

The Role of *Monosporascus eutypoides* in a Collapse of Melon Plants in an Arid Area of Israel

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ABSTRACT

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The role of the loculoascomycete *Monosporascus eutypoides* in a collapse of melon (*Cucumis melo*) plants was evaluated. Field trials and inoculation experiments showed that the organism was a primary pathogen in an extremely hot and arid area of Israel. The fungus caused a root rot that led to an early collapse of the plants. Fumigation of soil with methyl

bromide, metham-sodium, and ethylene dibromide with or without chloropicrin delayed symptom expression, the extent of which depended on the efficacy of the treatment. The relation between the thermophilic nature of the pathogen and the environment in which it is found is discussed.

Additional key words: irrigation, solarization.

In a recent note, we described the occurrence on melon roots of the loculoascomycete *Monosporascus eutypoides* (Petra) v. Arx, its possible relation to a mature plant collapse, and the geographic range of the organism in Israel (6). This fungus originally was described by Sivanesan et al (8) as *Bitrimonospora indica* found on *Achyranthis asperi* in India. The same year, Pollack and Uecker (5) described a similar fungus, originally isolated from melon (*Cucumis melo* L.) in Arizona. The culture was provided by J. Troutman who, with J. C. Matejka (9), described it forming unidentified fruiting structures on melon roots that were rotted. Pollack and Uecker established a new genus, *Monosporascus*, and their isolate, *M. cannonballus*. Under controlled inoculations this fungus caused a rot of secondary roots of melon (5). More recently, Hawksworth and Ciccarone (1) described *M. eutypoides* isolated from discolored stem bases of wheat in Libya. They showed that the ascospores germinated only above 30 C. As pointed out by the latter, collections of the above species derive from the "hotter areas in the world," viz., Arizona, India, Iran, Libya, and Pakistan. In our report, we pointed out that our collections of the fungus were made from an extremely arid and hot region of Israel, a portion of the African Rift Valley, which also encompasses the Dead Sea (6).

The purpose of this study was to determine the role of this fungus in a mature plant collapse of melon observed in this region by using various soil fumigation and other treatments.

MATERIALS AND METHODS

Field trial 1. The experiment was conducted at the Gilgal Experiment Station, 15 km north of the Dead Sea. Severe melon collapse had occurred on the plot in the previous season. Six treatments were evaluated:

- 1) Untreated control.
- 2) A mixture of 80% ethylene dibromide + 20% chloropicrin (w/w) at 150 L/ha (Bromine Compounds Ltd., Beersheba, Israel).
- 3) Metham-sodium at 1,000 L/ha (37.2% a.i.).
- 4) Methyl bromide at 500 kg/ha.
- 5) Ethylene dibromide at 150 L/ha (750 g/L) (Bromine Compounds Ltd.).
- 6) Soil solarization—plots kept moist and covered with clear polyethylene for 2 mo as described by Katan (2).

Treatments 2 and 5 were applied by a tractor-drawn rig and shanked in (shanks were 30 cm apart); treatment 3 was as described by Krikun and Frank (4) via sprinkler irrigation; treatment 4 as vaporized gas via the trickle irrigation system subsequently used to irrigate and fertilize the plants (7). Each plot consisted of three beds, each 1.8 m wide. Plot length was 25 m; thus, each plot was 25 × 5.4 m. There were four replications in a randomized complete block design. Two melon seeds were planted per hill, with six hills per meter. The cultivar used was Galia.

Isolations were made from roots 20, 35, 50, 70, and 90 days after sowing. Ten plants per plot were dug and washed, and portions of the root system were surface-disinfested in a 0.33% sodium hypochlorite solution for 2 min. Segments 3 mm long chosen at random were excised, plated on acidified potato-dextrose agar (PDA), and incubated at 28 C in the dark. The percentage of segments that yielded *M. eutypoides* was recorded 5–10 days after plating. Evaluation of plant collapse was begun 35 days after sowing, and was based on a scale of 0 to 5, reflecting the number and severity of collapsed plants from 0 = no collapse to 5 = all plants dead; 1 through 4 were intermediate degrees of collapse.

Field trial 2. This experiment was conducted at the Yotvata Experiment Substation situated 150 km south of the Dead Sea. Five treatments were evaluated:

- 1) Untreated control—trickle irrigation applied daily.
- 2) Untreated control—trickle irrigation applied every 3 days.
- 3) Methyl bromide—as in trial 1—trickle irrigation applied daily.
- 4) Methyl bromide—as above—trickle irrigation applied every 3 days.
- 5) Soil solarization—as in trial 1—trickle irrigation applied every 3 days.

Plots were 12.0 × 1.8 m, with one bed per plot. Seeds of cultivar Galia melon were planted four seeds per hill in hills 50 cm apart. Treatments were randomized in a complete block design with four replications. In this trial, only plant collapse evaluations were made during the growing season, as not enough plants were available for periodic root isolations.

At the end of the growing season, 10 plants from each plot were dug, the roots washed carefully, and ratings made on the extent of root rot and presence of perithecia of *M. eutypoides*. Yields in melon weight were determined from the middle 8 m of each plot. Collapse was rated as follows: 0 = no collapse, and from 1 = up to 25% collapse, to 4 = over 76% collapse. Root readings were made as follows: 0 = no rot symptoms present, 1–4 = increasing degrees of rot, 5 = root system severely rotted and bearing

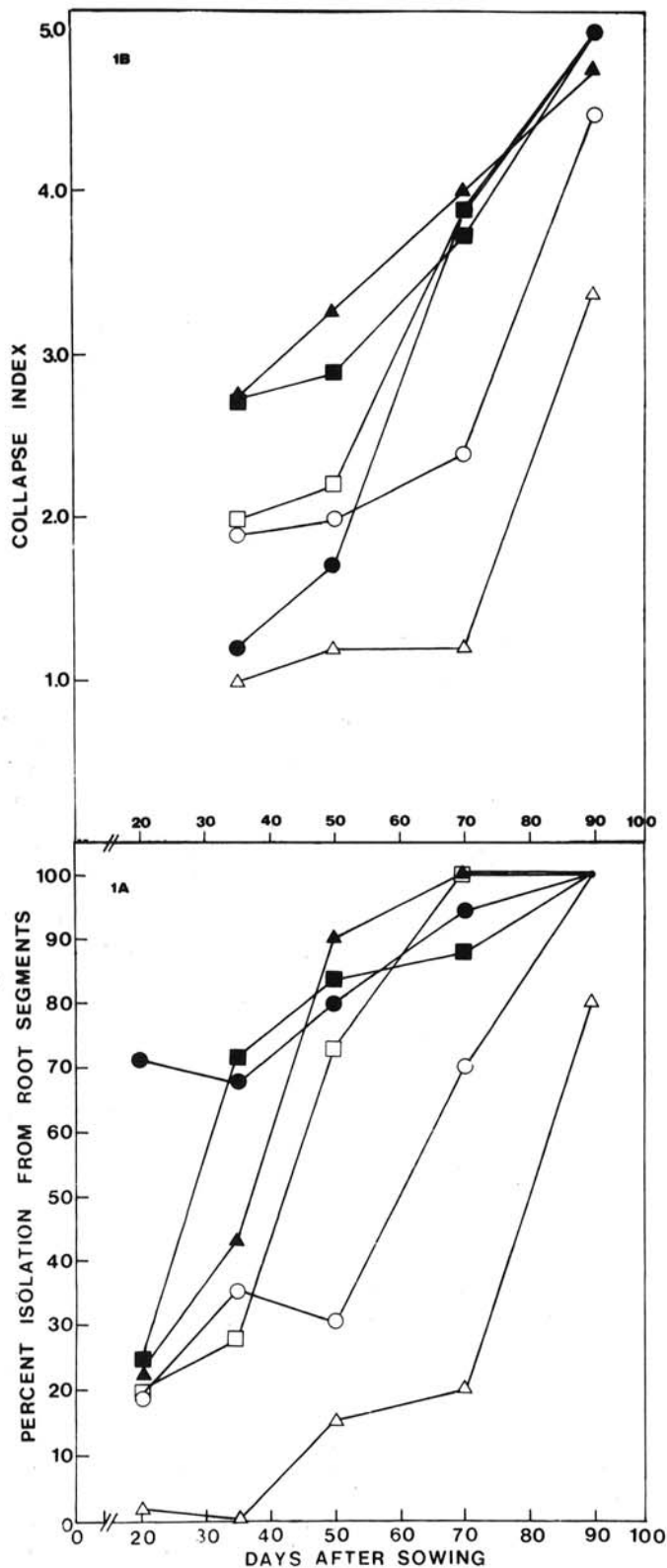


Fig. 1. A, Percent isolation of *Monosporascus eutypoides* from root segments of melon plants at different time intervals after sowing in the plots that received various treatments. B, Collapse index of melon plants at different time intervals after sowing in the plots receiving various treatments. Collapse index: 0 = no collapse; 5 = all plants dead; 1 through 4 = intermediate degrees of collapse reflecting the number and severity of collapsed plants. Symbols: ●—● control, ▲—▲ metham sodium, ■—■ EDB, ○—○ EDB + chloropicrin, △—△ methyl bromide, and □—□ soil solarization.

perithecia of *M. eutypoides*.

An analysis of variance was performed on all data, and means were separated by Duncan's multiple range test at $P = 0.05$.

Inoculation experiment. Twenty pots, each containing 3 kg of autoclaved soil, were infested with 100 ml of a mycelial and ascospore suspension that was mixed into the soil. The suspension contained 5×10^6 propagules per milliliter as determined by dilution plating. Inoculum was from 25-day-old cultures of *M. eutypoides* grown on PDA. Five seeds of cultivar Galia were sown per pot. Twenty noninfested pots served as controls. Half of the pots from each treatment were placed in each of two environmental chambers maintained at two thermoperiods: 25/20 C or 30/20 C, day/night. Photoperiod was 18/8 hr day/night with a light intensity of 10,000 lx (illuminated by Sylvania VHO, Gro-Lux fluorescent tubes and 40-W incandescent lamps). At various intervals, the onset and severity of symptom expression were recorded. When collapse was complete isolations from the roots were carried out to verify the presence of *M. eutypoides*.

RESULTS

Field experiment 1. The percent of root sections from which *M. eutypoides* was isolated is presented in Fig. 1A. The fungus penetrated the roots very soon after sowing in all treatments, but the highest frequency (72% at 20 days after sowing) was found in the control plots. Recovery increased with plant maturity and reached 82.5 and 94.5% at 70 days in the ethylene dibromide and control plots, respectively, and 100% in the metham-sodium and solarization treatments. Throughout the season, the lowest frequency was in the methyl bromide-treated plots.

The degree of collapse for the various treatments is shown in Fig. 1B. Symptom expression was obvious after 45 days, and very severe after 70 days in most treatments. The ethylene dibromide + chloropicrin and methyl bromide treatments delayed collapse. Onset of symptom expression coincided with recovery of *M. eutypoides* from the roots. Because of very early plant collapse, essentially no marketable yield could be collected from most treatments and, therefore, fruits not harvested.

Field trial 2. The kinetics of plant collapse is shown in Fig. 2. Onset of collapse began 56 days after sowing and was first seen in the control/3-day irrigation regime and soil solarization plots. Plant collapse in these plots proceeded at a rapid rate and, by 73 days after planting, complete plant collapse had occurred. In contrast, in the methyl bromide-treated and control plots that

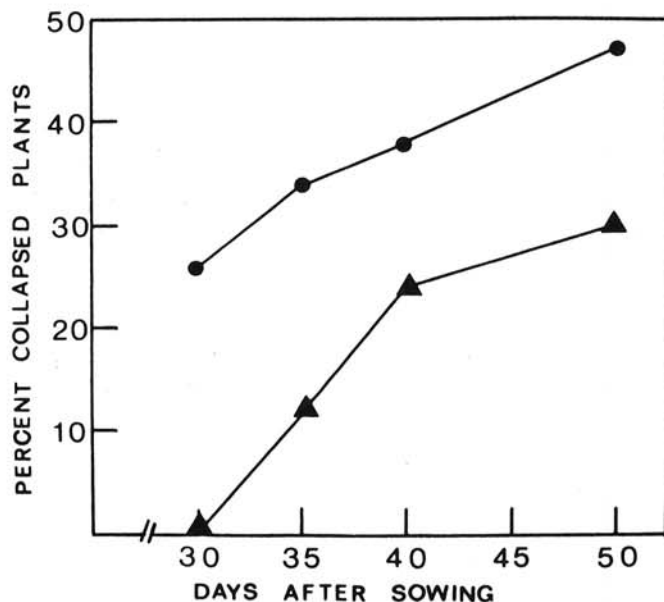


Fig. 2. Percent of collapsed plants as a result of growing in soil infested with *Monosporascus eutypoides* at two thermoperiod regimes: ●—● 30/20 C and ▲—▲ 25/20 C.

received daily irrigation, symptom onset was greatly delayed and reached very low levels. Plants in the methyl bromide treatment group that received alternate irrigations exhibited intermediate collapse. The root ratings and yield results are shown in Table 1. The roots from both methyl bromide treatments were the least rotted; the control-daily irrigation group was next in severity, whereas those of plants in the control-alternate irrigation and soil solarization groups had severely rotted roots. Percent of roots bearing perithecia of *M. eutypoides* coincided with the degree of root rot. The highest yields and highest percentages of large fruit were obtained in the plots with either no or limited plant collapse.

Inoculation experiment. The degree of collapse as affected by soil infestation at the two temperature regimes is shown in Fig. 3. Symptom onset was at 30 days after sowing. Examination of the roots in infested soil showed that they were rotted, and rot also developed on the root-stem transition zone and lower stem. Symptoms appeared earlier on plants maintained in the higher temperature regime (30/20 C) and their expression was accelerated in contrast to those appearing in the plants kept at the 25/20 C regime. *M. eutypoides* was recovered from the roots of plants growing in infested soil and not from those in control pots.

DISCUSSION

M. cannonballus has been reported from *C. melo* from the United States and India (1). We have shown that *M. eutypoides* is commonly recovered from roots of collapsed melon plants in a certain area in Israel. Fulfillment of Koch's postulates in our inoculation study and the results of our field experiments provide evidence that this fungus is a major pathogen of melon in Israel (6). As shown in Fig. 1, the degree of collapse was associated with the frequency of recovery of the fungus from the roots, which was a reflection of the degree of efficiency of the various treatments. Thus, when the inoculum density was markedly reduced, as by the methyl bromide treatment, the collapse was delayed markedly. When some collapse did occur early, it was seen at the border edges, suggesting that reinfestation had been initiated by inoculum from the adjacent plots.

In field trial 2, the methyl bromide-treated plots had either no early collapse, or it was delayed. The roots of plants from these plots were virtually free of rot; the yields of marketable fruit were high.

The results of the control plots that received daily irrigation are especially interesting. Two possibilities for the observed results may be operative: either the daily watering reduced the soil temperature enough to reduce the growth of the pathogen to a certain degree and, thereby, reduce rot; or the roots, even though suffering some rot, benefited enough from the more readily available water that collapse was prevented. It is also possible that both factors may act in unison.

The results of the inoculation experiment agree with our earlier data on the optimal temperature for mycelial growth of the pathogen, ie, 30 C (6). Another thermophilic characteristic of the fungus is the high temperature requirement needed for ascospore germination, which was shown for Hawksworth and Ciccarone (1) to be about 30 C, and could germinate even at a temperature of 40 C. In this regard, the results obtained in the soil solarization treatments should be considered. This method of soil pathogen control, as shown by Katan (2), should have been extremely useful in this region due to the high ambient temperature. In fact, we must now consider that in desert areas, where the existence of thermophilic fungal pathogens may have evolved, such a treatment may in fact be stimulatory, or perhaps even result in the selection of more highly thermophilic biotypes.

Thus, at Yotvata, the locale of field trial 2, Katan (3) reported that soil temperatures reached 53–59, 43–47, and 40–43 C at depths of 5, 20, and 30 cm, respectively, due to solarization. In our experiments, this treatment delayed infection and collapse somewhat, but after this initial stage, infection and collapse proceeded rapidly.

That methyl bromide fumigation is an excellent method for

controlling melon collapse in all areas of Israel is shown by the fact that it has been widely adopted to control such pathogens as *Macrophomina phaseolina* (7), which is found in other areas of Israel, and perhaps unknowingly, to control *M. eutypoides* in the locales under discussion.

That other hosts may be involved in inoculum increase of *Monosporascus* may be inferred from the results of Troutman and Matejka (9), who correlated the melon collapse implicated with *M. cannonballus* with small grain-melon rotations, and the findings of Hawksworth and Ciccarone (1), who found *M. eutypoides* on wheat. In the region reported upon here, small grains are not grown, and it may be that other cultivated species are acting as hosts. It should be pointed out that before the introduction of irrigation, the native vegetation was very sparse, and although the pathogen may have been colonizing species adapted to desert environments, it may be expected that high inoculum levels would not have been reached on native species. Our studies (6,7) suggest that this species is especially adapted to extremely arid environments, a point to be considered when it is planned to place

TABLE 1. The effect of soil fumigation, solarization, and irrigation treatments on rot of melon roots, presence of perithecia of *Monosporascus eutypoides* on the roots, and yield of fruit (field experiment 2)

Treatment	Root rot index ^y	Roots with perithecia (%)	Large and intermediate-sized fruit (t/ha)
Control daily irr.	1.7 b ^z	5.0 a	32.09 b
Control alternate irr.	3.4 c	40.0 b	27.51 b
Methyl bromide 500 kg·ha ⁻¹ daily irr.	0.6 a	2.5 a	42.20 a
Methyl bromide 500 kg·ha ⁻¹ alternate irr.	0.9 a	2.5 a	40.40 a
Solarization	2.8 c	30.0 b	27.65 b

^y0 = No rot symptoms on any of the roots, 5 = all roots severely rotted and bearing perithecia of the pathogen, 1–4 = intermediate degrees of root rot.

^zWithin columns, values followed by a common letter do not differ significantly according to Duncan's multiple range test ($P=0.05$) (means of four replicates).

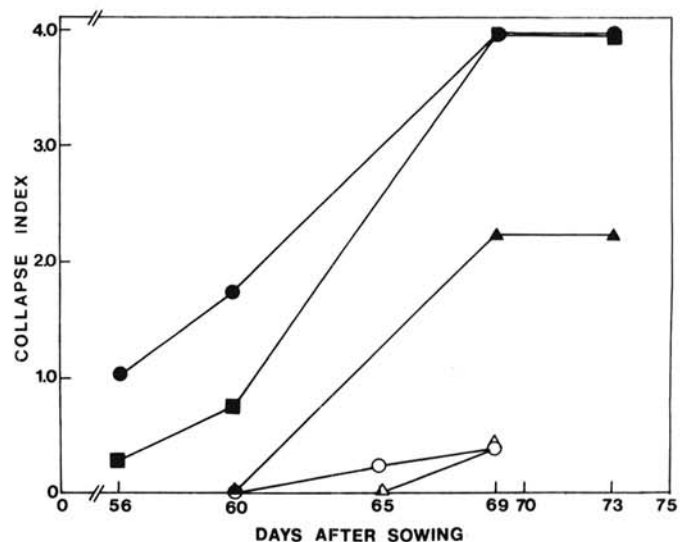


Fig. 3. Collapse index of melon plants at different time intervals after sowing in plots receiving various soil treatments and irrigation regimes. Collapse index: 0 = no collapse; 1 = up to 25%; 2 = 25–50%; 3 = 51–75%; 4 = over 76% plants collapsed. Curves: daily irrigation (open symbols) ○—○ control and △—△ methyl bromide; alternate irrigation (solid symbols) ●—● control, ▲—▲ methyl bromide, and ■—■ soil solarization.

such areas under cultivation by the introduction of irrigation. We should emphasize that in other areas of Israel where the temperature would have been favorable for the fungus, but the moisture relatively higher, and melons widely grown, the organism has not been found (7). Supporting evidence for the above is suggested by the results obtained with the daily irrigation treatment in field trial 2.

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