Boletín Micológico Vol. 13 (1-2) : 103 -110 1998

IN VITRO ANTAGONISTIC POTENTIAL OF *Trichoderma* spp. AGAINST *Colletotrichum gloeosporioides* AGENT OF ANTHRACNOSE IN THE PASSION FRUIT (*Passiflora*)

(Potencial antagónico in vitro de ***Trichoderma spp.*** *sobre* ***Colletotrichum gloeosporioides*** *agente de la antracnosis en Pasionaria (****Passiflora****))*

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Palabras clave: *Colletotrichum gloeosporioides, Trichoderma spp.*,control biológico, antracnosis, pasionaria.

Key words: *Colletotrichum gloeosporioides, Trichoderma spp.*, biocontrol, anthracnose, passion fruit.

RESUMEN

Diecisiete cepas de ***Trichoderma*** *aisladas del suelo de plantaciones de pasionaria, fueron seleccionadas por su tasa de crecimiento y capacidad de esporulación, evaluándose su potencial antagónico sobre un aislamiento de* ***C. gloeosporioides,*** *agente de la antracnosis de la pasionaria. Mediante la utilización de la técnica de cultivos apareados se comprobó que todas las cepas de* ***Trichoderma*** *causaron alteraciones morfofisiológicas en las hifas del fitopatógeno. Este tipo de actividad fue evidenciado por plasmolisis, vacuolización y enroscamiento de hifas. En 13 apareamientos hubo sobreposición de* ***Trichoderma*** *sp., causando inhibición del crecimiento micelial de las colonias del fitopatógeno, no sobreviviendo este en 10 apareamientos. De las tres especies evaluadas,* ***T. koningii*** *reunió el mayor número de aislamientos con características favorables para su utilización en el control biológico de* ***C. gloeosporioides,*** *lo que sugiere la posibilidad de controlar la antracnosis de esta planta en el campo.*

SUMMARY

Seventeen strains of ***Trichoderma*** *isolated from a soil planted with passion fruits were selected because of its rate of growth and sporulation ability, their antagonistic potential against an isolate of* ***C.gloeosporioides,*** *an agent causing anthracnose in passion fruit, being evaluated. By means of the paired culture technique it could be seen that all the* ***Trichoderma*** *strains induced morphophysiological alterations in the phytopathogen hyphae. This kind of phenomenon became apparent through plasmolysis, vacuolization and coiling of hyphae. An overlapping of* ***Trichoderma*** *occurred in thirteen pairings what inhibited the mycelial growth of the phytopathogen colonies and caused death of the latter in 10 pairing. Of the three species examined,* ***T. koningii*** *exhibited the highest number of isolates meeting the desirable requirements in order to perform the biocontrol of* ***C. gloeosporioides,*** *meaning that there is a chance to control anthracnose of this plant in the field.*

INTRODUCTION

Passion-fruit is subject to be attacked by various diseases and pests so to fight against them the systematic use of defensive means is necessary. This must be done, however, using strict criteria, since, to guarantee its fertilisation, the plant needs the action of pollinising insects (43).

Passion-fruit anthracnose has ***Colletotrichium gloeosporioides*** (Penz.) Penz. & Saccardo as ethiological agent. The disease attacks all the organs of the aerial part

*Part of dissertation to obtain a Msc degree in Biology of Fungi at the UFPE, Brazil.

of the plant. It occurs in all orchards throughout Brazil and its controll is sometimes very difficult when climatic conditions favour it (49).

In order that passion-fruit can receive the greatest market approval, in addition to adequate packaging, transport and conservation, the fruit must be presented in a well-matured state, with a smooth peel, and free of anthracnose (23). The control of the passion-fruit anthracnose, is made basically by means of the application of preventive fungicides (32, 37). The fungicides may promote an increase in the incidence of diseases due to the interference and death of antagonists or because of the action on the beneficial microbiota (27).

The research for alternative strategies to the use of chemical products used in the control of plant diseases, continues as a long-term goal of research in agriculture. Biological methods to reduce disease levels already exist in many traditional handling techniques and storage processes. What is usually absent, however, is a detailed understanding of the microbial ecology which forms the base of this natural method of suppression. This information is essential so that, the antagonistic interactions which occur between the saprophytic microbiota and the invading fungus which causes the disease in the plant can be explored (29). This natural method is known as biological control and can be defined as the occurrence of antagonistic interactions between pathogenic and non-pathogenic organisms which result in the suppression or control of the pathogenic organisms, by eliminating or decreasing their effects on the hosts.

The use of microorganisms as a recognised antagonistic ability and non-resident in the phyloplan is also a common technique in the biological control of diseases in the aerial portion (8). Among the potential agents of biocontrol, the *Trichoderma* Pers. has been one of the most studied, considering its peculiar antagonistic characteristics in natural conditions, mainly in the soil (5, 13, 24, 34, 42). Another advantage, of being a biocontrol agent is the fact that the *Trichoderma* species rapidly become resistant to fungicides when exposed to them. Resistant strains, antagonistic to a particular pathogen, may be used together with fungicides for the control of other diseases (1, 26, 34, 47).

The natural occurrence of *Trichoderma*, in commercial passion-fruit plantations in Brazil, has already been cited (36), and it has also been isolated from the root and stem of this plant (31). *C. gloeosporioides*, isolated from the leaves of the cashew tree, showed high sensitivity to *Trichoderma* which behaved as a promising agent in the control of this important pathogen under laboratory conditions (33).

Because of *Trichoderma*'s antagonistic potentiality in the biological control of *C. gloeosporioides*, it has become necessary to come to know its antagonistic behaviour and to verify the possibility of using it in the biological control of the passion fruit anthracnose. This study has been developed with the objective of selecting isolates of *Trichoderma* with a better antagonistic activities over *C.gloeosporioides*, by means of the study of in vitro antagonistic interactions.

MATERIALS AND METHODS

A high pathogenic isolate of *C.gloeosporioides*, obtained from a passion-fruit plant, 41 isolates of *Trichoderma* obtained from soils of passion-fruit plantations, and 2 isolates of *Trichoderma* (TR2 and T-25) obtained from URM-UFPE), in the State of Pernambuco, Brazil, were evaluated in the tests.

Mycelial growth rate and capacity of sporulation. To characterize the isolates as to their mycelial growth rate and capacity of sporulation, cultures were made in PDA (potato-dextrose-agar) medium, transfering 4 mm/diameter discs of mycelia-agar, with 3 day incubation withdrawn from the edge of the cultures, in to the centre of Petri dishes with PDA in triplicate. These were incubated at 26°C .

To evaluate the mycelial growth, daily measurements were made of the diameter of the colonies in two diametrically opposed directions, until the moment when the entire surface of the culture medium was colonised by the fungi, in any one of the repetitions used. To calculate the linear rate of growth of the mycelium the formula described by Lilly & Barnet was used (30).

The capacity of sporulation was visually estimated, after 10 days of incubation. The sporulation was classified as "abundant" when the sporulation was very visible all across the surface of the colony; average "medium" when the sporulation was visible in an area corresponding to about half the surface of the colony; and "sparse" when the sporulation was visible on lesser than half the surface of the colony.

Antagonism test. The antagonists which had the greatest rate of mycelial growth and sporulation were grown in paired culture with the phytopathogen under the method described by Dennis & Webster (18). Initially, the *C. gloeosporioides* isolate was innoculated on 9 cm/diam. Petri dishes, with PDA and incubated for 6 days at 26°C. After this period, 4 mm diameter discs of mycelia-agar were removed from the edges of the colonies where growth was vigorous, and transferred into Petri dishes with PDA. One disc of mycelia-agar was placed about 1 cm from the edge of each Petri dish and incubated for 3 days at 26°C.

After incubation, a disc of mycelia-agar of

Trichoderma, removed from the colony edge after being incubated for 3 days was transferred in to the opposite side of the Petri dish, about 1 cm from the edge, in a position diametrically opposed to that which had been occupied by the disc of mycelia-agar of *C. gloeosporioides*. The dishes, with the two discs 7 cm apart from each other, were incubated at 26°C, with three repetitions. The measures of the daily mycelial growth of the colonies one towards the other were noted. Their meeting, the inhibition of mycelial growth and the overlapping of the colonies were observed. The isolates were classified according to their antagonism agreeing with Bell *et al.*'s scale. (6).

Antagonic interactions. For studies of the antagonic interactions between hyphae of the antagonists and phytopathogen, paired cultures were made and cover slip placed in the central region of the Petri dishes, where the meeting of the colonies would take place. After the meeting , the cover slip were withdrawn and placed on a glass lamina for microscopy with a drop of Amann's blue colouring. Thereafter they were observed under an optical microscope and the occurred interactions among the hyphae were analysed.

Survival of *C.gloeosporioides*. The survival of the phytopathogen, after paired culture, was verified through observation of the development of the colonies beginning with mycelia-agar, 4 mm in diameter, removed from the colony of the phytopathogen in the zone of the antagonism, at a distance of 2 cm from the line of initial contact between the colonies. Five discs of mycelia-agar were transferred to Petri dishes with PDA medium and incubated at 26°C, with three repetitions, as a modification of the technique described by Tronsmo & Dennis (44).The analysis of the survival of the phytopathogen was carried out after 14 days of incubation by observing the number of colonies that had been formed.

RESULTS AND DISCUSSION

Data about the average of mycelium growth rate and sporulation capacity of the isolates of ***Trichoderma*** are showed in Table 1. The isolates Ti1, Ti2, Ti3, Ti5, Ti8, Ti9, Ti10, Ti13, Ti15, Ti17, Ti18, Ti19, Ti25, Ti26, Ti35, TR-2 and T-25, presented mycelium growth rates greater than the average rate with "abundant" or "average" sporulation and were selected for the antagonism test in paired cultures. The mycelial growth rate of the phytopathogen was, approximately, twice lessed than the average mycelial growth rate of the antagonists. *C. gloeosporioides* was inoculated three days earlier so that the meeting of the colonies could occur in the centre of the Petri dish. The mycelial growth rate of the antagonists affects their interactions with other fungi (25). It was observed that the isolates of ***Trichoderma*** which presented the greatest speed of mycelial growth showed greater antagonistic potential over *C. gloeosporioides* (33). The rapid mycelial growth winning the competition for nutrients and the rapid colonization of the substract, associated with the production capacity of a large number of spores for dispersion, are factors which, when added to a promising antagonistic potential, determine that an isolate has characteristics of being a good biocontroller (14).

Table 1. - Mycelial growth rate and sporulation of the strains of *C. gloeosporioides* (Cg) and of *Trichoderma* (Ti), in PDA medium at 26°C.

Isolate	Specie	Mycelial growth rate (mm/day)[1]	Sporulation[2]
Cg3	*C. gloeosporioides*	14.88	Abundant
Ti1	*T. harzianum*	24.48	Medium
Ti2	*T. koningii*	24.62	Abundant
Ti3	*T. harzianum*	46.87	Medium
Ti4	*T. koningii*	8.54	Sparse
Ti5	*T. harzianum*	40.46	Medium
Ti6	*T. harzianum*	15.43	Sparse
Ti7	*T. harzianum*	17.42	Sparse
Ti8	*T. aureoviride*	41.35	Medium
Ti9	*T. aureoviride*	43.82	Abundant
Ti10	*T. aureoviride*	46.87	Medium
Ti11	*T. aureoviride*	16.68	Medium
Ti12	*T. aureoviride*	15.26	Medium
Ti13	*T. aureoviride*	42.24	Medium
Ti14	*T. harzianum*	9.91	Medium
Ti15	*T. harzianum*	42.24	Abundant
Ti16	*T. koningii*	14.78	Medium
Ti17	*T. koningii*	39.60	Medium
Ti18	*T. harzianum*	39.43	Medium
Ti19	*T. aureoviride*	48.86	Abundant
Ti20	*T. koningii*	13.44	Sparse
Ti21	*T. koningii*	9.91	Medium
Ti22	*T. koningii*	15.90	Medium
Ti23	*T. harzianum*	9.91	Medium
Ti24	*T. harzianum*	11.19	Medium
Ti25	*T. koningii*	40.15	Medium
Ti26	*T. koningii*	39.74	Medium
Ti27	*T. koningii*	19.20	Medium
Ti28	*T. harzianum*	10.56	Medium
Ti29	*T. aureoviride*	12.24	Abundant
Ti30	*T. aureoviride*	14.79	Medium
Ti31	*T. aureoviride*	17.42	Medium
Ti32	*T. koningii*	19.20	Abundant
Ti33	*T. koningii*	8.54	Abundant
Ti34	*T. aureoviride*	17.42	Medium
Ti35	*T. aureoviride*	39.98	Medium
Ti36	*T. aureoviride*	17.91	Abundant
Ti37	*T. koningii*	12.87	Medium
Ti38	*T. koningii*	9.91	Abundant
Ti39	*T. koningii*	15.43	Medium
Ti40	*T. koningii*	19.20	Medium
Ti41	*T. koningii*	19.03	Sparse
TR-2	*T. viride*	32.95	Abundant
T-25	*T. harzianum*	41.12	Abundant

1) Average of three repetition.
2) Visual evaluation after 10 days of incubation.

In the pairing of the cultures, after the meeting of the colonies, overlapping occurred. The measures of these are showed in Table 2. The antagonistic behaviour of the

isolates was very diverse. The paired colonies were found after 48 hours of incubation, except for the two isolates TR-2 and T-25, which found the colony of the phytopathogen after 72 hours. The Ti 17 isolate was the one that overgrew most rapidly the phytopathogen after 72 hours. The Ti 19 isolate which had the greatest mycelial growth rate, started overlapping only after 72 hours, while the Ti 2 of least mycelial growth began overlapping before 72 hours. The Ti 10 and Ti 26 isolates of high mycelial growth rates did not overgrew the phytopathogen colonies. The Ti 2, Ti 17, T-25 isolates completly overgrew the colonies of *C. gloeosporioides*, with 144 h of incubation. After meeting of the colonies, there was an inhibition of the mycelial growth of the phytopathogen, in all the pairings. The varied antagonistic behaviour reflects the action of several antagonism mechanisms. Various types of antagonistic interactions used by the fungi were observed and they are detailed in Table 2.

Sporulation of the antagonistic occurred on the phytopathogen, generally in compact masses of conidia. The Ti 8, Ti 13, Ti 17, Ti 25, and T-25 isolates produced "abundant" sporulation on the phytopathogen, but the Ti 35 isolate, which overlapped the phytopathogen colony after some delay did not sporulate. The sporulation of the phytopathogen decreased and was "sparse" in 10 pairings, while "medium" sporulation of the phytopathogen occurred, even on the colony of antagonists, in the paired cultures with Ti 3, Ti 9, Ti 13, Ti 18, Ti 19, Ti 35 and TR-2 isolates.

The last column of Table 2 presents the classification results of the isolates as to antagonism based on the overlapping of the colonies and in accordance with Bell *et al.* (6). The Ti 2, Ti 17, TR-2 and T-25 isolates presented the greatest overlapping and were placed in class 1; most isolates were placed in class 2; the Ti 10 and Ti 26 isolates in class 3; and no isolates in classes 4 and 5.

Overgrowth on the phytopathogen colony is an advantageous characteristic of the antagonists in the dispute for the colonized area, winning the competition for space and nutrition. This is one of the ways of exercising biocontrol on the other organism which has its growth reduced or paralysed (18). The inhibition of mycelial growth and decrease of the sporulation of the phytopathogen colony is another important antagonistic characteristic exercised by the *Trichoderma* isolates. These are capable of producing antibiotics, besides enzymes and other toxic products which may interfere in the deve-lopment of the phytopathogen, inducing an undesirable condition for growth and sporulation (9, 10, 11, 28, 35, 46).

The interactions that occured between the hyphae are shown in Table 3. All the *Trichoderma* isolates affected the *C.gloeosporioides* through various types of antagonistic interactions, including those which do not cause overlapping, suggesting that there are mechanisms of biocontrol for antibiosis, competition and micoparasitism. The plamolysis of hyphae occurred in almost all paired cultures, except on Ti 2. Vacuolation was evident in many cells, which also showed a granulous aspect of the protoplasm without existing, however, a disintegration of the cell wall. In many cases the *Trichoderma* species can be classified as necrotrofic micoparasites, for they kill the host, and make use of the nutrients spilling from the dead hypha (4, 14).

In mycoparasitism, the hypha of the host may primarily suffer action of toxic metabolic products, including enzymes, before disorganization and dead occur (35).

Table 2. - Overgrow of the *Trichoderma* and classification of the antagonism.

	Overgrow (cm)[1]					
Trichoderma	72h	96h	120h	144h		Class of antag.[2]
Ti1	-	-	1.50	2.75	Me*	2
Ti2	0.80	1.80	3.45	4.50	Ab	1
Ti3	-	1.80	2.45	2.90	Me	2
Ti5	-	1.22	2.66	2.80	Me	2
Ti8	0.73	1.57	2.80	3.60	Me	2
Ti9	-	0.86	1.10	2.75	Ab	2
Ti10	-	-	-	-	Me	3
Ti13	0.90	1.80	2.80	3.70	Me	2
Ti15	-	-	1.83	2.85	Ab	2
Ti17	2.10	2.57	3.54	4.50	Me	1
Ti18	-	-	0.90	2.21	Me	2
Ti19	-	1.12	2.53	3.10	Ab	2
Ti25	0.75	1.61	3.20	3.67	Me	2
Ti26	-	-	-	-	Me	3
Ti35	-	-	-	1.93	Me	2
TR2	-	-	1.13	2.75	Ab	2
T-25	-	2.50	3.20	4.50	Ab	1

* Me = Medium Sporulation, Ab= Abundant

1) Average of three repetitions.

2) Bell *et al.* 's scale of classes of antagonism (6): 1= *Trichoderma* completely overgrew the pathogen and covered the entire medium surface; 2= *Trichoderma* overgrew at least two-thirds of the medium surface; and 3= *Trichoderma* and the pathogen each colonized approximately one-half of the medium surface and neither organism appeared to dominate the other; 4= The pathogen colonized at least two-thirds of the medium surface and appeared to withstand encroachment by *Trichoderma* and 5= The pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface.

Table 3.- Antagonistic interactions between hyphae of *Trichoderma* (Ti) and *Colletotrichum gloeosporioides* strains.

	Strains								
Interaction	Ti1	Ti2	Ti3	Ti5	Ti8	Ti9	Ti10	Ti13	Ti15
Plasmolysis of hyphae	+	-	+	+	+	+	+	+	+
Vacuolation of hyphae	+	+	+	-	-	+	-	+	+
Parallel growth of hyphae	+	+	+	+	+	-	+	+	+
Penetration of hyphae	-	+	+	-	-	+	-	-	-
Deformation of apressoria Cg	-	+	-	-	-	-	+	-	-
Hook-hyphae of Ti	-	+	-	+	+	+	-	+	+
Fragmentation of hyphae Cg	-	-	+	+	-	-	-	-	-
Rings of hyphae Ti	-	-	-	+	-	+	+	+	-
Coiling of hyphae	+	+	+	+	+	+	+	+	+

	Ti17	Ti18	Ti19	Ti25	Ti26	Ti35	TR2	T-25
Plasmolysis of hyphae	+	+	+	+	+	+	+	+
Vacuolation of hyphae	-	+	+	+	+	+	+	+
Parallel growth of hyphae	+	+	+	+	+	+	+	+
Penetration of hyphae	-	-	-	-	+	-	-	+
Deformation of apressoria Cg	+	-	-	-	+	+	-	+
Hook-hyphae of Ti	+	+	+	-	+	+	-	+
Fragmentation of hyphae Cg	-	-	+	-	-	-	-	-
Rings of hyphae Ti	-	+	+	+	-	-	-	-
Coiling of hyphae	+	+	+	+	+	+	+	+

(+) = presence; (-) = absence.

The coiling of the segments of hyphae with thickening of cell walls in the phytopathogen was observed in somes cases. The parallel growth of the hyphae, common in ***Trichoderma*** culture, occurred very often in paired cultures and only the Ti 19 isolate did not present parallel growth of hyphae. The penetration into hyphae of phytopathogen occurred with Ti 2, Ti 3, Ti 9 and Ti 26 isolates and, apparently, after desintegration of the protoplast, the cell wall remained intact.

The deformation of the *apressoria* of the phytopathogen was induced by the Ti 2, Ti 10, Ti 17, Ti 26, Ti 35 and T-25 isolates, which made them longer and more slender.

Appressoria is essential for the infection of *C. **gloeosporioides***, the chief function of the *apressoria* is direct penetration into the host (22). Deformation, probably, makes complete development difficult and limits the performance of the *apressoria* in the infection process of the phytopathogen, in the tissue of the host. The occurrence of short hyphae around the phytopathogen, which was similar to *haustoria* was observed, but the function of these structures, which are called hook-hyphae, cannot be certainly proved with assurance. The fragmentation of phytopathogen hyphae was observed with Ti 3, Ti 5, Ti 17 and Ti 19 isolates. Some isolates (Ti 9, Ti 10, Ti 13 and Ti 19) of ***T. aureoviride*** and the isolate (Ti 18) of ***T. harzianum***, formed characteristic rings of hyphae. Elad *et al.* (21), observed the same phenomenon and associated it

with parasitism of ***R. solani*** by ***T. harzianum***, for they formed hyphae rings in the interaction zone and they believed that there was not any explanation for the role of these structures in their life cycle and that they were related to micoparasitism, for they were not produced by ***T. aureoviride*** nor by ***T. harzianum*** in the absence of host mycelium. It was observed that, even in the absence of host mycelium, there was ring formation, suggesting that their formation was not directly related to mycoparasitism. When ***Trichoderma*** hyphae showed themselves to be in vigorous growth, thicker and less sinuous than the other mycelium ones, hyperparasitism through direct contact with phytopathogen hyphae did not occur. Contact between the hyphae of the fungi determined coiling at various points and at different intensities. Parallel growth and coiling of hyphae are cited by various authors as the most common antagonistic interactions, which later make possible the manifestation of the ones (9, 18, 21, 44, 47). ***C. gloeosporioides*** conidia did not become parasites.

Table 4, shows the survival data of the ***C. gloeosporioides*** after 7 days in paired culture with ***Trichoderma***. Mycelia-agar discs of the antagonistic phytopathogen were incubated for 14 days on Petri dishes with PDA. After incubating for 7 days some isolates permitted the survival of the phytopathogen to 100%. The Ti 9 and Ti 18 isolates, permitted only 40% and 20%, respectively, but after incubating for 14 days, they permitted 100% survival. In the remaining isolates Ti 1, Ti 8, Ti 15, Ti 19, Ti 25, TR2 and T-25 isolates, there was no survival of the phytopathogen. In the cases in which there was no survival of ***C. gloeosporioides*** from the beginning of incubation, it is possible that a fungicidal effect has occurred, caused by the antagonist in through the release of diffusible antibiotics in the medium (16, 17). In cases where survival occurred, with revival of phytopathogen colonies after a few days of incubation, a fungistatic effect may have occurred, in which, after the action of the antagonists stopped, the phytopathogen recovered its growth and sporulation capacity.

The results indicate that isolates of ***Trichoderma***, in *in vitro* tests, in relation to its antagonistic capacity to ***C.gloeosporioides***, in general, showed themselves to be promising in the biocontrol of the anthracnose agent of the passion fruit plant.

The tests made it possible to observe the changeability in the antagonistic ability of the isolates. The Ti 2, Ti 17 and Ti 25 isolates of the ***T. koningii*** and the T-25 isolate of the ***T. harzianum*** presented the bestperformances in antagonism to ***C. gloeosporioides***. The high level of intraspecific variability which can occur was emphasised by Bell *et al.*, (6).

Table 4.- Survival of *C. gloeosporioides* after paired culture with *Trichoderma* (Ti).

	Survival of *C. gloeosporioides* isolate (%) [1]	
Isolate	**7 days**	**14 days**
Ti1	0	0
Ti2	0	0
Ti3	100	100
Ti5	100	100
Ti8	0	0
Ti9	40	100
Ti10	100	100
Ti13	0	0
Ti15	0	0
Ti17	0	0
Ti18	20	100
Ti19	0	0
Ti25	0	0
Ti26	100	100
Ti35	100	100
TR2	0	0
T-25	0	0

1) Average of three repetitions.

The variation in the ability of ***T. koningii*** in biocontrol has already been observed (48), the Ti 17 isolate was the one which showed the greatest number of characteristics desirable for use in biocontrol, having rapid growth, overlap capability, coiling around the phytopathogen hyphae with the formation of hook-hyphae, induction of plasmolysis and vacuolation of hyphae and deformation of *appressoria*. ***T. koningii*** has been cited as a biocontrol agent of many phytopathogens (38, 39, 40, 41, 48). It produces a variety of antifungal metabolites, including antibiotics (3, 7, 19, 39, 41, 48) and enzymes which degrade the cell wall (12).

A better understanding of the factors which affect the interactions between species of ***Trichoderma*** and ***C. gloeosporioides***, is necessary for the development of an effective programme for the biological control of anthracnose in passion-fruit. *In vitro* tests in artificial media have the advantage of speed and control of the factors investigated, but are shown to be derivated from field tests results *in situ*. A good performance in *in vitro* tests does not guarantee that the isolate selected will have the same performance in the field, where many enviromental factors may influence the behaviour of the isolate and alter its antagonistic capacity. Nevertheless, *in*

vitro tests are necessary for a preliminary understanding of the basic action methods of the fungus, keeping in mind the obvius limitations imposed by the artificial conditions in which the study has been undertaken (2, 6, 25, 27).

ACKNOWLEDGMENTS

We thank Sidney Turyassu Gomes Bastos and José Soares Silva Filho, for their helpful comments and suggestions that improved the manuscript.

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