

PRODUCTION OF THREE *Hirsutella thompsonii* VARIETIES ON  
SEMI-SOLID MEDIA AND DIFFERENTIAL EFFECTS  
OF TWO FUNGICIDES

Daniel R. Sosa Gomez<sup>1</sup>

RESUMO

Produção de três variedades de *Hirsutella thompsonii* em meios semi-sólidos e sua compatibilidade com dois fungicidas

Foi avaliada a produção de três variedades do fungo patógeno de ácaros, *Hirsutella thompsonii* em 4 meios semisólidos (arroz, arroz-sacarose-peptona, arroz-sacarose-farinha de soja, e aveia), não encontrando-se diferenças significativas entre meios de cultura. *Hirsutella thompsonii* var. *synnematos*a (isolado H t R = ARSEF 255) apresentou a maior produção de conídios em todos os meios; a produção máxima foi obtida no meio arroz-sacarose-farinha de soja ( $19,13 \times 10^7$  conídios/g). Não foram encontradas diferenças significativas entre *H. thompsonii* var. *thompsonii* (isolado H t T1 = ARSEF 2012) e *H. thompsonii* var. *vinacea* (isolado H t NC = ARSEF 254). Nos ensaios de compatibilidade com fungicidas o oxicleto de cobre impediu a conidiogênese de todos os isolados. A produção de conídios dos isolados H t1 e H t NC foi menos afetada pelo enxofre, não havendo diferenças entre os mesmos. O crescimento micelial de todos os isolados foi reduzido, mas não inibido totalmente.

ABSTRACT

The conidia production of three *Hirsutella thompsonii* varieties on 4 semisolid media (rice; rice with sucrose and peptone; rice with sucrose and soybean meal; and oat) was evaluated.

Recebido em 18/01/90.

<sup>1</sup> EMBRAPA/Centro Nacional de Pesquisa de Soja (CNPSo), Cx. Postal 1061, 86001 Londrina PR.

Differences were not found among media. *Hirsutella thompsonii* var. *synnematos*a (isolate H t R = ARSEF 255) presented the highest conidia production in all media. The maximum production was obtained on rice-sucrose-soybean meal media ( $19.13 \times 10^7$  conidia/g). No significant differences were found among *H. thompsonii* var. *tompsonii* (isolate H t T1 = ARSEF 2012) and *H. thompsonii* var. *vinacea* (isolate H t NC = ARSEF 254). Copper oxychloride suppressed the conidiogenesis of all isolates. The isolates H t T1 and H t NC were less affected by sulphur, and no significant differences were observed among them. The mycelial growth was reduced, but not totally inhibited for both fungicides.

## INTRODUCTION

*Hirsutella thompsonii* is a cosmopolitan fungus which has been found to attack different species of mites on citrus, blueberry, avocado, guava, coconut and oil palm. This variation in habitats and hosts results in multiple physiological patterns.

SANSON *et al.* (1980), described three varieties of *H. thompsonii*. These varieties have great variability in terms of isozyme contents (BOUCIAS *et al.*, 1982), susceptibility to agrochemicals (SOSA GOMEZ *et al.*, 1984; 1987) and conidia production (WINKELHOFF & McCOY, 1984).

*Hirsutella thompsonii* is the only mite pathogen which has been produced in large scale to induce artificial epizootics (McCOY *et al.*, 1975, 1978; WINKELHOFF & McCOY, 1984). Taking into account this natural variability, it is important to know which is the response of the different isolates to semisolid media commonly used by the industry.

Both artificial and natural epizootics would be curtailed by applications of fungicides, and such applications simultaneous with the occurrence of epizootics are common in citrus. Therefore, it is necessary to evaluate the effect of some fungicides commonly used on citrus orchards on three pathotypes of *H. thompsonii* and to determine which are the more productive on semisolid media.

## METHODS AND MATERIALS

### Fungal isolates:

*Hirsutella thompsonii* var. *tompsonii* (H t T1) was isolated by Sosa Gomez (SOSAZ GOMEZ & NASCA, 1983) and deposited as ARSEF 2012 in the culture collection of the Boyce Thompson Institute (Ithaca, NY); *H. thompsonii* var. *vinacea* (H t NC = ARSEF 254 = CBS 555.77) and *H. thompsonii* var. *synnematos*a (H t R = ARSEF 255

= CBS 556.77A) were provided by Dr. C.W. McCoy, IFAS, University of Florida, U.S.A.

#### Conidia Production in Different Media

The isolates were grown in one liter Erlenmeyer flasks on four different media: 1) 100g of boiled rice, 2) 100g of boiled rice supplemented with 1.0g of sucrose and 2.0g of peptone, 3) 100g of boiled rice supplemented with 1.0g of sucrose and 1.4g of soybean meal, and 4) 100g of boiled oat.

Each treatment was replicated 4 times. The medium was autoclaved for 30 minutes at 15 psi before inoculation. Inoculum was obtained from 12-day-old cultures, from which conidia and mycelia from 4 colonies were taken and incorporated in each Erlenmeyer flask, and incubated at  $28 \pm 2^\circ\text{C}$  and 12 hours of photophase for two weeks. The conidia were dislodged from the medium by agitation on a rotary shaker in 250ml of sterile distilled water. Conidial densities were determined by counting in a hemocytometer. The data were transformed in square root and analyzed as a factorial experiments (4 x 2).

#### Effect of Fungicides

Table 1 shows the fungicides used in this work and respective concentrations, based on the recommended dosages for application.

Each fungicide was added to PSA (potato-sucrose-agar) medium according to the methods described by SOSA GOMEZ *et al.* (1984).

The experimental design was completely randomized and the data were analyzed as factorial experiments. The data were expressed as percentages of conidia production and mycelial growth in relation to the performance of the control and were transformed in  $\text{arc. sin } \sqrt{x/100}$  before the analysis. Means were compared by Tukey's test.

## RESULTS AND DISCUSSION

#### Conidia Production in Different Media

Pathotypes of *H. thompsonii* differed in their ability to produce conidia. As shown in Table 2, there are significant differences in conidiation among isolates, but no significant differences among culture media. *H. t.* var. *synnematos*a (isolate H t R) was about 10 times more productive than *H. thompsonii* var. *thompsonii* (isolate H t T1) or *H. t.* var. *vinacea* (isolate H t NC) (Fig. 1). WINKELHOFF & McCOY (1984) also found high

production of another pathotype of *H. t. var. synnematos*a in submerged fermentation. In this work, the production was  $19.13 \times 10^7$  conidia/g of rice-sucrose-soybean meal medium, while WINKELHOFF & MCCOY (1984) mentioned a production of  $9.7 \times 10^7$  conidia/ml in what was called medium "E".

#### Effect of Fungicides

Both fungicides reduced conidiogenesis and mycelial growth -Copper oxychloride caused complete inhibition of the conidiogenesis (Table 3).

Since copper oxychloride is one of the most commonly used fungicide in citrus orchards and it is mixed with different coadjuvants, with the purpose of extending the tenacity of the deposits (STEIN *et al.*, 1983), the risk of resurgence of mite population could be increased because of its deleterious effect on *H. thompsonii*, which is often found on citrus attacking different mite species, especially *Phyllocoptruta oleivora*, a common pest in the more humid areas.

Conidia production of H t T1 and H t NC was equally affected by sulphur in both concentrations. For all varieties conidiation was significantly decreased at the maximum sulfur concentration.

Mycelial growth was reduced, but not totally inhibited. When growth between varieties was compared, at the same fungicide concentration, significant differences were found between mycelial growth of the 3 isolates, for both minimal and maximal concentrations of copper oxychloride. For each fungal isolate, significant differences in mycelial growth were found among concentrations. With copper oxychloride the higher concentration always produced more mycelial growth than the lower (Table 3). Sulphur was significantly less toxic than copper oxychloride for all isolates. This fact was also reported by URUE TA (1980) in a strain of *H. thompsonii* probably *var. thompsonii*.

Mycelial growth in response to sulphur did not differ significantly between pathotypes, although it was significantly affected by both concentrations.

The relative low sensitivity of certain pathotypes of *H. thompsonii* to a particular fungicide might permit selection of those pathotypes for use in citrus groves when such fungicide is to be applied.

#### CONCLUSIONS

The isolate H t R was about ten times more productive than H t T1 and H t NC.

Copper oxychloride had a strong inhibitory effect on conidia production for all 3 varieties of *H. thompsonii* tested, but not suppressed mycelial growth.

The conidia production of the isolates H t T1 and H t NC were less affected by the sulphur.

#### ACKNOWLEDGMENTS

This work was financially supported by the National Research Council Republic of Argentina. Thanks are extended to Dr. G.J. de MORAES, Dr. F. MOSCARDI and E. ASSIS MENEZES for critical review of the manuscript and CIRPON (Pest Control Research Center), Tucuman, Argentina for providing research facilities.

TABLE 1 - Fungicides tested against *H. thompsonii* and respective concentrations, based on recommended dosages.

Fungicide	Formulation	Concentration in 100ml of culture medium	
		min.	max.
Sulphur	Wettable powder (92.5% a.i.)	0.970 g	1.950 g
Copper oxychloride	Wettable powder (84% a.i.)	0.149 g	0.398 g

TABLE 2 - Conidia production of 3 *H. thompsonii* varieties grown on different culture media ( $n \times 10^9$  conidia/Erlenmeyer flask).

	Media				Mean (1)*
	rice	rice, sucrose and peptone	rice, sucrose and soyb. meal	oat	
Fungus					
<i>H. t. thompson.</i>	1.56	1.42	2.28	2.12	1.84 b
<i>H. t. vinacea.</i>	1.23	2.88	1.89	0.89	1.72 b
<i>H. t. synnemat.</i>	18.99	19.04	19.13	10.55	16.93a
Mean (2)*	7.26 A	7.78 A	7.77 A	4.52 A	

(\*) Means followed by the same low case letters in a vertical line, or the same capital letters in a horizontal line are not significantly different at the 0.05 level (Tuckey test).

(1) mean number of conidia for all culture media.

(2) mean number of conidia for all all 3 varieties.

TABLE 3 - Conidia production and mycelial growth of 3 *H. thompsonii* varieties growing on media incorporated 2 with fungicides. (Parameters expressed as percentage in relation to control = 100%).

Fungicide	<i>H.t. thompsonii</i>		<i>H.t. vinacea</i>		<i>H.t. synnematososa</i>	
	concentration					
	min.	max.	min.	max.	min.	max.
Copper oxychloride						
Coind. prod.	0.00	0.00	0.00	0.00	0.00	0.00
myc. grow.	21.84a	27.91 b	18.17a	23.90 b	27.27a	31.82 b
Sulphur						
conid. prod.	27.28a	8.13 b	17.62a	1.79 b	2.81	0.00
myc. grow.	51.57a	27.50 b	48.30a	31.12 b	48.86a	28.78 b

Within each variety means followed by the same letters in a horizontal line are not significantly different at the 0.05 level (Tuckey test).

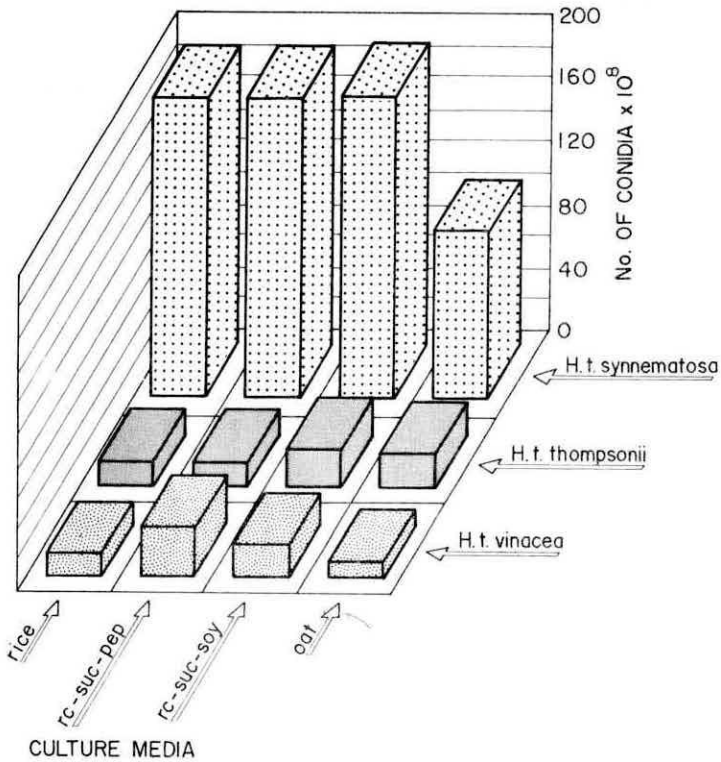


FIGURA 1 - Sporulation of *Hirsutella thompsonii* var. *synnematoso* (isolate H t R), *H. t.* var. *thompsonii* (isolate H t T1) and *H. t.* var. *vinacea* (isolate H t NC) on different semisolid media (rice, rc-suc-pep = rice-sucrose-peptone, rc-suc-soy = rice-sucrose-soybean meal, and oat).



## LITERATURE CITED

- BOUCIAS, D.G.; McCoy, C.W.; JOSLYN, D.J. Isozyme differentiation among 17 geographical isolates of *Hirsutella thompsonii* J. *Invertebr. Pathol.* 39 (3):329-337, 1982.
- McCOY, C.W.; COUCH, T.L.; WESTHERWAX, R. A simplified medium for the production of *Hirsutella thompsonii*. *J. Invertebr. Pathol.* 37 (1):137-139, 1978.
- McCOY, C.W.; HILL, A.J.; KANAVAL, R.F. Large scale production of the fungal pathogen *Hirsutella thompsonii* in submerged culture and its formulation for application in the field. *Entomophaga* 20 (3):229-240, 1975.
- SAMSON, R.A.; McCoy, C.W.; O'DONNELL, K.L. Taxonomy of the acarine parasite *Hirsutella thompsonii*. *Mycologia* 72:359-377, 1980.
- SOSA GOMEZ, D.R. & NASCA, A.J. Primera cita del hongo patógeno de ácaros, *Hirsutella thompsonii* Fisher, 1950 para la República Argentina. *Cirpon Revista de Investigación* 1 (3): 137 - 141, 1983
- SOSA GOMEZ, D.R.; MANZUR, J.; NASCA, A.J. Efecto del clorobencilato, dicofol-tetradifon, carbofenotion y metidation sobre tres variedades de *Hirsutella thompsonii*. *Cirpon Revista de Investigación* 2 (3-4): 115-126, 1984.
- SOSA GOMEZ, D.R.; MANZUR, J.; NASCA, A.J. Influencia de algunos agroquímicos sobre tres variedades de *Hirsutella thompsonii* Fisher (Hyphomycetes: Moniliales). *An. Soc. ent. Brasil* 16 (2): 399-408, 1987.
- STEIN, B.E.; GONZALES, J.L.; FOGUET, J.L. Persistencia de residuos de cobre en hojas de cítricos en relación a los adhesivos empleados como coadyuvantes en las pulverizaciones. *Revta Ind. Agr. Tucumán* 60(2):29-40, 1983.
- URUETA, E.J. Control del ácaro *Retracrus elaeis* Keifer (Eriophyidae) mediante el hongo *Hirsutella thompsonii* Fisher e inhibición de este por dos fungicidas. *Revta Augura* 6(2): 25-31, 1980.
- WINKELHOFF, A.J. VAN & McCoy, C.W. Conidiation of *Hirsutella thompsonii* var. *synnematosus* in submerged culture. *J. Invertebr. Pathol.* 43: 59-68, 1984.