Interaction between *Rhizoctonia solani* and *Meloidogyne incognita* on *Capsicum annuum* from Sequentially Infested Soil

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"Ah, New Mexico, how do I love thee? Let me count the ways: The sunshine; the dry, light air; the cultural diversity; and, oh yes, THE CHILE! Whether it's the delicious Green Chile of late Summer, or the rich Red Chile of Autumn, here in the heart of chile country, we lovers of New Mexico Red and Green are truly blessed!"


Santa Fe
TEXAS
25
17
40
Albuquerque
ARIZONA
27
NEW MEXICO
Portales
Phoenix
Lubbock
Roswell
Seminole
Hobbs
Animas
Columbus
Dell City
Fabens
Coyanosa
10
America's Chile Belt
= Geographical range of
commercial chile production

Introduction

- **Root knot nematode (RKN) by itself is known to be a powerful plant parasite which kills chile.**

- ***Rhizoctonia solani* by itself causes root rot and it kills chile seedlings early in the season and also infects mature plants leads to chile wilting and death.**

- **Results form studies in New Mexico chile fields indicated a wide distribution of root knot nematodes (*Meloidogyne incognita*) in sandy soils.**
- **Additionally, *Rhizoctonia solani* was isolated from chile in most fields.**

Hypothesis

- **Sequential inoculation of *R. solani* and *M. incognita* had a synergistic effect on chile injury in greenhouse experiments**

Objectives

- **To determine if sequential inoculation of *R. solani* and *M. incognita* affects reproduction rates of *M. incognita* and frequency of recovery of *R. solani* from chile roots.**

- **To investigate whether this sequential inoculation affects plant growth parameters more than either microorganism alone.**

Material and Methods

- **Describe 2 experiments under greenhouse conditions (pot experiments). These experiments are replicates.**

Experimental Design

- **Each experiment was conducted in a randomized complete block design with five replications per each of the nine treatments.**

Treatments

1. **Control: Un-inoculated soil.**

2. ***M. incognita* 1: Soil inoculated with *M. incognita* alone 57 days after seed germination.**

3. ***R. solani* 1: Soil inoculated with *R. solani* alone 57 days after seed germination.**

4. *M. incognita* 1 + *R. solani* 1: Soil inoculated with both *M. incognita* and *R. solani* 57 days after seed germination.

5. *M. incognita* 2: Soil inoculated with *M. incognita* alone 70 days after seed germination.

6. ***R. solani* 2: Soil inoculated with *R. solani* alone 70 days after seed germination.**

7. ***M. incognita* 2 + *R. solani* 2: Soil inoculated with both *M. incognita* and *R. solani* 70 days after seed germination.**

8. *M. incognita* 1 + *R. solani* 2: Soil inoculated with *M. incognita* 57 days after seed germination and then inoculated with *R. solani* 70 days after seed germination.

9. *R. solani* 1+ *M. incognita* 2: Soil inoculated with *R. solani* 57 days after seed germination and then inoculated with *M. incognita* 70 days after seed germination.

Treatment	Description
Control	Un-inoculated soil
M. incognita 1	Soil inoculated with *M. incognita* alone 57 days after seed germination
R. solani 1	Soil inoculated with *R. solani* alone 57 days after seed germination.
M. incognita 1 + *R. solani* 1	Soil inoculated with both *M. incognita* and *R. solani* 57 days after seed germination
M. incognita 2	Soil inoculated with *M. incognita* alone 70 days after seed germination
R. solani 2	Soil inoculated with *R. solani* alone 70 days after seed germination
M. incognita 2 + *R. solani* 2	Soil inoculated with both *M. incognita* and *R. solani* 70 days after seed germination.
M. incognita 1 + *R. solani* 2	Soil inoculated with *M. incognita* 57 days after seed germination and then inoculated with *R. solani* 70 days after seed germination
R. solani 1+ *M. incognita* 2	Soil inoculated with *R. solani* 57 days after seed germination and then inoculated with *M. incognita* 70 days after seed germination

Treatment Application

Pasteurized soil (82-93 °C) was placed into pots

Chile seeds planted (cv. Nu Mexico-64)

Soil was inoculated when plants were at the 10 to 12 leaf growth stage (57 day after germination) & 12-14 leaf growth stage (70 day after germination) according to treatments

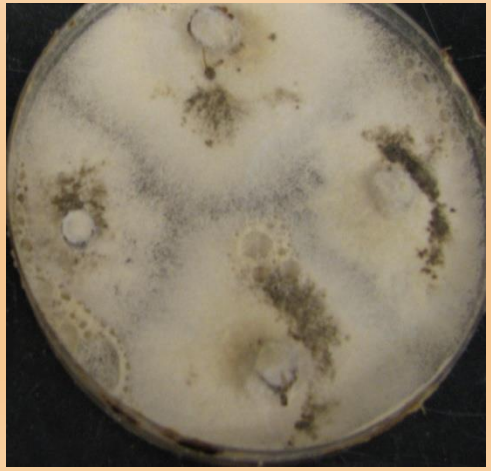

CAUTION

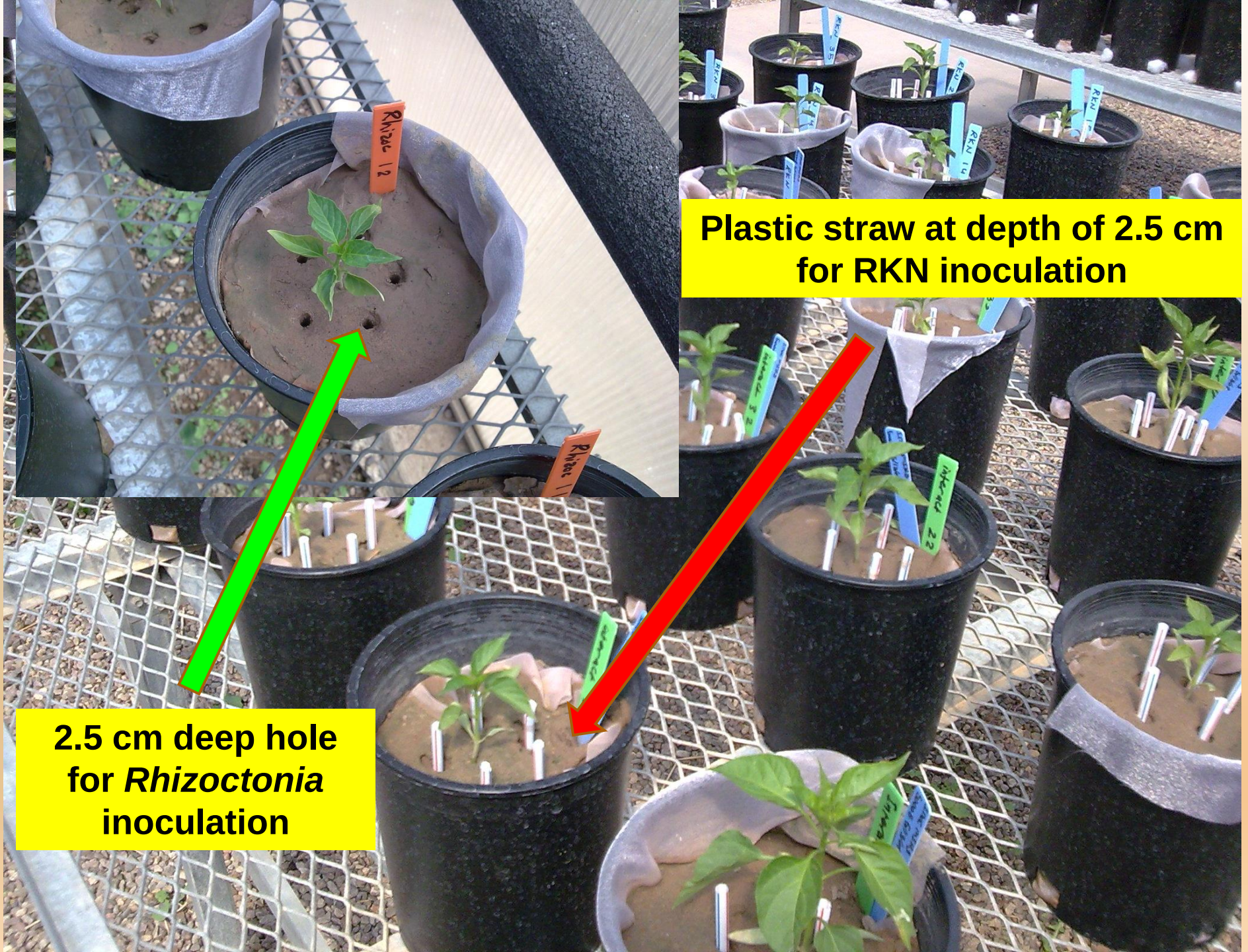
Treatment Application…

Inoculation of RKN (5,000 eggs per plant)

Inoculation of *Rhizoctonia solani*

(5 culture pellets per plant)



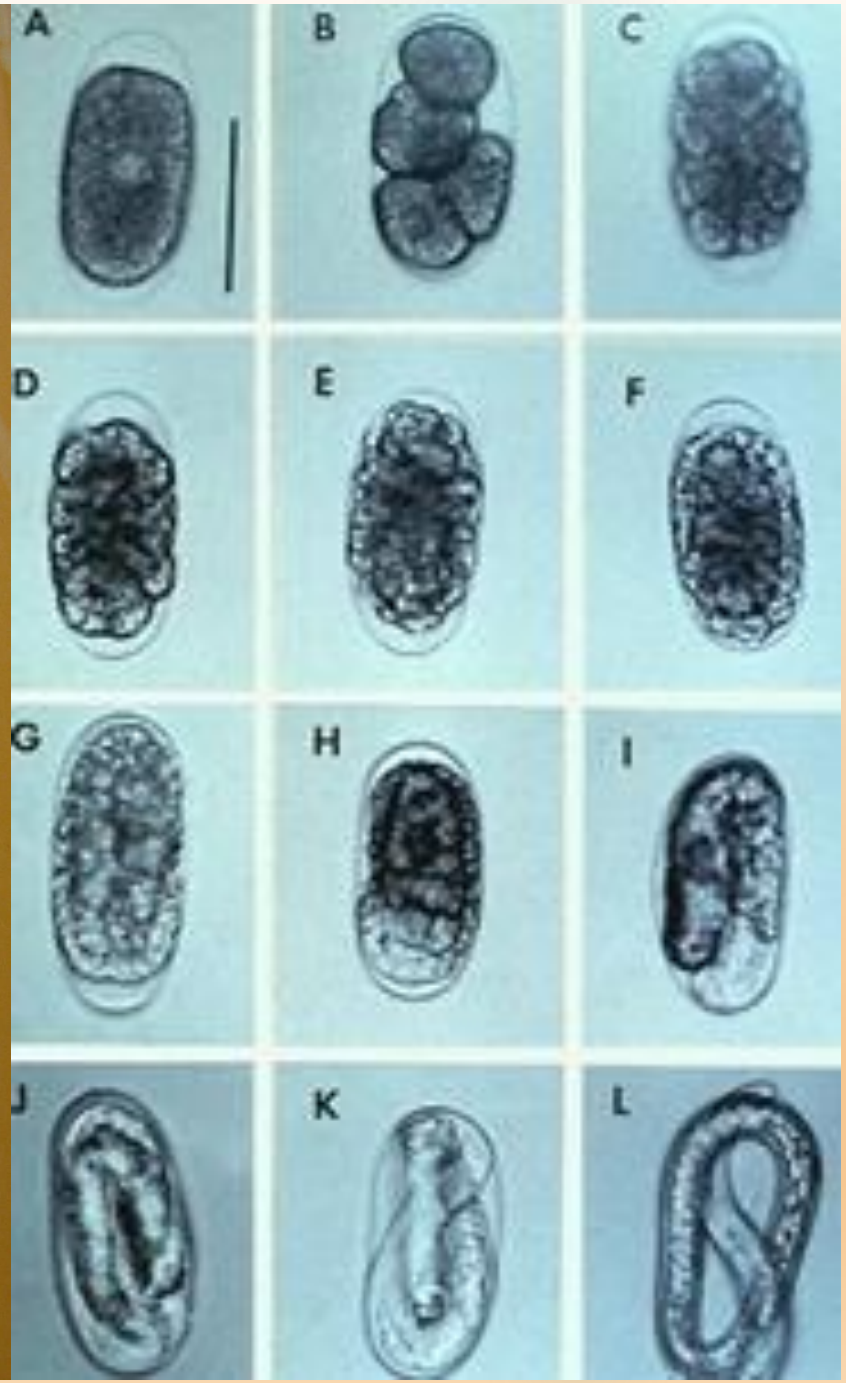

Plastic straw at depth of 2.5 cm for RKN inoculation
2.5 cm deep hole for *Rhizoctonia* inoculation


Ahmed

RKN Assessment:

- **Chile root system harvested and tap roots separated from lateral roots.**
- **Roots clipped into small pieces, placed into sodium hypochlorite solution, and macerated in rotary stirrer.**

- **Root suspension filtered and collected through 2mm, 74µm, & 30µm sieves.**
- **RKN eggs counted using chambered counting microscopic slide.**


A
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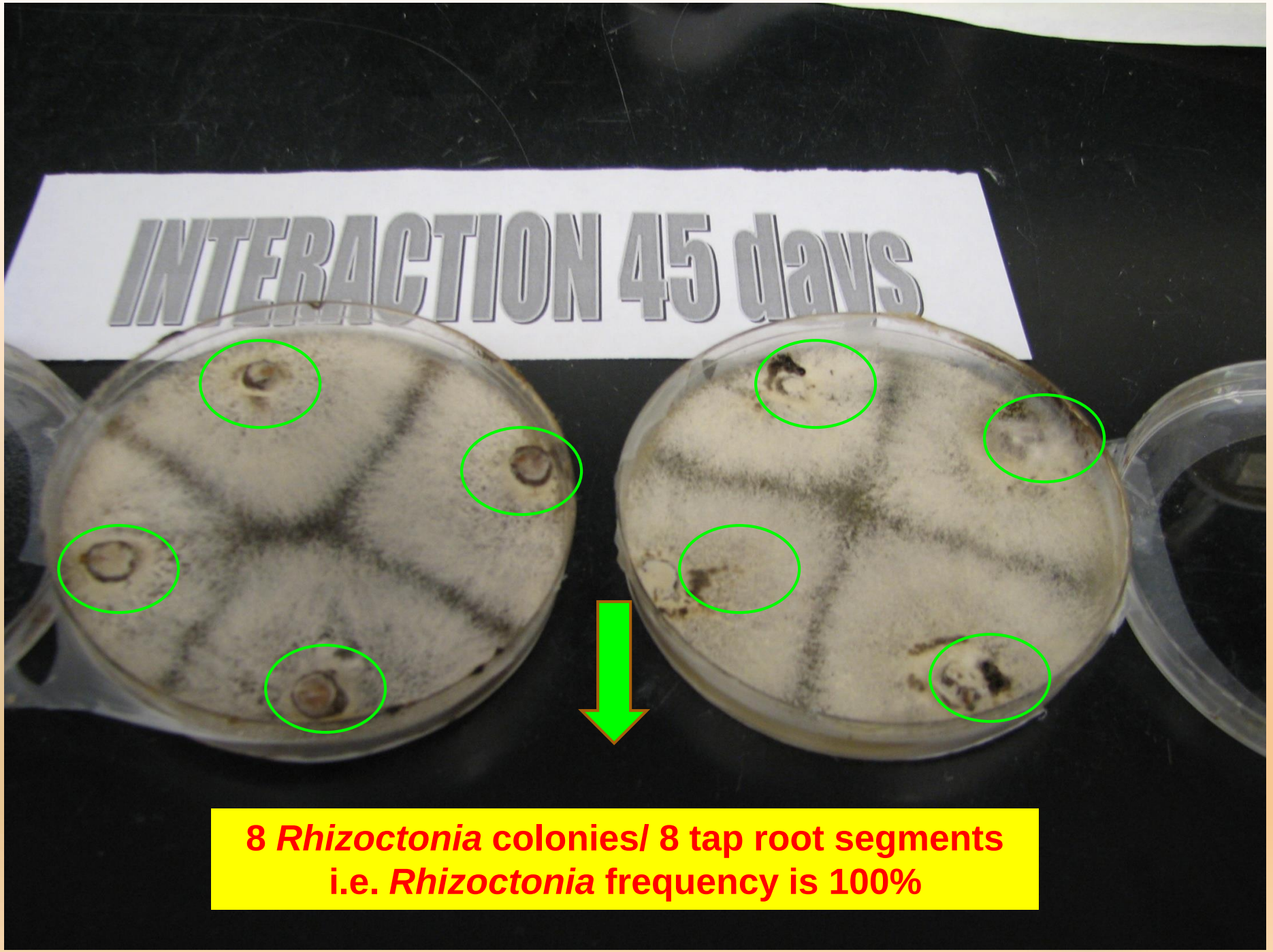
Rhizoctonia Assessment

- **The tap root & stem for each plant was clipped into homogeneous segments under aseptic conditions.**
- **Colony development of *Rhizoctonia* was evaluated by placing 4 pieces of tap root/ stem on a Petri plate containing acidified potato dextrose agar.**

- **All plates were incubated at room temperature. Two weeks after incubation, colonies of *Rhizoctonia* were counted to give *Rhizoctonia* frequency based on 8 tap root segments.**



8 *Rhizoctonia* colonies/ 8 tap root segments
i.e. *Rhizoctonia* frequency is 100%


I2
R1 TREN2
Control

Studied Parameters

- **Root knot nematode eggs recovery:**
- **eggs count expressed as:**

  (log (X + 1)/ g dry root weight).
- **Reproduction Factor (RF): X/ PI. Where X is the number of RKN eggs per plant and PI is the rate of RKN eggs inoculum = 5000 egg.**

- **Frequency of recovery of *Rhizoctonia solani* from root & stem segments.**
- **Plant dry weights were taken at the end of the experiment (oven drying at 68 °C).**

- **Physiological plant measurements were taken using Li-Cor instrument.**
- **Plant heights & fruit numbers were taken.**
- **Statistical analysis: Using analysis of variance SAS Proc GLM....LSD, α = 0.05**

Results

Figure 1. Dry weight of plant above ground biomass in experiment 1.

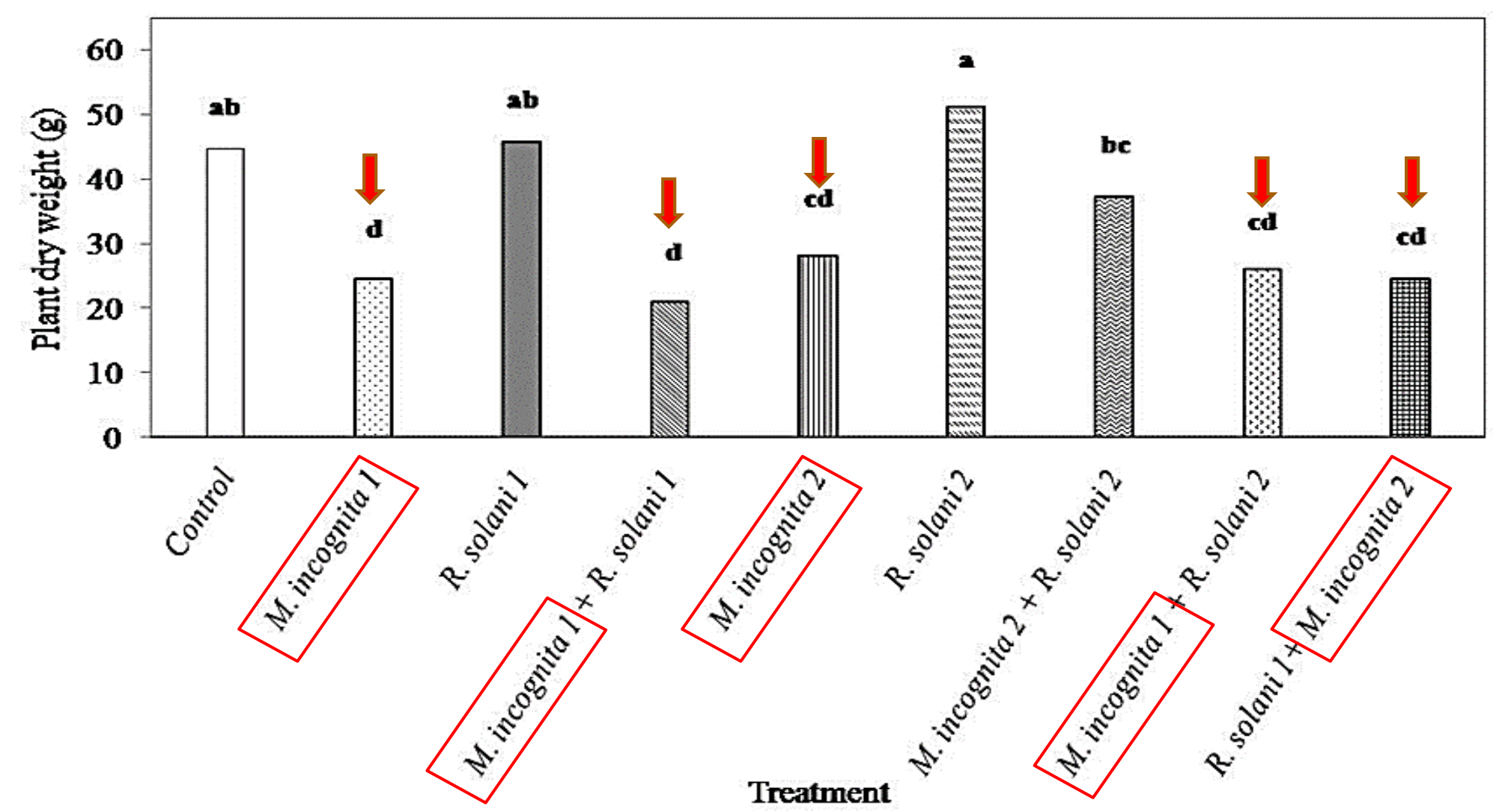


Figure 2. Dry weight of plant above ground biomass in experiment 2

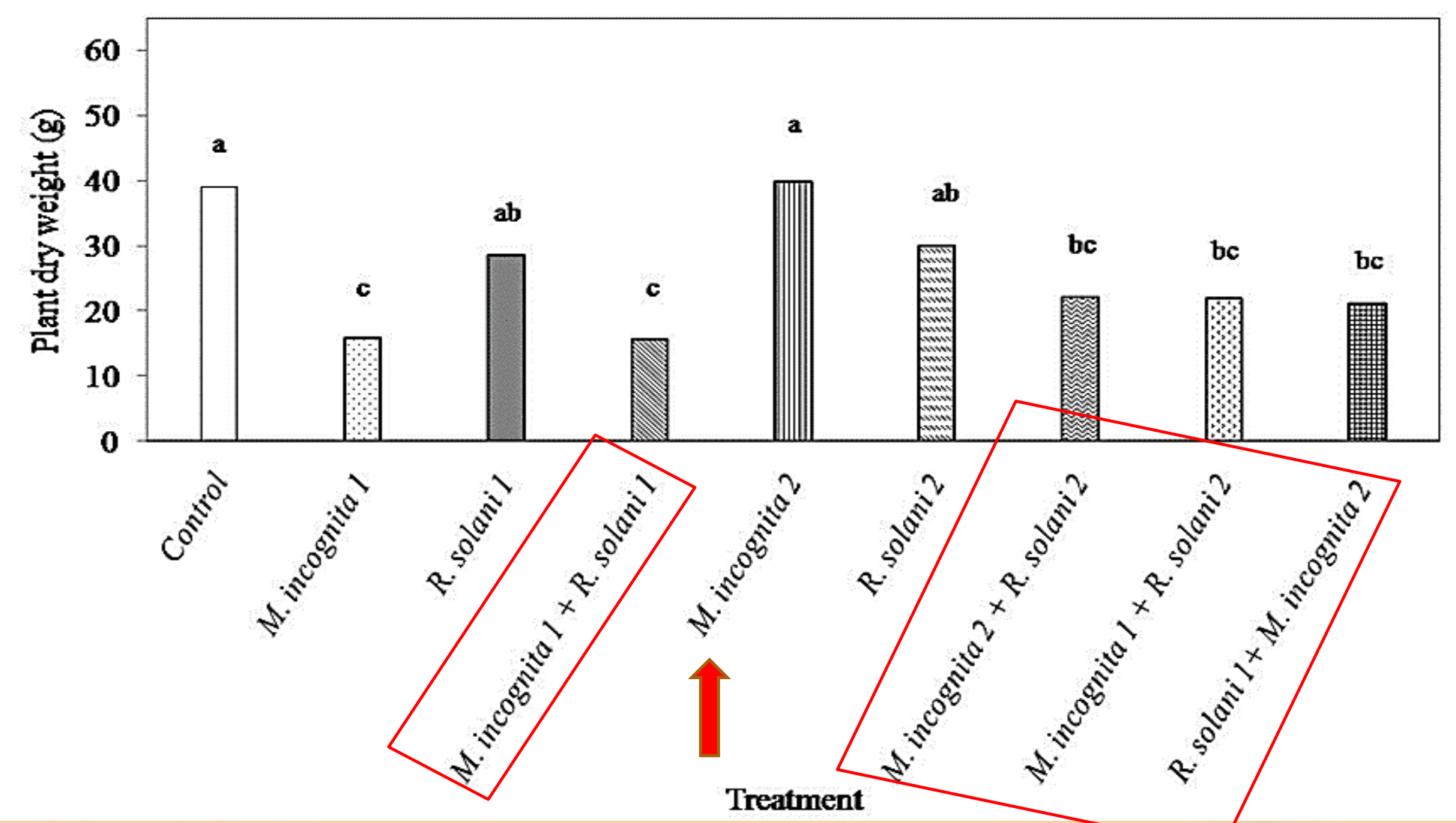


Figure 3. Photosynthesis rates of chile plants in experiment 2.

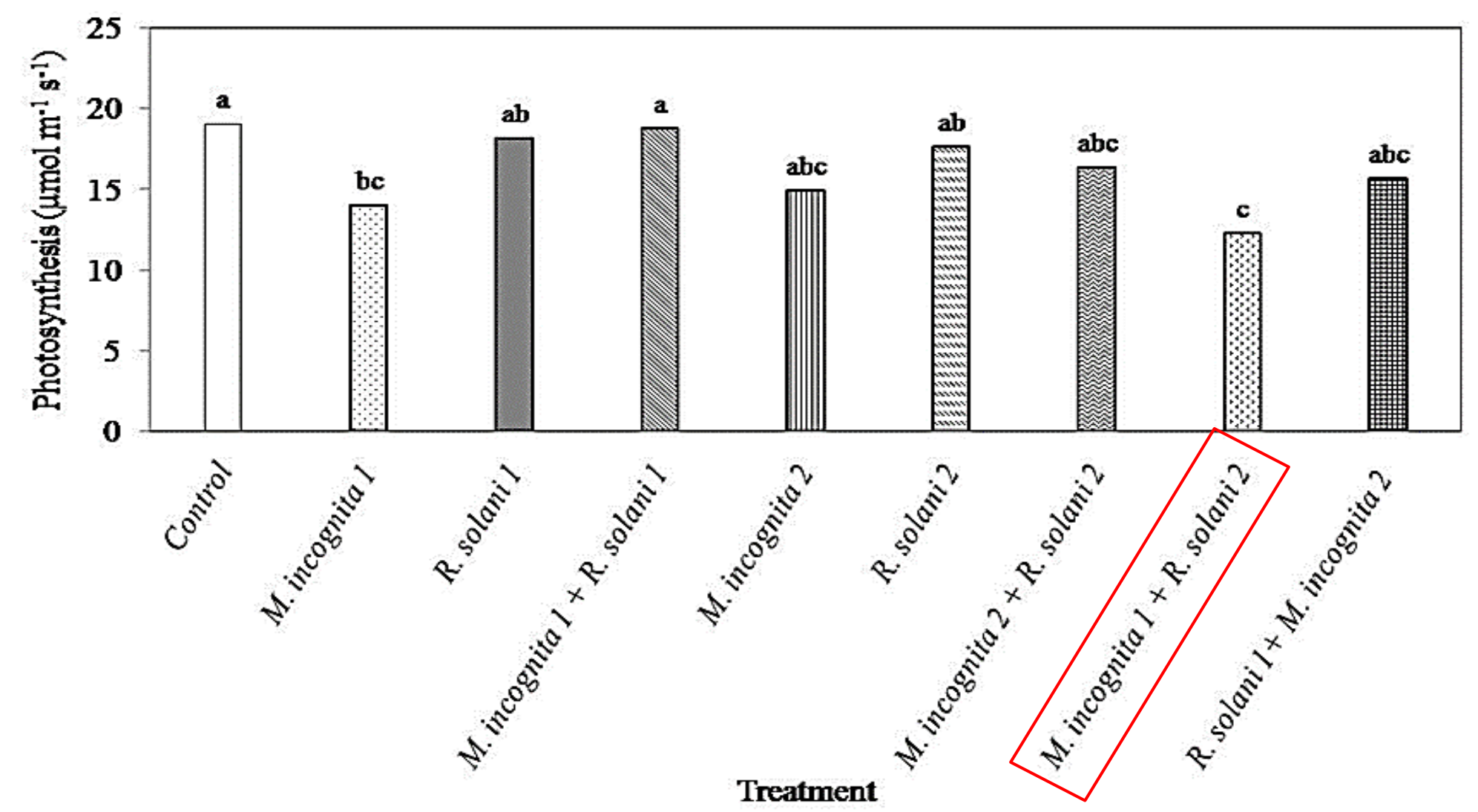


Figure 4. Transpiration rates of chile plants in experiment 2

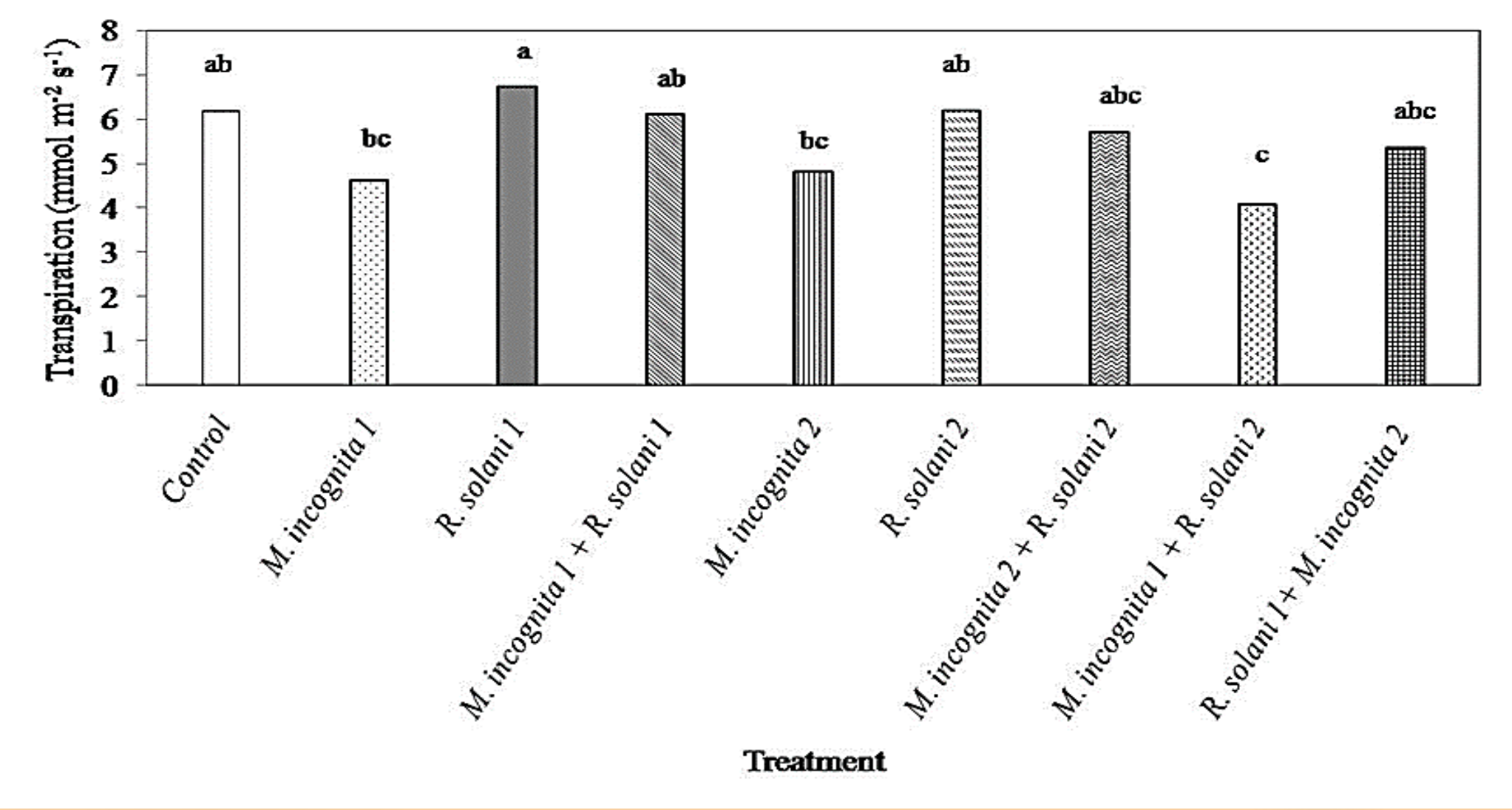


Table 2. *Meloidogyne incognita* reproduction factor (RF) and egg counts.

Treatment	*Meloidogyne incognita*			
	Reproduction factor (RF)		Total egg counts/ g dry root	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	0.1 c	0.3 c	0.10 b	0.94 c
M. incognita 1	267.7 a	348.6 bc	2.35 a	3.85 ab
R. solani 1	0.0 c	0.1 c	0.00 b	0.50 c
M. incognita 1+ *R. solani* 1	194.2 ab	470.7 ab	2.03 a	4.76 a
M. incognita 2	291.1a	796.3 a	1.74 a	1.74 bc
R. solani 2	0.0 c	0.0 c	0.15 b	0.68 c
M. incognita 2 + *R. solani* 2	97.6 bc	633.3 ab	2.00 a	1.97 bc
M. incognita 1 + *R. solani* 2	158.8 ab	578.1 ab	1.99 a	2.44 bc
R. solani 1+ *M. incognita* 2	184.5 ab	354.0 bc	1.75 a	3.38 ab

Treatments	*Meloidogyne incognita*			
	Reproduction factor (RF)		Number of eggs Log(X + 1)/g dry root wt	
	Experiment 3	Experiment 4	Experiment 3	Experiment 4
Control	0.1 c	0.3 c	0.10 b	0.94 c
M. incognita 1	267.7 a	348.6 bc	2.35 a	3.85 ab
R. solani 1	0.0 c	0.1 c	0.00 b	0.50 c
M. incognita + *R. solani (1)*	194.2 ab	470.7 ab	2.03 a	4.76 a
M. incognita 2	291.1a	796.3 a	1.74 a	1.74 bc
R. solani 2	0.0 c	0.0 c	0.15 b	0.68 c
M. incognita + *R. solani* (2)	97.6 bc	633.3 ab	2.00 a	1.97 bc
M. incognita 1 + *R. solani* 2	158.8 ab	578.1 ab	1.99 a	2.44 bc
R. solani 1+ *M. incognita* 2	184.5 ab	354.0 bc	1.75 b	3.38 ab

Table 3. Frequency of recovery of *Rhizoctonia solani* from stem and tap root segments of chile.

	Rhizoctonia solani frequency %			
	Experiment 1		Experiment 2	
Treatments	roots	stems	roots	stems
Control	73 NS	88 NS	65 a	80 ab
M. incognita 1	63 NS	45 NS	98 a	45 bc
R. solani 1	75 NS	67 NS	90 a	25 c
M. incognita 1 + *R. solani* 1	80 NS	80 NS	90 a	100 a
M. incognita 2	33 NS	20 NS	60 a	83 ab
R. solani 2	100 NS	60 NS	60 a	20 c
M. incognita 2 + *R. solani* 2	100 NS	100 NS	70 a	75 ab
M. incognita 1 + *R. solani* 2	40 NS	40 NS	0 b	0 c
R. solani 1+ *M. incognita* 2	80 NS	100 NS	78 a	100 a

Discussion

- **Under field conditions, chile plants are exposed to numerous soilborne pathogens such as *R. solani* and *M. incognita*.**
- **Generally the nature of the interaction between soilborne fungi and root-knot nematodes does not necessarily have one pronounced effect on a host plant.**

- **The interaction between soilborne pathogens depends on different factors such as those involving field and experimental conditions.**

- **Variations in these conditions can result in variations in measured parameters in interaction studies such as nematode reproduction rate and fungal growth rate.**

- **Our study showed death of a few chile seedlings three weeks after soil inoculation with *R. solani* alone or in combination with *M. incognita*.**

- **Early death of chile seedlings caused by *R. solani* indicated the pathogenic effect of the fungus on chile seedlings.**

- **However, other chile seedlings continued growing without showing symptoms of infection by the fungus even though later analysis showed they were infected.**

- **Assessment of *M. incognita* in our study showed no consistent trends of RF and egg counts regarding either simultaneous or sequential inoculation of *M. incognita* and *R. solani.***

- **The reasons for lower RF in the presence of *R. solani* in our study may be attributed to the damage to chile plants caused by the fungus.**

- **Lower RF of *M. incognita* in plants inoculated 57 days after seed germination compared to plants inoculated 70 days after soil inoculation may be attributed to soil temperature.**

- **Higher RF in our study when plants were inoculated with *M. incognita* alone 70 days after seed germination than when plants were inoculated with *M. incognita* alone 57 days after seed germination in experiment 2 can be explained by the fact that:**

- **Chile plants at 70 days after inoculation were larger in size than younger chile plants at the earlier inoculation time.**
- **Mature chile plants contain more photosynthates and nutrients available for *M. incognita* reproduction.**

- **Sequential inoculation when *M. incognita* was inoculated after *R. solani* produced lower *M. incognita* egg counts than the sequential inoculation when *M. incognita* was inoculated before *R. solani* in experiment 1.**

- **Presence of *R. solani* two weeks earlier than *M. incognita* may have adversely affected chile roots, made roots less suitable for *M. incognita* development, and consequently reduced root-knot nematode egg counts.**

- **In addition, *R. solani* has vigorous hyphae which might suppress *M. incognita* reproduction.**
- **Furthermore, the adverse effect of *R. solani* on *M. incognita* could be a result of the fungus disturbing *M. incognita* feeding sites and producing fungal toxins.**

- **Lower frequency of *R. solani* in the sequential inoculation when root-knot nematode preceded the fungus could be due to compounds toxic to *R. solani* excreted from chile roots in response to infection with *M. incognita*.**

- **Generally, treatments with *M. incognita* alone or in combination with *R. solani* produced significant reduction in the dry biomass of chile plants.**

- **The small variability in plant dry weight among treatments in our study could be attributed to the experimental conditions as we supplied plants with irrigation water and fertilization over the growing season.**
- **Plant stress was minor.**

Conclusion

- **Our experiments showed a slight indication that *R. solani* and *M. incognita* act synergistically if inoculated sequentially to soils planted with chile.**

- **Under conditions of sequential inoculation, *M. incognita* and *R. solani* may infect chile plants without causing death and the interaction has some pronounced effect on frequency of *R. solani*, reproduction of *M. incognita*, and plant growth parameters.**

Acknowledgment to

- New Mexico Chile Association.
- The Hashemite University-Jordan.
- The 24th International Pepper Conference Organizers.

Thank You

Questions???

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