Agar) to allow the fungal growth. After seven days, fungi were identified on the basis of morphology following the key for imperfect fungi (Barnett et al. 1972).

Disease progress in strawberry fruits with infested anthers

Forty strawberry fruits with anthers naturally infested with Cladosporium sp. were incubated in a humid chamber for nine days. During this period, the number of strawberry fruits rotten by Cladosporium sp. was accounted for to establish the relationship between the Cladosporium found infesting anthers and the rot of the strawberry fruits caused by this fungus.

Proposal of chemical control of the most recurrent pathogen

Cladosporium mycelial growth was measure under influence of the following chemical fungicides azoxystrobin + diphenocanazole (Dose 120 mL active ingredient/ha + 75 mL a. i./ha; spray volume 600 L), trifloxystrobin + tebuconazole (0.075 L a.i./ha + 0.15 L a.i./ha; 1000 L), metconazole (90 mL a.i./100 mL; 1000 L), tebuconazole (15 mL a.i./100 mL; 1000 L), methyl thiophanate (49 mL a.i./100 L; 1000 L), pyrimethanil (60 mL a.i./100 mL; 600 L), bosalide + cresoxim-methyl (400 mL a.i./ha + 200 mL a.i./ha; 1000 L), diphenocanazole (10 mL a.i./100 L; 200 L), bosalide (400 g a.i./ha; 1000 L), fluazinam (50 mL a.i./100 L; 1000 L), azoxystrobin (8 g a.i./100 L; 800 L) iprodione (75 g a.i./100 L; 1000 L) and procimidone (50 g a.i./100 L; 1000 L) to recommend the most efficient fungicide to prevent the rot of the strawberry fruit. The experiment was performed in vitro with Petri dishes containing PDA medium. The fungus was sown in the center of the Petri dishes, which were incubated at 25°C and 12 h photoperiod for 2 weeks. At the end of this period, the mycelial growth was measure.

Statistical analyses

The results of in vitro fungicides efficiency were submitted to analysis of variance. When significant by the F test, the means were compared by the Scott-Knott statistical test at 5% error probability.
Results

**Fungi found in colonized anthers**

We isolated 3 genera of fungi, *Cladosporium*, *Fusarium* and *Penicillium*. *Cladosporium* sp. was found in 100% of the plants and in approximately 65% of the flowers during strawberry cultivation in a semi-hydroponic system. This fact had not been observed in the previous two years of cultivation. Occasionally, *Fusarium* sp. and *Penicillium* were also found infested the anthers (Figs 1, 2). From 148 anthers without fungal signs placed into Petri dishes containing PDA, 94 were contaminated with *Cladosporium* and two of them had *Penicillium*. All 40 strawberry fruits which had *Cladosporium* infesting their anthers were rotten by this fungus after nine days (Fig. 3).

As the most recurrent fungus was *Cladosporium*, the most efficient fungicides to control this fungus *in vitro* were azoxystrobin + diphenoconazole, trifloxystrobin + tebuconazole, metconazole, tebuconazole, methyl thiophanate, pyrimethanil and boscalide + cresoxim-methyl at the recommended doses for *B. cinerea* and *M. fragariae* (Fig. 4).

![Fig. 1](image1.png) – Anthers colonizing fungi in strawberry flowers. A Anthers without apparent fungal colonizations. B Anthers colonized by fungi of the genus *Cladosporium*. C Anthers colonized by fungi of the genus *Fusarium*.

![Fig. 2](image2.png) – Mycelial growth of strawberry anther colonizing fungi. A *Fusarium* sp. mycelial growth in PDA (Potato-Dextrose-Agar) culture medium. B *Cladosporium* sp. mycelial growth in PDA culture medium.
Discussion

Cladosporium cladosporioides can cause the disease strawberry blossom blight, which is characterized by necrosis of flower that may lead to the production of small and malformed or misshapen fruits due to poor pollination (Gubler 1999, Nam et al. 2015). Black scab and dark brown sooty patches of leaves were also attributed to Cladosporium macrocarpum, Cladosporium tenuissimum and Cladosporium limoniforme (Ayoubi et al. 2017, Nam et al. 2015). On the basis of our results, the case of the fungus discussed herein seems to play a saprobic role (Koike et al. 2003) without causing any damage to the strawberry plants and its production.

Despite the negative visual appearance of the fungus-infesting anthers, there is no proven relationship between the incidence of fungus on the anthers and the increase in the number of deformed fruits or lack of pollination (Koike et al. 2003). We did not observe any fruit deformed and malformation related to the incidence of Cladosporium in the anthers. Neither any symptom related to leaves black scabs or typical blossom blight.

One question that has been raised by our research team is whether the presence of this fungus in flowers, even in a saprobic condition, could serve as a source of inoculum that would incite postharvest rot. Indeed, anther colonization may serve as a source of inoculum to incite postharvest disease, because colonized anthers remain attached to fruits. In our experiments, strawberries whose anthers were colonized by Cladosporium sp. showed rot caused by this fungus from five days after harvest.

Besides Cladosporium and Fusarium, fungi belonging to the genus Alternaria, Botrytis and Penicillium (also herein) have been found colonizing anthers in strawberry flowers (Koike et al. 2003). Since there are no active ingredients registered to control Cladosporium, other fungicides registered to B. cinerea and M. fragariae should be considered for flowers pulverization.

Conclusion

Cladosporium sp. was the most recurrent fungi isolated from the anthers. Fungi that grow in the anthers can serve as a source of inoculum for postharvest diseases. Azoxyostrobin + diphenocanazole, boscalide + cresoxim-methyl, metconazole, methyl thiophanate, pyrimethanil, tebuconazole and trifloxystrobin + tebuconazole were the most efficient chemical fungicides against the mycelial growth of Cladosporium sp.
Fig. 4 – Proposal for chemical control of Cladosporium based on fungicides recommended to Botrytis cinerea and Mycosphaerella fragariae. A Fungicides against the mycelial growth of the
Cladosporium at the recommended doses. B One of three replicates of fungicides treatments and their influence on the Cladosporium mycelial growth.

* The means were compared by the Scott-Knott statistical test at 5% error probability.

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