

Effect of Soil Fungicides on *Macrophomina phaseolina* Sclerotium Viability in Soil and in Soybean Stem Pieces

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ABSTRACT

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The effectiveness of 10 soil fungicides in reducing viability of *Macrophomina phaseolina* sclerotia in soil depended upon the concentration of the fungicide used and exposure time. The most effective fungicides in laboratory tests in descending order were: benomyl, thiophanate-methyl, thiram, thiabendazole, triforine, and captan. There was greater reduction of sclerotium viability by all fungicides in low than in high organic-matter-containing soils. In

laboratory tests, benomyl, thiophanate-methyl, thiabendazole, and thiram were effective in reducing sclerotium viability in soybean stem pieces placed in fungicide-treated soil. The reduction in viability was greater when free sclerotia were used, least when sclerotia were in fresh stem pieces, and intermediate when sclerotia were in partially decomposed stem pieces.

Additional key words: *Rhizoctonia bataticola*, charcoal rot, systemic fungicides.

Macrophomina phaseolina (Tassi) Goid. [*M. phaseolina* (Maubl.) Ashby, *Rhizoctonia bataticola* (Taub.) Butler] causes charcoal rot of over 400 species of plants (2, 9, 11, 14). The fungus is a soil- and root-inhabiting pathogen with sclerotia as the principle means of survival (8). Populations in U.S. soils were reported as high as 108 propagules per gram of oven-dried soil (8). It causes a seedling blight, root rot and stem decay of soybean [*Glycine max* (L.) Merr.] and can reduce soybean yields (8). There are no known commercial soybean cultivars resistant to *M. phaseolina* (2).

Soil fumigation prior to planting is effective for controlling charcoal rot of coniferous seedlings (14), but may not be economical for soybeans. Glucono- δ -lactone was shown to decrease the severity of charcoal rot of soybean, but its use is not practical (9). The disease on soybeans was not controlled with certain systemic fungicides (7). We present results on the effect of 10 fungicides on the viability of *M. phaseolina* sclerotia in soil or in soybean stem pieces.

MATERIALS AND METHODS

Preparation of inoculum.—The isolate of *M. phaseolina* used was recovered from silty-loam soil from a field which had been in soybean monoculture for 6 years. After growth at 30 C, stock cultures on Difco potato-dextrose agar (PDA) were held at 4 C. All experiments were conducted at 30 C unless otherwise indicated.

Sclerotia were produced by placing four to six, 6-mm-diameter PDA disks, cut with a sterile cork-borer, from a

5-day-old stock culture of *M. phaseolina* into each of several 6,000-ml Erlenmeyer flasks containing 1,000 ml soybean-seed-extract broth (SSEB). SSEB was prepared by boiling 100 g of dry soybean seeds in 1,000 ml of distilled water for 10-15 minutes, filtering the extract through eight layers of cotton gauze, adjusting the filtrate volume to 1 liter with distilled water, then adding 20 g sucrose and autoclaving (121 C for 15 minutes). After 15 days, the mycelial mats with sclerotia were homogenized in a VirTis mixer with sterile distilled water to break up the mat and separate the sclerotia from the mycelium. The homogenate was filtered through Whatman No. 42 filter paper. Sclerotia were washed with sterile distilled water three to four times. Sclerotia were dried for 48 hours, clumps separated by grinding in a mortar and screened through a 149- μ m sieve to separate the individual sclerotia. Sclerotia were stored at 4 C. The viability of sclerotia was tested on a selective medium for *M. phaseolina*, the chloroneb-mercury-rose bengal-agar (CMRA) medium (8), and on PDA supplemented with 30 μ g/ml streptomycin sulfate.

Types and preparation of soils.—Two soil types were used: (i) silty-sand loam, consisting of 34% sand, 51% silt and 15% clay, pH 6.3, total nitrogen of 0.0273%, total organic matter of 0.6%, and a maximum moisture holding capacity (MHC) of 30% water by weight; and (ii) silty loam, consisting of 13% sand, 65% silt, 22% clay, pH 5.4, total N of 0.175%, total organic matter of 3.5%, and a maximum MHC of 42% water by weight.

Soil was air-dried at room temperature (25 ± 2 C), crushed, and screened through a 2-mm sieve. Dry

sclerotia were added at a rate of 1 g/kg and mixed in a twin-arm mixer for at least 40 hours. An approximate concentration of 1,750 sclerotia/g on an oven-dry weight basis was determined by plating samples of infested soil on CMRA medium in 15 replications.

Effect of soil fungicides on *Macrophomina phaseolina* sclerotia in soil.—Ten fungicides were evaluated at 50, 100, 200, and 500 $\mu\text{g/g}$: benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, Benlate 50% WP of E. I. duPont de Nemours and Co., Inc., Wilmington, Delaware]; thiophanate-methyl [dimethyl 4,4-*o*-phenylenebis(3-thioallophanate), Topsin-M 70 WP of Pennwalt Corp., Tacoma, Washington]; thiabendazole [2-(4-thiazolyl)benzimidazole], Mertect 60 WP of Merck Chem. Div., Rahway, New Jersey]; thiram (tetramethylthiuram disulfide, Thylate 65 WP of E. I. duPont de Nemours and Co., Inc., Wilmington, Delaware); captan [N-(trichloromethyl) thio-4-cyclohexene-1,2-dicarboximide, Captan 80 WP of Stauffer Chem. Co., Mountain View, California]; triforine [N,N'-(1,4-piperazinediylbis(2,2,2-trichloroethylidene) bis(formamide)), W-524 80 WP of CibaMerck GMBH and Co. KG and FMC Corp., Middleport, New York]; hymexazole (3-hydroxy-5-methyl isoxazole, calcium salt, Hymexazole 70 WP of Sankyo Co., Ltd., Tokyo, Japan); ethazol (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, Terrazole 35 WP of Olin Corp., Little Rock, Arkansas); and carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide, Vitavax 75 WP of UniRoyal Chem. Co., Bethany, Connecticut).

Three-hundred grams of the sclerotia-soil mixture (based on air-dried weight) were placed into each of three glass containers (replications). The fungicides were mixed as suspensions with silty-sand loam soil. Containers were covered with Parafilm to reduce moisture loss but it was punctured several times with a needle to allow for aeration. Soil moisture was maintained at 33% MHC (10). All containers were incubated at 30 C and 15-g samples were removed after 1, 3, 4, 6, and 8 days.

Soil samples were air-dried for not more than 10 hours, then ground in a mortar. A 50-mg sample was sprinkled evenly over the surface of five CMRA plates. If no *M. phaseolina* colonies were formed, or if the colony count was low (less than 10 per five plates), then 100-200 mg of soil was sprinkled over 10-20 CMRA plates. Colony counts were made at 10-12 days. Colony counts from any one treatment were combined to calculate differences between populations among treatments.

The counts from all plates of any one fungicide treatment were combined and percent germination of sclerotia based on the controls was calculated. The percent viability was plotted against time of exposure for each fungicide concentration used. The dosage-response curves for the 10 fungicides increased with increasing concentration and exposure time. All data were subjected to factorial experiment analysis. Statistical F-values for fungicides, dosage rates, exposure time, and their interactions were significant $P = 0.05$ and 0.01 . Least significant differences for F-values between fungicides, between dosages, and between exposure times were also calculated (1).

Influence of soil type.—Four-hundred grams of the sclerotia-soil mixture for each soil type were placed into each of four glass containers (replications). The 10 fungicides were used at a rate of 200 $\mu\text{g/g}$ and applied as

aqueous suspensions. Samples were taken at 2-day intervals for 8 days and plated on CMRA medium. Colony counts were recorded after 10-12 days. Statistical F-values were calculated for fungicides, soil types, and exposure time.

Effects of fungicides on *Macrophomina phaseolina* sclerotia in soybean crop residues.—Soybean stems (cultivar Amsoy-71) containing sclerotia of *M. phaseolina* were collected from an experimental field plot and stored at 4 C. Infested stems were washed with running tap water and cut into 1-cm pieces approximately 5 mm thick.

One-hundred grams of soil was placed into each 150-ml beaker for each soil type. One-hundred-twenty-five stem pieces with *M. phaseolina* sclerotia were mixed with the soil in each beaker. Aqueous suspensions of each fungicide were mixed with the soil at 200 $\mu\text{g/g}$ in each beaker. There were three replications for each fungicide per soil type. Nontreated, noninfested; nontreated, infested; and treated, noninfested soils served as controls. Each beaker was covered with punctured Parafilm. Every 2 days for 10 days and at the end of 14 days, 20 stem pieces were removed from each treatment, washed under tap water, surface sterilized, and plated on CMRA medium.

In another experiment free sclerotia or stem pieces containing sclerotia were mixed in soil which contained benomyl, thiophanate-methyl, thiabendazole, or thiram at either 50, 100, or 200 mg/g. Free sclerotia were mixed with nontreated and treated soil at a rate of 1 g/kg. Fresh and old (partially decomposed) soybean stem tissues containing sclerotia were used. Old stem pieces were collected 8 months after harvest from a field plot that contained diseased soybean plants. Both types were mixed with nontreated and treated soils at a rate of 1 g of tissue per 100 g of soil. Stem pieces were randomly selected and assayed on CMRA medium to determine the viability of sclerotia.

The stem pieces were removed after 8 days, washed with tap water, and comminuted in 50 ml of sterile tap water in a Waring Blendor for 2 minutes at high speed. Appropriate dilutions were made from the comminuted sample on CMRA medium and colony counts were made after 10 days. The percent viable sclerotia in soil was determined by sprinkling 50 mg containing free sclerotia from each treatment onto CMRA medium. Fresh and old stem pieces without fungicide gave approximate populations of ~30,000 and ~50,000 sclerotia/g stem pieces, respectively,

RESULTS

The most effective fungicides for reducing sclerotia viability in soil in descending order were: benomyl, thiophanate-methyl, thiram, triforine, and captan (Fig. 1, 2, 3). The time required to reach 50% mortality for 50 $\mu\text{g/g}$ benomyl was 4 days (Fig. 1-A), for thiophanate-methyl it was 7 days (Fig. 1-B), and for thiram it was 8 days (Fig. 1-D) after treatment. The time required to reach 50% mortality for 200 $\mu\text{g/g}$ benomyl was less than 24 hours, for thiophanate-methyl it was 4 days, for thiram it was 5 days, for thiabendazole it was 6 days, and for triforine and captan it was 7 days after treatment (Fig. 1-C, 2-A,B). Less than 50 percent of the sclerotia were killed after 8 days in soil treated with thiabendazole and triforine at

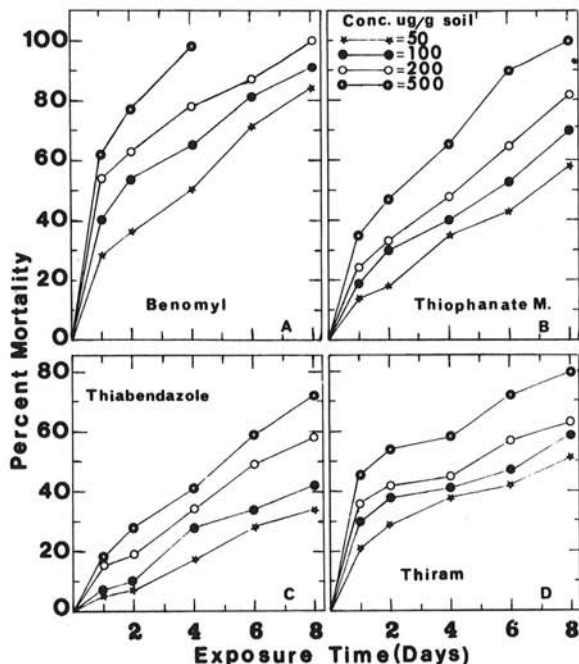


Fig. 1-(A to D). Viability of *Macrophomina phaseolina* sclerotia in silty-loam soil at various days after treatment with one of the following fungicides: A) benomyl, B) thiophanate-methyl, C) thiabendazole, and D) thiram. FLSD values, where X = fungicide, Y = rates, and Z = time, at 5 and 1%, respectively, for differences between two: (i) X means, 2.1 and 2.9; (ii) Y means, 0.8 and 1.03; and (iii) Z means, 0.7 and 0.91.

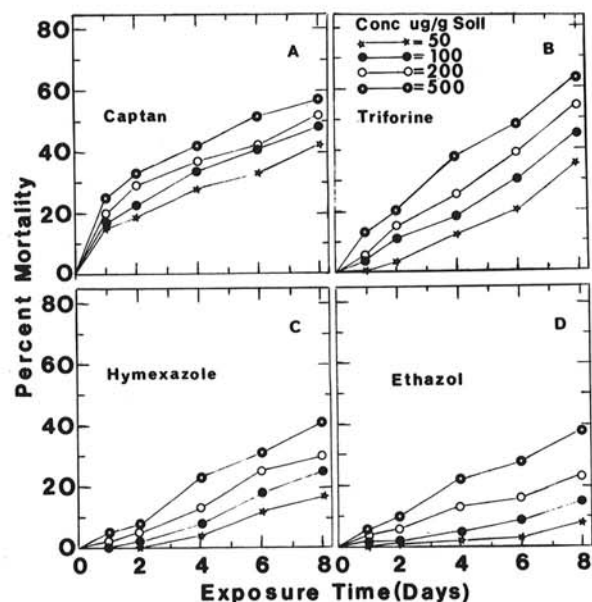


Fig. 2-(A to D). Viability of *Macrophomina phaseolina* sclerotia in silty-loam soil at various days after treatment with one of the following fungicides: A) captan, B) triforine, C) hymexazole, and D) ethazol. FLSD values, where X = fungicide, Y = rates, and Z = time, at 5 and 1%, respectively, for differences between two: (i) X means, 2.1 and 2.9; (ii) Y means, 0.8 and 1.03; and (iii) Z means, 0.7 and 0.91.

TABLE 1. Recovery of *Macrophomina phaseolina* from infected soybean stem pieces at various days after treatment with various fungicides at 200 µg/g of soil, in two soil types

| Fungicide ^a | Recovery of <i>M. phaseolina</i> (%) ^b | | | | | |
|------------------------|---|--------|--------|--------|---------|--------|
| | 4 days | | 8 days | | 14 days | |
| | Soil A | Soil B | Soil A | Soil B | Soil A | Soil B |
| Benomyl | 78 | 88 | 48 | 62 | 7 | 33 |
| Thiophanate-methyl | 93 | 100 | 67 | 82 | 32 | 47 |
| Thiabendazole | 100 | 100 | 77 | 85 | 48 | 62 |
| Thiram | 100 | 100 | 100 | 100 | 82 | 92 |

^aThe following fungicides were ineffective: captan, triforine, Hymexazole, ethazol, pentachloronitrobenzene, and carboxin. A = silty-sand-loam, B = silty-loam.

^bPercentage of stem pieces giving rise to *M. phaseolina* when plated on a selective agar medium. A = silty-sand-loam, B = silty-loam.

either 50 or 100 µg/g and in soil treated with PCNB, ethazol, hymexazole, or carboxin at 500 µg/g (Fig. 1, 2, 3).

The effectiveness of the six active fungicides at 100 and 200 µg/g increased with increasing exposure time (Fig. 1, 2). Sclerotium viability was reduced more by benomyl than by the other fungicides at each exposure time. At the same rates, reduction of sclerotial viability by thiram exceeded that of thiophanate-methyl until about 3 days after treatment, after which time the reduction of sclerotial viability by thiram exceeded that of thiophanate-methyl.

Reduction of sclerotial viability by all fungicides increased with increased exposure time in both soil types and was greater in silty-sand-loam than in silty-loam except for ethazol and carboxin which showed equal effectiveness in both soil types.

Benomyl, thiophanate-methyl, and thiabendazole were effective in reducing viability of sclerotia in fresh soybean stem pieces (Table 1). Thiram had a slight effect after 10-14 days. The other fungicides tested were ineffective after 14 days. Benomyl, thiophanate-methyl, thiabendazole, and thiram were more effective in silty-sand-loam than in silty-loam (Fig. 4). The time required to reach 50% mortality for thiabendazole occurred after 14 days in silty-sand-loam (Fig. 4-C).

The reduction in sclerotial viability by benomyl, thiophanate-methyl, thiabendazole, and thiram was greater when free sclerotia were used, least when sclerotia were in fresh stem pieces, and intermediate when sclerotia were in old stem pieces (Fig. 4-A, B, C, D).

There were no significant differences in sclerotial viability within fresh stem pieces treated with thiram at any rate or in old stem pieces treated with thiram at 50 and 100 µg/g (Fig. 4-D). There was a greater reduction of sclerotial viability in old stem pieces in soil treated with thiram at 200 µg/g.

DISCUSSION

Benomyl and thiophanate-methyl hydrolyze to methyl-2-benzimidazole carbamate (MBC) (3, 4, 13, 15). Fuchs et

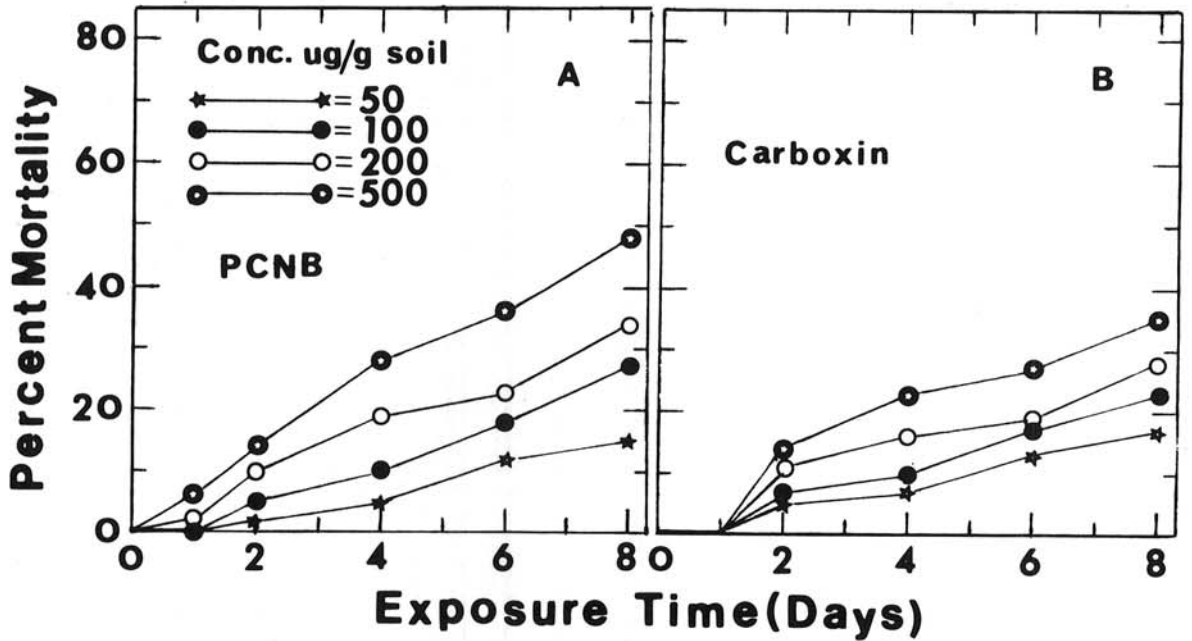


Fig. 3-(A, B). Viability of *Macrophomina phaseolina* sclerotia in silty-loam soil at various days after treatment with one of the following fungicides: A) PCNB and B) carboxin. FLSD values, where X = fungicide, Y = rates, and Z = exposure time, at 5 and 1%, respectively, for differences between two: (i) X means, 2.1 and 2.9; (ii) Y means, 0.8 and 1.03, and (iii) Z means, 0.7 and 0.91.

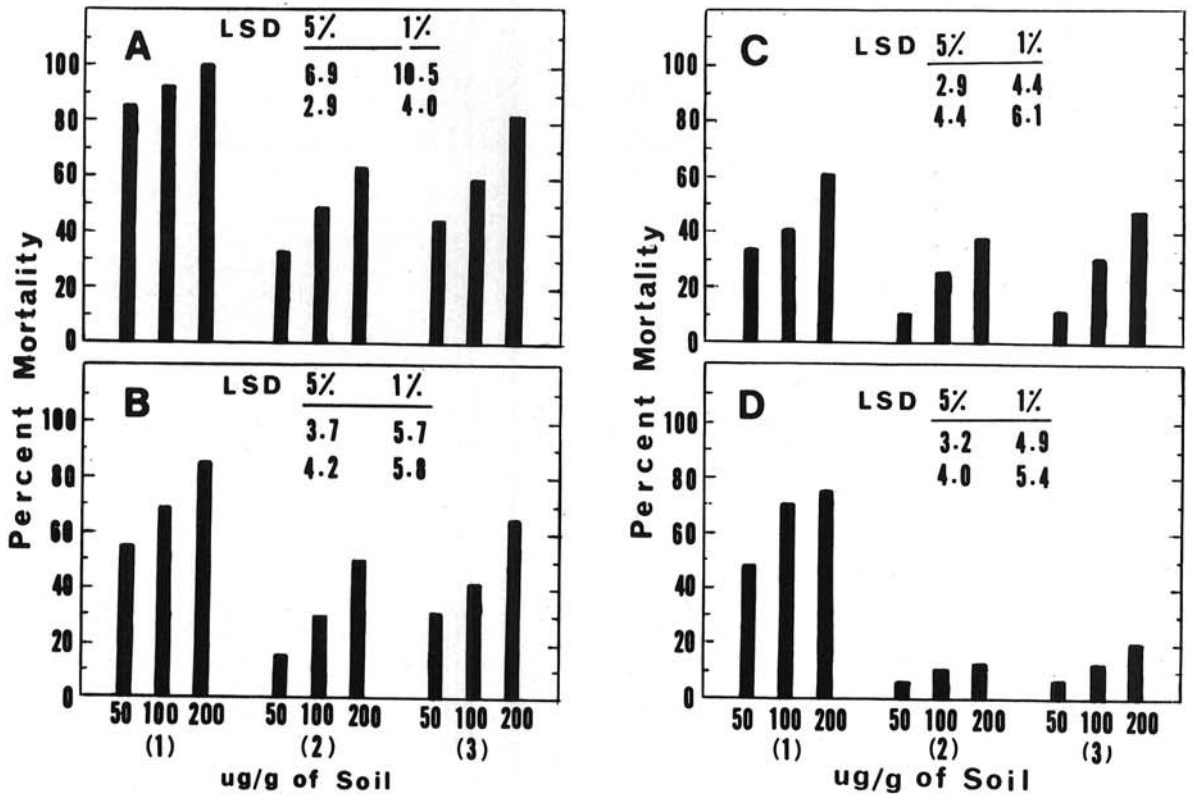


Fig. 4-(A to D). Viability of *Macrophomina phaseolina* sclerotia after 8 days mixed with soil either as free sclerotia (1) or contained in fresh (2) or in partially decomposed (3) soybean stem pieces. The silty-and-loam soil used was treated with: A) benomyl, B) thiophanate-methyl, C) thiabendazole, or D) thiram at 50, 100, or 200 $\mu\text{g/g}$ soil.

al. (5) reported that the transformation rate was greater for benomyl than for thiophanate-methyl. This difference in conversion rate may help explain the differences between the two compounds in the time required to reach 50% mortality of *M. phaseolina* sclerotia. Thiabendazole has a fungitoxic spectrum similar to that for benomyl but it is not hydrolyzed (4). This may explain in part the activity of thiabendazole being lower than that for benomyl and thiophanate-methyl.

The influence of soil type on the availability and effectiveness of benomyl and thiabendazole has been reported (6, 12, 16). In general, the higher the soil organic matter, the less active is the fungicide. The higher activity obtained with the more effective fungicides in low organic-matter soil compared to the high-organic-matter soil suggests that the fungicides become bound to or inactivated by organic particles.

The reduction of sclerotial viability in partially decomposed stem pieces was greater than in fresh stem pieces treated with benomyl, thiabendazole, thiophanate-methyl, and ethazol. This may have been due to the greater uptake and accessibility of MBC to sclerotia within the spongy, partially decomposed stem tissues. Also, the organic matter content of partially decomposed stem pieces would be expected to be lower than in fresh stem pieces.

The amount of fungicide required to reduce sclerotium viability in a field containing fresh crop residue would be greater than for a field containing old crop residue. A soil treatment at sowing would be more economical in terms of lesser amounts of fungicide required than one at an earlier date. A field where soybeans have been grown in successive years may require higher dosages of fungicide than a field where soybeans are grown in rotation with a nonhost crop.

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