Quantitative Analysis of the Resistance to *Phytophthora cinnamomi* in Five Avocado Rootstocks Under Greenhouse Conditions

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ABSTRACT

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The level of resistance to *Phytophthora cinnamomi* in the avocado (*Persea americana*) rootstocks Thomas, Barr Duke, and Topa Topa and the avocado hybrid (*P. americana* × *P. schiedeana*) rootstocks Martin Grande and UCR 2023 was analyzed quantitatively under greenhouse conditions. Rootstocks were planted in U.C. mix C, either not infested or artifically infested with *P. cinnamomi*, and grown in the greenhouse. Resistance was assessed at 6, 12, and 24 wk after inoculation. The percent of reduction in root or shoot dry weights and the root growth potential were found to be useful parameters for analyzing resistance to *P. cinnamomi* in these rootstocks. The moderately resistant Thomas had the lowest percent of reduction in root and shoot dry weights compared to the other rootstocks. However, Thomas supported a higher soil population of *P. cinnamomi* than the other moderately resistant rootstocks Martin Grande and Barr Duke. Both Thomas and Martin Grande had a higher root growth potential than Barr Duke and the susceptible rootstock Topa Topa. On a quantitative basis, Thomas expressed a level of resistance to *P. cinnamomi* equal to or greater than Martin Grande, while Barr Duke expressed a level between Martin Grande and the susceptible rootstocks Topa Topa and UCR 2023.

Phytophthora root rot, caused by *Phytophthora cinnamomi* Rands, is a serious disease of avocado (*Persea americana* Miller) in California (5). Avocado rootstocks that express a moderate level of resistance are an important element in the integrated control of this disease (5,18). Resistance in avocado rootstocks has been described as field resistance (5,18); however, its biological basis has been difficult to identify. Quantitative measurements of infection efficiency, lesion growth, and sporangia production were useful for identifying general resistance in foliage of potatoes to *P. infestans* (Mont.) de Bary (16). Kellam and Coffey (13) analyzed different components of the avocado-*P. cinnamomi* interaction with the moderately resistant rootstocks Duke 7 and G6 and susceptible Topa Topa. They found that the amount of root infection, apparent infection rate, and root growth potential were useful parameters in quantitative comparisons of resistance in these rootstocks.

The experimental clonal rootstocks Thomas, Martin Grande, and Barr Duke, which possess moderate resistance to Phytophthora root rot, have performed well in recent field trials (5,10). However, little is known about the mechanisms of resistance operating in these new rootstocks. The rootstock UCR 2023 is a vigorous seedling of Martin Grande that has not been tested for its resistance to *P. cinnamomi*. To further our understanding of the mechanisms and levels of resistance operating in these rootstocks, components of the host-pathogen interaction were analyzed quantitatively.

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MATERIALS AND METHODS

Rootstocks. The avocado rootstocks Topa Topa (18), Barr Duke (4), Thomas (4,5), and the avocado hybrid (*P. americana* × *P. schiedeana* Nees) rootstocks Martin Grande (G755c; 4–6) and UCR 2023 (Martin Grande seedling) were vegetatively propagated as previously described (7,9). The ungrafted rootstocks were planted into U.C. mix C (1) in 10-L pots when a well-developed root system was visible (9).

Phytophthora inoculum. The U.C. mix C was amended with *P. cinnamomi* inoculum (isolate P2428) which was grown on millet seed for 1 wk and then blended into a slurry before addition to the soil. The inoculum concentration used was 30 propagules per gram (ppg) dry weight of soil and was calculated as previously described (11). Inoculated plants were given a 3-cm buffer zone of noninfested soil to allow undamaged roots to grow into the infested soil. Control rootstocks were planted into noninfested U.C. mix C.

All rootstocks were grown in the greenhouse with natural light and a temperature range between 18 and 29 C. Rootstocks were arranged on benches so that one infested and one noninfested rootstock of each selection was randomized in each row. Rows were randomly selected at each assessment time. All rootstocks were watered with a dilute Hoagland's solution (15) and the soil was maintained at field capacity. The experiment duration was 24 wk, with assessments made at 6, 12, and 24 wk after inoculation. Each assessment included at least seven replicates of the infested and noninfested rootstocks. The experiment was conducted twice. In the first experiment Thomas, Martin Grande, and Topa Topa were compared. Barr Duke and UCR 2023 were added in the second experiment. UCR 2023 was assessed only at 24 wk after inoculation because of the limited availability of this rootstock.

Quantitative assessment of resistance. At each assessment, the *P. cinnamomi* soil population was determined by combining four soil cores (2.5-cm diameter and 30-cm length) from each pot (20-cm diameter and 45-cm length). The equivalent of 10 g of soil dry weight was mixed with 100 ml of 0.25% water agar, and 2 ml of the suspension were plated onto the *Phytophthora*-selective medium PARPH (13). The number of colonies per plate present after 3 days of incubation at 24 C was expressed as ppg. The percent of necrotic roots was determined by washing the root systems free of soil and visually rating them. The percent of root infection by *P. cinnamomi* was determined quantitatively by taking 30 1-cm long root segments selected randomly from the entire root system and plating them on PARPH. The number of root segments with *P. cinnamomi* growing from them was determined after 3 days in the dark at 24 C and calculated as a percent of the total root segments.

The percent of reduction (infested vs. noninfested) in trunk diameter was determined by marking a reference point on the trunk of all rootstocks and taking measurements after planting and at each assessment time. The percent of reductions (infested vs. noninfested) in root and shoot dry weights were determined by using the weights of the oven-dried (80 C) shoots removed at the soil line and washing the root systems free of soil. The percent of reductions in trunk diameter and root and shoot dry weights were determined using the following formula: ([noninoculated − inoculated]/noninoculated) × 100.

Root growth potential. The relative root growth potentials of Thomas, Martin Grande, Barr Duke, and Topa Topa were assessed by two techniques. The first involved removing one-half of the root system longitudinally with a knife before planting in 10-L pots with noninfested U.C. mix C. At 24 wk after planting, measurements of trunk diameter and root and shoot dry weights were determined as described above. The second technique involved planting rootstocks into infested or noninfested soil. At 8 and 14 wk after planting, 300 $\mu g/ml$ of phosphorous acid, buffered to pH 6.8 with KOH to give potassium phosphonate, was applied as a 1-L soil drench per pot to inhibit growth of *P. cinnamomi* (8). Twenty-four weeks after inoculation, the percent of root infection, the percent of necrotic roots, and the percent of reductions in trunk diameter and root and shoot dry weights were determined as described above.

The root growth potential experiments were conducted twice with Thomas, Martin Grande, and Topa Topa. Barr Duke was added to the second experiment. Both experiments included a minimum of eight replicates for each rootstock selection.

RESULTS

The results reported for resistance quantification and root growth potential are from the second experiment and are

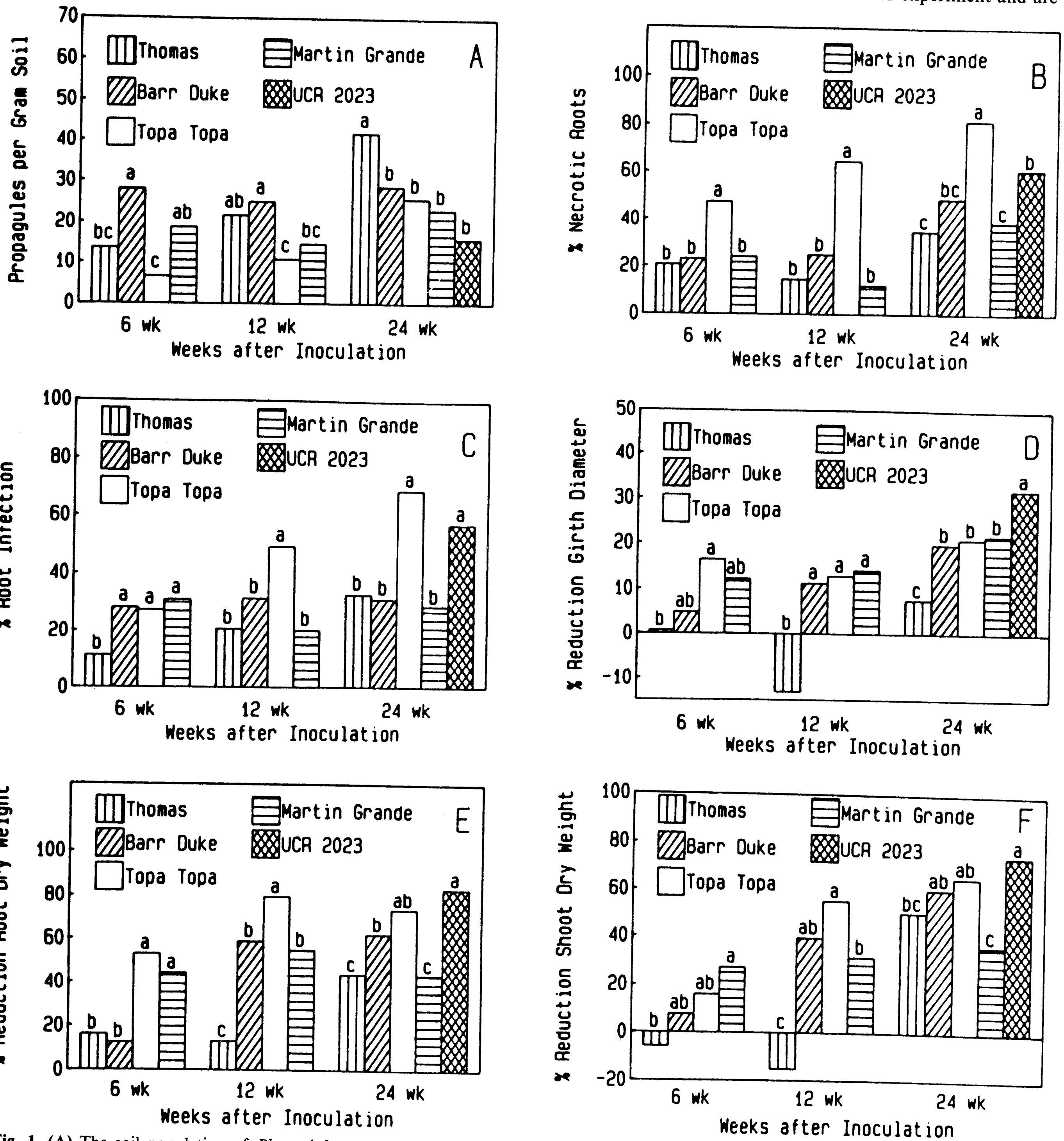

Fig. 1. (A) The soil population of *Phytophthora cinnamomi,* **(B)** the percent of necrotic roots determined by visual rating, **(C)** the percent of root infection measured by plating root segments on a *Phytophthora*-selective medium, PARPH, **(D)** the percent of reduction in trunk diameter, **(E)** the percent of reduction in root dry weight, and **(F)** the percent of reduction in shoot dry weight for the avocado rootstocks Thomas, Barr Duke, Topa Topa, and Martin Grande at 6, 12, and 24 wk, and UCR 2023 at 24 wk after inoculation with *P. cinnamomi.* Different letters above bars within each assessment time and parameter analyzed are significantly different according to Duncan's new multiple range test ($P = 0.05$).

similar to the results of the first experiment for Thomas, Martin Grande, and Topa Topa. An analysis of variance was used to determine significant differences between rootstock selections for all experiments. Duncan's new multiple range test was used to compare rootstock selection means for all experiments ($P = 0.05$).

Quantitative assessment of resistance. Six weeks after inoculation, the original inoculum in U.C. mix not planted to rootstocks had dropped to 1 ppg, while that of U.C. mix planted to rootstocks was considerably higher (6–28 ppg; Fig. 1A). By 24 wk after inoculation, a trend was apparent. Thomas supported a higher population of *P. cinnamomi* than either UCR 2023, Martin Grande, Topa Topa, or Barr Duke (Fig. 1A).

Topa Topa had a significantly higher percentage of necrotic roots, based on visual ratings, than Barr Duke, Martin Grande, and Thomas at 6, 12, and 24 wk after inoculation, and UCR 2023 at 24 wk after inoculation (Fig. 1B). The percent of necrotic roots, based on visual ratings, for the noninfested controls was less than 1% for all rootstock selections. Following plating on PARPH-selective medium, Topa Topa was found to have a significantly higher percentage of root infection than either Thomas, Martin Grande, or Barr Duke at 12 and 24 wk after inoculation (Fig. 1C). UCR 2023 was not significantly different from Topa Topa at this stage (Fig. 1C). The percent of reductions in trunk diameter (Fig. 1D) and root (Fig. 1E) or shoot (Fig. 1F) dry weights generally increased over the duration of the experiment for all of the selections. At 12 wk after inoculation, Thomas had significantly lower percent reductions in trunk diameter and root and shoot dry weights than Martin Grande, Barr Duke, and Topa Topa. These latter three rootstocks were not significantly different in their percent of reduction in trunk diameter. However, Martin Grande and Barr Duke had a significantly lower percent reduction in root dry weight than Topa Topa (Fig. 1E). At 24 wk after inoculation, Thomas again had a significantly lower percent of reduction in trunk diameter (Fig. 1D) than either Barr Duke, Topa Topa, Martin Grande, or UCR 2023. Both Thomas and Martin Grande had a significantly lower percent of reduction in root dry weight (Fig. 1E) than Barr Duke, Topa Topa, and UCR 2023. UCR 2023 had a greater percent of reduction in trunk diameter (Fig. 1D) than Topa Topa and was not significantly different in its percent of reductions in root and shoot dry weights (Fig. 1E and 1F).

Root growth potential. The percent of reductions in root and shoot dry weights of Thomas, Barr Duke, Topa Topa, and Martin Grande following either root pruning or potassium phosphonate treatment are shown in Figures 2A and B, respectively. In the root pruning experiment, Martin Grande showed significantly less reduction in root dry weight than Barr Duke and Topa Topa. Thomas was not significantly different from either Martin Grande or Topa Topa. Both Thomas and Martin Grande showed significantly less reduction in shoot dry weight than Barr Duke and Topa Topa.

Following potassium phosphonate treatment, the infested Thomas rootstocks showed significantly less reduction in both root and shoot dry weights than either Barr Duke, Topa Topa, or Martin Grande. The dry weights of the infested root systems of Martin Grande, Topa Topa, and Barr Duke were less than those of the noninfested root systems. In contrast, the root system of infested Thomas had a greater root dry weight when compared to the noninfested treatment. *P. cinnamomi* was reisolated from all infested rootstock selections; however, there were no significant differences in the ppg recovered. Thomas had a lower percentage of necrotic roots based on visual ratings (6%) than either Barr Duke (13%), Topa Topa (20%), or Martin Grande (21%). Finally, there was no significant difference in the percent of reduction in trunk diameter of the four selections in either experiment.

DISCUSSION

Our results, using quantitative component analysis, support previous field research in demonstrating that Thomas, Martin Grande, and Barr Duke expressed greater resistance to *P. cinnamomi* than the susceptible Topa Topa rootstock (5,10). A vigorous seedling selection of Martin Grande, UCR 2023, was also found to be susceptible to *P. cinnamomi.*

The percent reductions (infested vs. noninfested) in trunk diameter and root or shoot dry weights permitted useful comparisons between these rootstock selections. By 12 wk after inoculation, Thomas had significantly lower reductions in trunk diameter and root and shoot dry weights than the moderately resistant rootstocks Martin Grande and Barr Duke. This suggests that Thomas has a higher level of resistance to *P. cinnamomi* than these rootstocks. At 24 wk after inoculation, Martin Grande had lower reductions in root and shoot dry weights than Barr Duke, indicating that it has a higher level of resistance to *P. cinnamomi* than Barr Duke. This greater resistance may be attributable in part to the restricted development of *P. cinnamomi* in juvenile roots of Martin Grande (7).

Measurements of soil populations of *P. cinnamomi* and other *Phytophthora* species can be notoriously variable (14,17). Nevertheless, there was a trend for Thomas to support a higher population of *P. cinnamomi* than the other rootstocks. Similar results have been reported for moderately resistant and

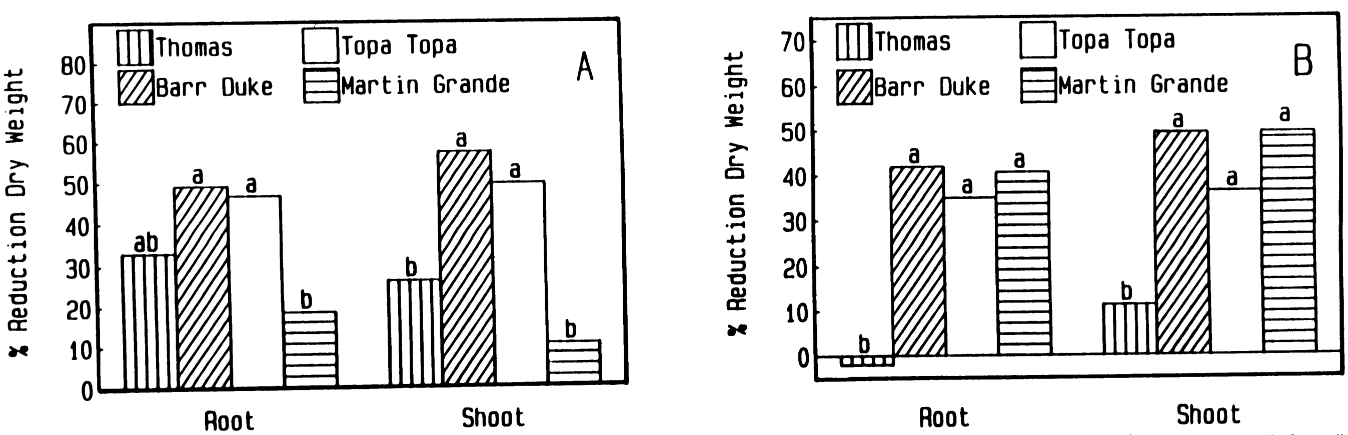

Fig. 2. Measurement of root growth potential by determining the percent of reductions (A: pruned vs. nonpruned, B: infected vs noninfected) in root and shoot dry weights for the avocado rootstocks Thomas, Barr Duke, Topa Topa, and Martin Grande at 24 wk after planting. **(A)** All rootstocks had 50% of their root system removed longitudinally before potting in noninfested U.C. mix C. **(B)** All rootstocks were treated with 300 μg/ml of phosphorous acid, buffered to pH 6.8 with KOH, at 8 and 14 wk after inoculation with *Phytophthora cinnamomi.* Different letters above bars within each experiment and parameter analyzed are significantly different according to Duncan's new multiple range test ($P = 0.05$).

susceptible *Eucalyptus* species. Halsall (12) found a more rapid build up of *P. cinnamomi* sporangia produced on the root surfaces of moderately resistant *E. maculata* Hooker compared to the susceptible *E. sieberi* L. Johnson.

The pruned, noninoculated root systems of Thomas and Martin Grande showed a greater ability to regenerate roots than either Barr Duke or Topa Topa. Similarly, Kellam and Coffey (13) found that the moderately resistant avocado rootstock Duke 7 had a greater root growth potential than susceptible Topa Topa under noninoculated conditions. A greater root growth potential has also been correlated with higher field resistance to *P. cinnamomi* in *Eucalyptus* spp. (12) and to *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian in *Citrus* spp. (2). Bruehl (3) reported that nonspecific host resistance to a pathogen is often related to agronomic characteristics such as a more vigorously growing root system. The higher root growth potential seen in Thomas and Martin Grande supports this observation.

We found Thomas had the lowest reduction in root weight when the fungicide potassium phosphonate (5,8) was applied to infected rootstocks to reduce or inhibit infection by *P. cinnamomi* and allow root growth to recover. This greater root growth probably reflects the higher level of resistance expressed in Thomas at 12 wk after inoculation (Fig. 1E), compared to the other rootstocks. The application of potassium phosphonate at 8 and 14 wk after inoculation probably reduces or restricts further disease development (8), and the lower reduction in root and shoot dry weights assessed for Thomas at 24 wk after inoculation may be a reflection of the stage of disease development at the time of potassium phosphonate application. Nevertheless, the enhanced root growth seen in Thomas treated with potassium phosphonate supports the use of fungicides in an integrated approach to the control of Phytophthora root rot (5).

The percent of root infection and the percent of necrotic roots are measurements based on the existing root system at the time of breakdown. However, those roots that are completely necrotic are lost during root washing and are not included in these ratings. Therefore, these measurements do not fully represent the amount of root damage present. However, with such measurements it was still possible to separate those rootstocks with moderate resistance (Thomas, Martin Grande, and Barr Duke) from the susceptible Topa Topa and UCR 2023.

Measurements of trunk diameter have proven useful for analyzing resistance to Phytophthora root rot in avocado rootstocks in the field (5,10). However, in these greenhouse experiments, the trunk diameter of Martin Grande was not significantly different from the susceptible Topa Topa. There was also no significant difference in the percent reduction in trunk diameter between the rootstock selections in the experiments with root growth potential, indicating that this parameter is not likely to prove useful in such short-term evaluations.

Higher soil populations of *P. cinnamomi* were recovered 24 wk after inoculation (16–42 ppg) compared to those normally found in the field (1–3 ppg; 13). It has been suggested that these high soil populations are caused by the restricted size of the containers used (13). The raised inoculum level might be expected to lead to a higher infection (14) of the rootstocks being screened, thus making this procedure a severe test of the level of resistance to *P. cinnamomi* in these rootstocks. However, the close correlation of these greenhouse results with previous field experiments (5,10) suggests that short-term quantitative analysis using container-grown greenhouse material may be useful in preliminary screening of new rootstocks before selecting them for longer term field trials.

We found that the level of resistance in Thomas was equal to or greater than that of Martin Grande, while that of Barr Duke appeared to be intermediate between Martin Grande and susceptible Topa Topa. The Martin Grande seedling, UCR 2023, was also found to be susceptible. Further, the higher root growth potentials of Thomas and Martin Grande may be important contributing factors in their resistance to Phytophthora root rot.

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