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Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions

J. INBAR, M. ABRAMSKY, D. COHEN and I. CHET
Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel

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Abstract. *Trichoderma harzianum* was applied to cucumber and pepper seedlings as a peat-bran preparation incorporated into the propagative mixture in a commercial production nursery. On marketing day (after 18 and 30 days for cucumber and pepper, respectively), significant increases of 23.8% and 17.2% in seedling height, 96.1% and 50% in leaf area, and 24.7% and 28.6% in plant dry weight were observed in cucumber and pepper seedlings, respectively, as compared to their non-treated counterparts. *Trichoderma*-treated seedlings were much more developed and vigorous and had higher chlorophyll contents. No significant differences were found in N, P or K content between treatments. Cucumber seedlings were then transplanted to a commercial greenhouse and analyzed over two successive growth cycles following soil fumigation with methyl bromide (500 kg/ha). Results revealed the *Trichoderma*-treated plants to be more resistant to damping-off disease. During the first cycle, immediately after soil fumigation, no damping-off was observed with either treatment, except in border beds where 4% of the non-treated plants died, as compared to no damping-off in the *Trichoderma*-treated plants. During the second growing cycle however, significant reductions in damping-off of 67% and 52% were obtained in middle and border beds, respectively, as compared to the non-treated controls.

Introduction

Trichoderma spp. are known antagonists of other fungi [Papavizas, 1985] and have been shown to be very potent biocontrol agents of several soilborne plant pathogenic fungi under both greenhouse [Chet, 1987; Sivan and Chet, 1992] and field conditions [Sivan and Chet, 1993; Sivan et al., 1987]. In some cases, application of species of *Trichoderma* to pathogen-free soil stimulated increased plant growth. *Trichoderma*-induced increased growth response has been reported for various plant species, including bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), pepper (*Capsicum annum* L.), periwinkle (*Vinca minor*), and petunia (*Petunia hybrida*) [Baker, 1989; Chang et al., 1986; Kleifeld and Chet, 1992; Paulitz et al., 1986; Windham et al., 1986]. In vegetables, the increased growth response can be expressed by shorter germination times and increased germination percentage, plant height, dry weight, and leaf area [Chang et al., 1986; Kleifeld and Chet, 1992; Paulitz et al., 1986]. In addition, in petunia and periwinkle, earlier flowering and increased numbers of blooms have been observed [Chang et al., 1986]. Several possible mechanisms have been suggested to explain this phenomenon including: control of minor pathogens, production of plant hormones, production of vitamins, conversion of nonutilizable materials into a form that can be utilized by the plant, and increased uptake and translocation of minerals [Baker, 1989; Kleifeld and Chet, 1992]. The increased growth response induced by *Trichoderma* spp. is not yet fully understood. However, more than one of the foregoing mechanisms appears to be involved [Windham et al., 1986].

To date, most of the work on induction of plant growth promotion by beneficial microorganisms and biological control has been carried out under controlled environmental conditions. In this study, the degree of plant growth promotion induced by *T. harzianum* Rifai isolate T-203 in vegetable seedlings, as well as its capacity to reduce disease incidence caused by soilborne plant pathogenic fungi, was analyzed under commercial conditions.

Material and methods

Application of Trichoderma

T. harzianum Rifai isolate T-203 [Elad et al., 1982] was used in all experiments. *T. harzianum* was applied in the nursery as a wheat bran-peat preparation [Sivan et al., 1984]. The wheat bran-peat mixture (1:1, v/v) was adjusted to 40% moisture (w/w) and autoclaved in autoclavable polyethylene bags (50 × 50 cm) for 1 h at 121 °C on three successive days. The sterile bran-peat mixture was then inoculated with blended (Waring commercial blender) mycelia of *T. harzianum* and incubated in a growth chamber for 14 days at 30 °C. This preparation, containing 5×10^9 conidia/g (dry weight), was mixed with a vegetable propagative mixture (peat and vermiculite, 1:1, v/v) at a concentration of 5% (v/v). Cucumber (*Cucumis sativus* L. 'Hasan') and pepper (*Capsicum annum* L.) seeds were sown in seedling-type preformed trays consisting of 180 conical compartments each measuring 3.7 × 3.7 × 10 cm. Trays were randomly distributed on the nursery bench. Seedlings were incubated at 24–30 °C under natural illumination and irrigated daily by spraying. Fertilizers (N, P and K, 'Deshen-col 20-20-20', Hafia Chemicals Ltd., Israel – a formulation containing KNO_3 0.44% (w/w); $NH_4H_2PO_4$, 0.32% (w/w), $Co(NH_2)_2$, 0.22% (w/w) plus microelements: Fe(EDTA) 0.05%, Zn(EDTA) 0.015%, Cu(EDTA), 0.011% and $(NH_4)_6$ Mo_7 0.007%) were applied twice a week via the irrigation water, in a concentration of 60 ppm N). Pesticides ('Sendakur' [a formulation containing cymoxanil, 14.2% (w/v), mancozeb, 48% (w/v) and oxadixyl, 6.9% (w/v)]; chlorothalonil 50% (w/w) was used in a final concentration of 0.35% (w/v) and cyromazine 75% (w/w) was used in a final concentration of 0.3% (w/v)] were applied three times a week. At the end of the rooting period (at the time of marketing – after 18 and 30 days for cucumber and pepper, respectively), 20 seedlings per treatment were randomly sampled for plant growth response measurements. The rest of the seedlings (730 *Trichoderma*-treated seedlings and 1980 non-treated seedlings) were transplanted to a commercial greenhouse for further observation.

Plant response measurements
The following plant response parameters were measured in each test plant from the various treatments: *plant height*, from soil to apical buds; *stem diameter; leaf area* – leaves were cut from the stem and their area was measured by an automatic leaf area-measuring apparatus (Hayashi Dienko, Tokyo, Japan); *plant dry weight* – plants were washed under running tap water to remove residual soil from the roots, then dried at 80 °C in a drying oven. After 72 h, plant dry weight was determined on an analytical balance (Sartorius, Gottingen, Germany); *chlorophyll concentration* of the leaves was measured with a SPAD-502 chlorophyll meter (Minolta Camera Co., Japan) [Shenker et al., 1992].

Mineral content of plants
For mineral content determination, leaves and roots of pepper plants were dried separately at 80 °C in a drying oven for 72 h and pulverized in Cyclotec – 1092 sample mill (Tecator, Sweden). The ground plant material (100 mg) was then treated with 2 ml of H_2SO_4 (36 N) for 5 h at room temperature. After cooling, 1.5 ml of H_2O_2 were added to the reaction tubes and incubated at 130–140 °C. This step was repeated a few times until the solution became clear. The incubation temperature was then raised to 280 °C for an additional 20 min of incubation. This solution, after cooling, was used for further analysis of nitrogen (N), phosphorus (P) and potassium (K) content. Potassium was measured by flame photometer (Corning Flame Photometer). Nitrogen and phosphorus were measured by autoanalyzer (Technicon).

Greenhouse experiments
Treated and non-treated cucumber seedlings were transplanted to a commercial greenhouse (red-brown sandy loam soil, pH 7.2), spaced 1 m apart in one row per bed (104 seedlings per row). The experiment consisted of seven *Trichoderma*-treated rows (replications) and 19 non-treated rows. Treated and non-treated rows were randomly distributed in the commercial greenhouse. Damping-off of cucumber seedlings was assessed one week after transplanting, over the course of two successive growing cycles (one growing cycle, from transplant to harvest, lasted 90 days). For each cycle,

seedlings were prepared as described above. Before the first cycle, the soil was fumigated with methyl bromide (500 kg/ha).

Diseased plants were sampled and analyzed for the presence of pathogenic fungi. Segments of the diseased plants were surface disinfected with 1% sodium hypochlorite for 1 min, washed with sterile tap water three times and placed on Potato Dextrose Agar (PDA) medium and incubated at 28 °C for 48 h.

Plant growth response and disease incidence data were statistically analyzed by ANOVA, and means were separated by Duncan's multiple range test at a significance level of $P = 0.05$. All experiments were conducted twice and data from the first trial are presented. Similar results were obtained in the second trial.

Results

Growth response in cucumber and pepper seedlings

In cucumber (Figs. 1 and 2), a significant increase was observed for each of the parameters measured (plant height, leaf area, plant dry weight, stem diameter and chlorophyll content), compared to the non-treated seedlings. *Trichoderma*-treated seedlings were twice as tall as the non-treated controls, with double the leaf area and increased chlorophyll content (absolute values for the controls – i.e., 100% = 2.5 cm height, 28.8 cm^2 leaf area, 0.26 gr dry weight, 4.3 mm stem diameter and 35.3 spad units of chlorophyll).

In pepper (Figs. 3 and 4), a significant increase was observed for all parameters measured except chlorophyll content, which was slightly, but not significantly higher in the *Trichoderma*-treated pepper plants than in the controls. *Trichoderma*-treated pepper seedlings were 17.2% higher, with a 50% increase in leaf area. No significant differences between treatments were observed in N, P, and K contents of either roots or leaves. The average values of N, P and K in roots of both treated and non-treated seedlings were 2.8%, 0.5%, 3.7% (of dry weight) respectively, and in leaves – 3.9%, 0.7% and 5.3% (of dry weight), respectively (absolute values for the controls – i.e., 100% = 10.2 cm height, 54.3 cm^2 leaf area, 0.35 gr dry weight, 3.3 mm stem diameter and 44.5 spad units of chlorophyll).

Disease reduction

The effect of *Trichoderma* application on damping-off of cucumber plants was measured seven days after transplanting the seedlings to a commercial greenhouse. In the middle beds, no significant differences were found in disease incidence between treatments during the first growing cycle (Fig. 5A). Due to soil fumigation, the level of disease was low (below 0.5%). At the border beds however, where soil fumigation was less effective, 4% of the non-treated plants were diseased, whereas in the *Trichoderma*-treated

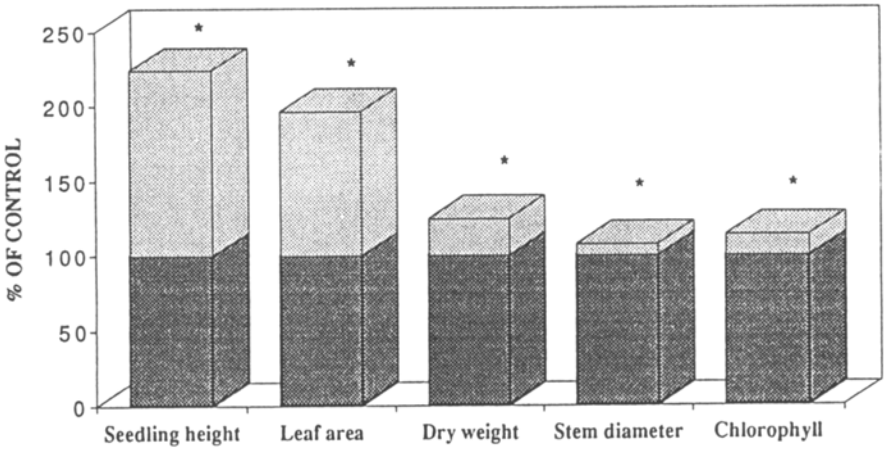

Fig. 1. Increased growth response of cucumber seedlings (cv. 'Hasan') induced by *Trichoderma harzianum* applied as a peat-bran preparation into the propagative mixture. On day 18, seedling height, leaf area, plant dry weight, stem diameter and chlorophyll content of the leaves were measured and compared with those of the non-treated seedlings. * Significant increase (P = 0.05).

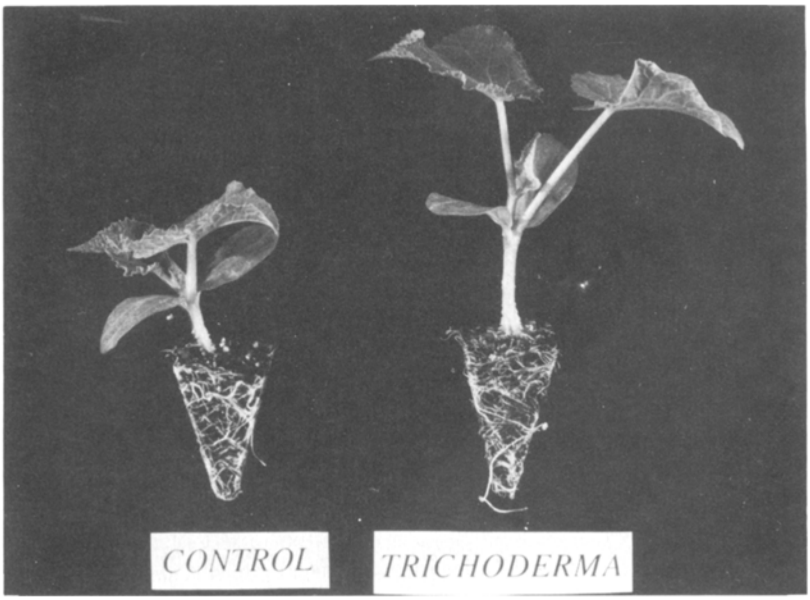

Fig. 2. Cucumber seedlings, normal (control) or treated with *Trichoderma harzianum* (Trichoderma) applied as a peat-bran preparation via the seedling propagative mixture. On marketing day, 18-day-old *Trichoderma*-treated seedlings were twice as tall as the non-treated controls and looked much more developed and vigorous, with a higher leaf area.

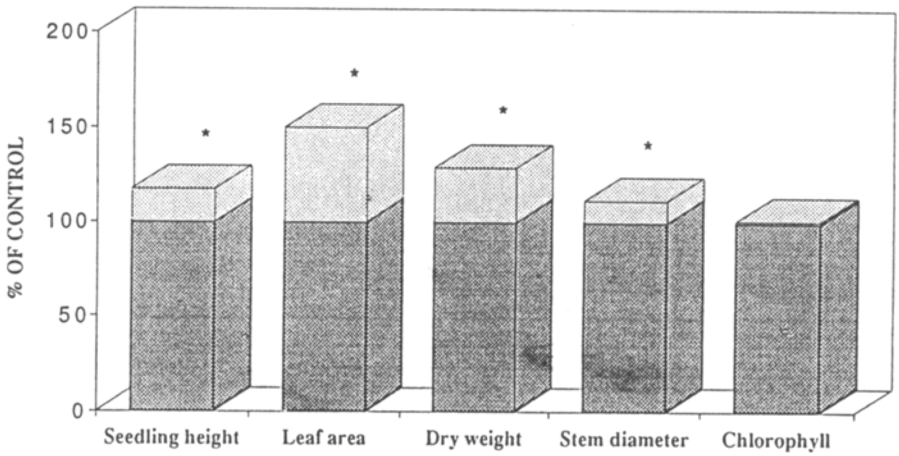

Fig. 3. Increased growth response of pepper seedlings induced by *Trichoderma harzianum*. On marketing day, seedling height, leaf area, plant dry weight, stem diameter and chlorophyll content of the leaves were measured and compared with those of the non-treated seedlings. * Significant increase ($P = 0.05$).

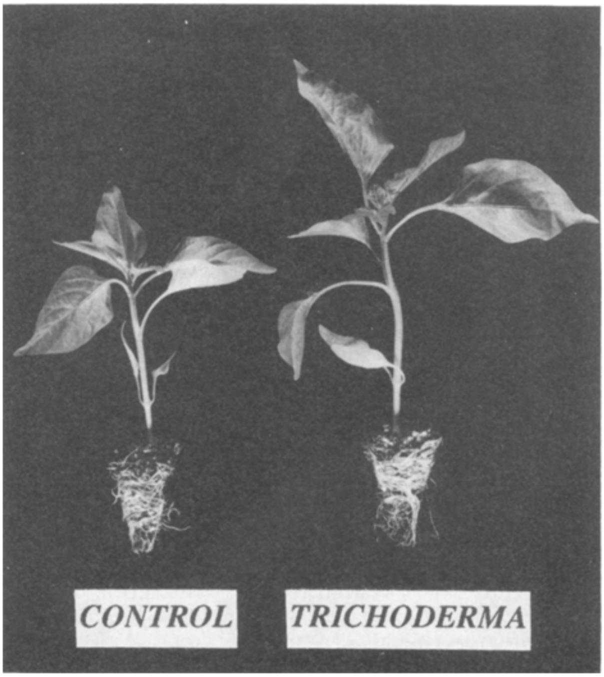

Fig. 4. Thirty-day-old pepper seedlings, normal (control) or treated with *Trichoderma harzianum* (Trichoderma) applied as a peat-bran preparation via the seedling propagative mixture. *Trichoderma*-treated seedlings exhibited increased leaf area and stem diameter, were higher, and appeared more developed than the non-treated controls.

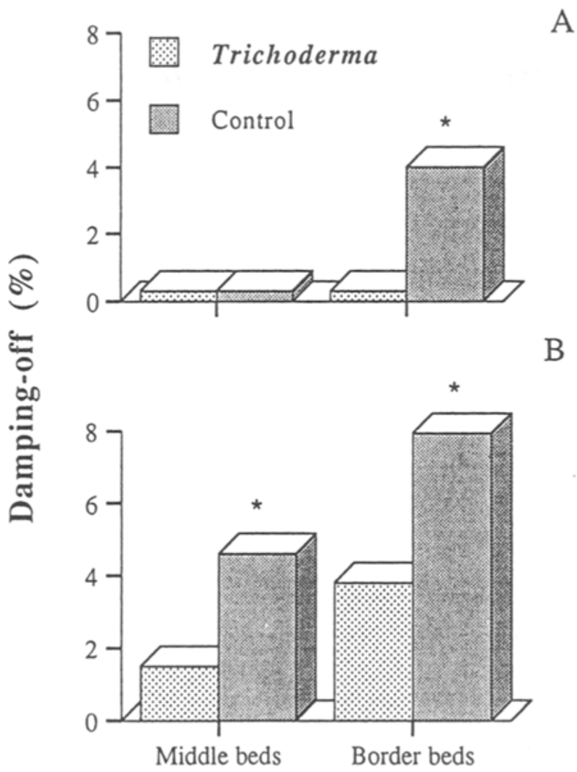

Fig. 5. Incidence of damping-off in non-treated (control) or *Trichoderma*-treated cucumber seedlings, in middle and border beds, seven days after transplanting to a commercial greenhouse. Measurements were carried out during two successive growing cycles. (A) First cycle, immediately after the soil was fumigated with methyl bromide (500 kg/ha); (B) second successive growing cycle. For each cycle, treated and non-treated seedlings were prepared as described in Materials and methods. * Significantly different ($P = 0.05$).

beds, the level of damping-off remained below 0.5%. The differences in plant height, leaf area and chlorophyll content between the two treatments remained constant throughout the growing period (data not shown).

At the end of the first cycle, plants were uprooted and the beds were conserved. For the second growing cycle, cucumber seedlings were transplanted to the same beds as in the first cycle, i.e., non-treated seedlings to control beds and *Trichoderma*-treated seedlings to treatment beds. Seven days after transplanting, the number of dead plants in each bed was determined (Fig. 5B). In the middle beds, a significant 67% reduction in the damping-off of cucumber seedlings was observed. In the *Trichoderma*-treated beds, only 1.5% of the plants died, as compared to 4.6% in the non-treated beds. In the border beds, 7.9% of the non-treated plants died, as compared to 3.8% in the *Trichoderma*-treated beds (a 52% reduction). Differences in plant growth response between treatments were as in the first cycle.

Analysis of the diseased plants revealed the presence of *Pythium* spp. (in 60% of diseased plants) and *Rhizoctonia solani* (in 25% of the diseased plants). In 15% of the plants, both pathogens could be isolated.

Discussion

Trichoderma spp. are well known as biocontrol agents of soilborne plant pathogenic fungi [Chet, 1987; Chet, 1990; Harman and Lumsden, 1990; Sivan and Chet, 1992]. The increased growth response of several plants, including vegetables, following application of *Trichoderma* spp. to a pathogen-free soil has also been documented [Baker, 1989; Chang et al., 1986; Kleifeld and Chet, 1992]. In the present work, *T. harzianum* isolate T-203 was applied as a peat-bran preparation to a cucumber and pepper seedling propagative mixture. Seedlings were grown in a commercial nursery, then transplanted to a commercial greenhouse. The increased growth response in plants is usually determined by measuring their length, leaf area and dry weight [Chang et al., 1986; Kleifeld and Chet, 1992]. Applying *T. harzianum* as a peat-bran preparation to both cucumber and pepper seedlings under commercial conditions significantly increased their length (by 24% and 17%, respectively), leaf area (by 96% and 50%, respectively), and dry weight (by 25% and 29%, respectively). In this work, the chlorophyll content of the leaves and the stem diameter were also measured, to further validate the advantage of the *Trichoderma*-treated seedlings over the normal, non-treated seedlings: increases in both were indeed observed. In all parameters, *Trichoderma*-treated plants appeared to be much more developed and vigorous than their non-treated plants appeared to be much more developed and vigorous than their non-treated counterparts. Overall, they had a more mature appearance than that of normal, non-treated plants. It is clear that this effect results from the presence of *Trichoderma* in the mixture. The uses and effects of autoclaved peat-bran mixture itself or autoclaved *Trichoderma* preparation on plants have already been demonstrated and discussed [Baker et al., 1984; Kleifeld and Chet, 1992]. Application of an autoclaved *Trichoderma* preparation to pepper increased seedling length and leaf area by only 20 and 70%, respectively, whereas the non-autoclaved *Trichoderma*-preparation caused a 120 and 180% increase, respectively, over the non-treated control [Kleifeld and Chet, 1992]. Similar effects were observed when an autoclaved preparation was applied to radish [Baker et al., 1984].

Under controlled conditions, without the addition of fertilizers, a significant increase in P and K content of *Trichoderma*-treated tomato leaves and roots has been detected [Kleifeld and Chet, unpublished data].

However, in our experiments in the nursery, all plants were fertilized according to the grower's practice. Under these conditions, no significant differences were found in N, P and K contents of either roots or leaves of pepper plants between normal, non-treated seedlings and *Trichoderma*-treated seedlings. It appears, therefore, that the increased growth response observed under commercial conditions is not due to increased mineral uptake by the *Trichoderma*-treated plants.

The effects obtained with *Trichoderma* have important economic implications, in terms of shortening the plant's growth period and time in the nursery, thereby increasing production capacity. Moreover, the *Trichoderma*-treated seedlings were found to be more resistant to damping-off diseases, caused by *Pythium* spp. and *R. solani*, occurring in the greenhouse during the first week after transplanting. During the second growing cycle, a significant reduction in the death of seedlings was achieved by pretreating the plants with *T. harzianum*. Using the soil for successive growing of the same crop results in a build-up of soilborne plant pathogens, thereby increasing damping-off during the growing season. Soil fumigation with methyl bromide is commonly used to solve this problem. However this method, besides being time-consuming and uneconomical, pollutes the atmosphere and is environmentally harmful, as the chemicals accumulate in the soil and water. The use of *Trichoderma*-treated seedlings may be combined with reduced levels of methyl bromide [Sivan and Chet, 1993] thereby prolonging the effect of the soil fumigation and minimizing damage to the environment [Chet, 1990].

The ability of *Trichoderma* spp. to reduce disease caused by soilborne plant pathogenic fungi is well known [Chet, 1987; Chet, 1990; Chet et al., 1993; Harman and Lumsden, 1990; Sivan and Chet, 1992]. This ability is related mainly to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere, mainly iron and carbon [Sivan and Chet, 1986]. Recently however, another possible mechanism has been suggested by Kleifeld and Chet [1993, unpublished], namely, *Trichoderma*-induced resistance in plants to fungal attack. *Trichoderma* has been found to penetrate and live in the plant root cortex [Kleifeld and Chet, 1992]. In response, the level of lignin in roots and shoots is increased. Increases have also been found in the activities of the hydrolytic enzymes β-1-3-glucanase and chitinase, suggesting that *Trichoderma* stimulates the plant's defense mechanisms [Kleifeld and Chet, 1993, unpublished].

Production of vigorous seedlings which are more resistant to soilborne plant pathogenic fungi is advantageous to the producer as well as to the farmer. Application of a beneficial microorganism (e.g. *Trichoderma*) to the propagative mixture during seedling production in the nursery makes the use of such microorganisms for both plant growth enhancement and biological control more feasible.

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