Comparative Efficacy of 2-Chloro-6-Methoxy-4(Trichloromethyl) Pyridine and Ethazole for Control of Phytophthora Root Rot of Rhododendron and Soybean

H. A. J. Hoitink and A. F. Schmitthenner

Associate Professor and Professor, respectively, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster 44691.

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ABSTRACT

Dowco 269 [2-chloro-6-methoxy-4-(trichloromethyl) pyridine] effectively controlled *Phytophthora cinnamomi* on rhododendron. Soil drenches (300 μg/ml) before inoculation prevented root rot, whereas drenches applied after inoculation did not. Dexon drenches were ineffective. Ethazole was less effective than Dowco 269, but a 375-μg/ml drench prevented root rot. In greenhouse tests, a 100-μg/ml drench or 1.12 kg/hectare(ha) furrow treatment of Dowco 269 effectively controlled soybean root rot caused by *Phytophthora megasperma* var. *sojae*. Furrow treatment with ethazole (1.12 kg/ha) was equally effective, but phytotoxic. Slight stunting was caused by 2.24 kg/ha Dowco 269. Foliar sprays with Dowco 269 delayed appearance of rhododendron and soybean root rot symptoms, indicating that systemic uptake of Dowco 269 by leaves and stems, and movement into roots may occur. Dowco 269 does not eradicate *P. cinnamomi* or *P. megasperma* var. *sojae*, race 1, from infected rhododendron or soybean plants.

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Phytophthora root rot (*P. cinnamomi* Rands) is a serious disease of rhododendron in both nursery and landscape plantings (4). An effective chemical control procedure is not available. Drenches with Dexon and ethazole have not been adequate (2, 7). A comparison of the efficacy of Dexon and ethazole revealed that ethazole was more active against *P. palmivora* Butl. than Dexon (8). Neither fungicide, however, eliminated *Phytophthora* spp. from soil (8, 10).

Soybean root rot (*P. megasperma* var. *sojae* Drechs, Hildb.) has become more serious in recent years due to appearance of new races (6) for which there is no resistance in agronomically acceptable cultivars. Chemical control, therefore, may become desirable.

In this study, we report the comparative efficacy of Dexon, ethazole, and Dowco 269 (a new fungicide) for control of Phytophthora root rots of rhododendron and soybean. A new formulation of Dowco 269 now is known as Nurelle.

MATERIALS AND METHODS.—*Fungicides.*—Dowco 269 [2-chloro-6-methoxy-4-(trichloromethyl)pyridine] was obtained from Dow Chemical, Midland, Michigan and supplied as S1805, 95% EC (D269); M3858, 7.2% EC soil drench (D269D); and M3860, 12.4% EC topical spray (D269S). To prepare powders of Dowco 269 for seed treatment and furrow application, S1805 was mixed with Barden clay (w/w) to yield 2.5, 5.0, 10, and 20% formulations (D269C). The mixtures were made into a slurry with acetone, thoroughly stirred, and allowed to air-dry. Resulting powders were ground with a mortar and pestle, stored in air-tight containers, and bioassayed for activity against *P. cinnamomi*. No measurable activity was lost in preparation of the powder formulations. Ethazole (5-ethoxy-3-trichloromethyl-1, 2, 4-thiadiazole) was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. as the 25% EC and 30% WP Truban (Ter E and Ter W) formulations. Terrazole, 5% granular (Ter G), another ethazole formulation, was received from Olin Corp., Agricultural Division, P. O. Box 991, Little Rock, Ark. Dexon 35% WP (*p*-dimethylaminobenzenediazo sodium sulfonate) was used in soil drench trials only. All fungicide rates are expressed as active ingredient.

Minimal inhibitory concentrations.—The minimal inhibitory concentration (MIC) of Dowco 269 was determined in modified V-8 juice agar (VA) (3). The MIC of Dowco 269 is regarded as the lowest concentration (μg/ml) which completely inhibits growth of a *Phytophthora* sp. in VA agar, but does not kill the fungus. After transfer of an inoculum piece from a plate with the MIC for a particular isolate outgrowth does occur, indicating that the treatment was not fungicidal. Aliquots of varying concentration in sterile distilled water were added to VA agar (50 C) to yield 500, 250, 100, 50, 25, 5, 0.5, and 0 μg/ml Dowco 269/ml VA medium. Plates (20 ml/plate) were inoculated on the same day with an agar inoculum block (ca. 9 mm^2) of the following *Phytophthora* spp. (four replications/treatment): *P. cactorum* (Leb. and Cohn) Schroet., *P. cinnamomi, P. citricola* Sawada, *P. citrophthora* Sm., *P. cryptogea* Pethyb. and Laff., *P. erythroseptica* Pethyb., *P. gonapodyidis* (Pet.) Buis., *P. ilicis* Budd., *P. lateralis* Tucker and Milbr., *P. megasperma* Drechs., *P. nicotiana* var. *parasitica* (Dast) Waterhouse, and *P. palmivora* Butl.

Plates with the same concentration were enclosed in a polyethylene bag and incubated for 4 days at 25 C. After 4 days, agar blocks were transferred from plates with a growth-inhibiting concentration to VA plates without Dowco 269. Six days later, colony radii were measured.

Rhododendron root rot.—All plants, unless specified otherwise, were produced in containers in peat and muck and inoculated with hemp seed broth (HS) cultures of *P. cinnamomi,* as described previously (3). Inoculum was poured near roots of actively-growing plants. Under these conditions all plants died 2-3 weeks after inoculation. *P. cinnamomi* was reisolated from a number of plants to verify cause of losses. In preliminary greenhouse tests, drenches with Dowco 269 (100 μg/ml D269) and lower concentrations were compared with 150 and 300 μg/ml

Dexon and 125 μg/ml ethazole (Ter E) drenches. These were applied two days before and on a varying number of days after inoculation.

In additional greenhouse tests, one-year-old plants of the susceptibile hybrid cultivar Roseum Elegans were drenched two days before inoculation with Dowco 269 (D269D) at 75, 150, and 300μg/ml and with ethazole (Ter E) at 125, 250, and 375 μg/ml. In addition, 300 μg/ml drenches with Dowco 269 (D269D) were applied at 1, 2, and 3 days after inoculation. Each plant was treated with a 300 ml drench per 4-liter container (one plant/container). Each treatment was replicated three times (six plants/treatment). Six weeks after inoculation, roots were rated with a root rot index of 1 = healthy, 2 and 3 = mild and severe root rot, 4 = crown rot, and 5 = dead plant (3).

Outdoor-grown, container-produced (8-liter containers), highly susceptible plants (cultivar Lee's Dark Purple) were inoculated during July, 1973, with HS cultures of *P. cinnamomi* (two flasks per plant). Plants were drenched (500 ml/plant) with 38, 75, 150, and 300 μg/ml Dowco 269 (D269 and D269D) and 125 and 250 μg/ml ethazole (Ter E) 3 days before inoculation. Twenty plants were used per treatment in four replications of five plants each. Numbers of dead plants per treatment were determined at 6 and 10 weeks after inoculation.

A split-root technique (1) was used to examine uptake by, and systemic movement of Dowco 269 in, rhododendron roots. Roots and the lower 2 cm of stems of individual plants were divided longitudinally into two equal portions which were planted in separate, but adjoining, 2-liter containers. Care was taken not to splash water from one to the other. One root portion (five plants/treatment) was drenched (200 ml/2-liter container) with Dowco 269 10, 6, and 2 days before and 4 and 11 days after inoculation. Soil moisture in this container was maintained well below the saturation point. The other root portion was inoculated with HS cultures, and flooded daily with 200 ml water. Dowco 269 concentrations were 0, 75, and 100 μg/ml (D269D). Uninoculated controls were included to evaluate phytotoxicity.

To evaluate efficacy of topical sprays, plants of hybrid cultivar Roseum Elegans were inoculated in the greenhouse as was described under soil drenches. At 7 or 2 days before inoculation, new growth on plants was sprayed until runoff with 0, 200, 600, 1,200, and 2,400 μg/ml Dowco 269 (D269S). Control plants were not inoculated. Numbers of dead plants were determined weekly. Each treatment consisted of ten plants (two blocks of five). To avoid direct contact of fungicide with soil, the container mix surface was covered with absorbent paper before application of sprays. Plants were grown in a greenhouse with a low relative humidity (50-70%) to speed up drying of sprayed plant surfaces. Irrigation water was poured directly onto the soil and not poured on leaves or stems to avoid washing of fungicide into soil.

Soybean root rot.—Steamed greenhouse soil mix of Wooster silt loam, muck and Canadian peat (5:5:2,v/v) was used in all tests. Ten Harosoy seed were planted per 10- or 15-cm diameter polystyrene or styrofoam pots or 20 Corsoy seed per 32 × 12 × 11 cm polystyrene containers. Soil was infested after planting in some experiments by pouring HS cultures of *P. megasperma* var. *sojae* (Pms-1) into the root zone as specified for the rhododendron inoculations. In others, seed were planted in soil infested as above either immediately after infestation, or 2 months following a crop of Harosoy, Amsoy, and Wayne seedlings that had been planted to build up inoculum. The soil was stored wet between inoculum buildup and use. Fungicide drenches, seed treatments, foliar sprays, and furrow treatments were replicated five times unless otherwise specified. The number of dead and diseased plants was recorded 3-4 weeks following treatment. Root rot of surviving plants was rated by a system in which 1 = healthy roots, 2 = up to 20% root reduction, 3= 20-50% root reduction, 4 = 50-90% root reduction, and 5 = over 90% root reduction.

In preliminary drench experiments, 0, 1, 2, 5, 10, 20, 50, and 100 μg/ml Dowco 269 (D269) treatments were applied (200 ml/container) either 2 days before, 2 days after, or 7 days after inoculation. Plants were inoculated 2 weeks after planting. Dexon (150 μg/ml) and 125 μg/ml ethazole (Ter W) were used for comparison. Controls were water, 100 μg/ml Dowco 269, 150 μg/ml Dexon, and 125 μg/ml ethazole, in noninfested soil. Ten-day-old plants with fully expanded unifoliolates were sprayed to runoff with 0, 500, 1,000, or 2,000 μg/ml Dowco (D269D) under conditions described for the rhododendron spray treatments.

To examine effects of seed treatment, seed were treated with Dowco 269 powders (2.5, 5.0, 10.0, and 20.0% D269C) at a rate of 4 gm/kg seed. Seed were planted in steamed, infested soil in one set of treatments, and in steamed soil in two other sets which were infested one or two weeks following planting. Uninoculated controls were included.

For initial furrow treatments, Harosoy seeds in a 2-cm-deep furrow in infested soil were sprinkled with Barden clay or .019, .037, and .074 g Dowco 269 per 30/cm row (.37 g/30 cm row of 5, 10, and 20% D269C), equivalent to 0, 0.67, 1.34, and 2.68 kg/hectare(ha) Dowco 269. Calibrations were based on applications of 13.4 kg/ha of a 10% granular formulation in 90-cm rows. In a second experiment, Corsoy seed in furrows were sprinkled with a 10% D269C or 5% Ter G at rates equivalent to 1.12 and 2.24 kg/ha active (1 and 2 lbs/acre). Barden clay at 2.24 kg/ha was used as a control. After sprinkling with the fungicide powders or granulars, seed were covered with a 1:1 (v/v) mixture of vermiculite and infested soil, and watered.

RESULTS.—*Minimal inhibitory concentration.*—Concentration of 50 μg/ml Dowco 269 and higher completely inhibited growth of the *Phytophthora* spp. in VA medium. The concentrations of 5 and 25 μg/ml resulted in more than 50% inhibition of growth; 0.5 μg/ml had little or no effect on growth of any of the species. Transfer of agar inoculum blocks after 4 days, from VA plates with 500 and 250 μg/ml on which no growth occurred, to VA plates without Dowco 269 resulted in growth equal to that of the controls.

During initial trials, it was noted that the diameter of control colonies on VA plates was smaller if enclosed in a polyethylene bag with plates containing 500 μg/ml Dowco 269. In subsequent similar trials, it was verified that the fungicide apparently diffused out of plates with a

high concentration (500 μg/ml) into controls and caused inhibition.

Rhododendron root rot.—In preliminary soil drench tests, 100 μg/ml Dowco 269 and 125 μg/ml ethazole reduced root rot if applied before inoculation. A lower concentration delayed symptoms, but plants eventually died. Dexon drenches (up to 300 μg/ml) were not effective. No greenhouse plants were killed when Roseum Elegans plants were drenched with 300 μg/ml Dowco 269 (D269D) or 375 μg/ml ethazole (Ter E) 2 days before inoculation (Table 1). The mean root rot rating of this Dowco 269 treatment was not significantly different ($P = 0.01$) from uninoculated plants. Ethazole (375 μg/ml) was slightly less effective. Lower concentration of both fungicides were ineffective. A 300 μg/ml Dowco 269 (D269D) drench applied 1, 2, or 3 days after inoculation did not control root rot.

In an outdoor test with Lee's Dark Purple plants in containers, no plants were killed in treatments with 150 and 300 μg/ml Dowco 269 (D269) applied prior to inoculation (Table 2). Losses on plants treated with Dowco 269 (D269D) at 75 μg/ml and higher concentration of ethazole (Ter E) at 125 and 250 μg/ml were significantly lower than on untreated plants. Except for the 38 μg/ml Dowco 269 drench (D269D), losses in all treatments occurred later than 6 weeks after inoculation. Root growth on uninoculated plants treated with ethazole and Dowco 269 in both greenhouse and outdoor plants was similar to untreated plants, indicating that treatments were not phytotoxic.

In split-root tests several 75- or 150-μg/ml drenches with Dowco 269 (10, 6, and 2 days before, and 4 and 11 days after, inoculation) applied to uninoculated root portions did not reduce or prevent root rot in the

TABLE 1. Effect of ethazole (Ter E) and Dowco 269 (D269D) on rhododendron root rot in greenhouse plants

Inoculation	Fungicide formulation[c]	Rate (μg/ml)	Time of application[a]	Dead plants (no.)	Mean root rot rating[b]
No	...	...	...	0	1.2
Yes	Control	None	...	7	3.7
Yes	D269D	75	2^b	8	3.7
Yes	D269D	150	2^b	4	2.0
Yes	D269D	300	2^b	0	1.4
Yes	D269D	300	1^a	4	3.5
Yes	D269D	300	2^a	10	4.3
Yes	D269D	300	3^a	11	4.4
Yes	Ter E	125	2^b	4	3.6
Yes	Ter E	250	2^b	2	3.2
Yes	Ter E	375	2^b	0	1.8

[a] D269D (7.2% EC soil drench); and Ter E (25% EC ethazole). Drenched (300 ml per 4-liter container) 2 days before (2^b) or 1, 2, 3 days after (1^a, 2^a, 3^a) inoculation.

[b] LSD($P = 0.01$) = 0.2 based on 18 plants per treatment, 6 plants per replicate. Rating scale: 1 = healthy plant; 2 and 3, moderate and severe root rot; 4, crown rot and 5, dead plant; determined at 6 weeks after inoculation.

TABLE 2. Effect of Dowco 269 (D269 and D269D) and ethazole (Ter E) on rhododendron root rot in outdoor containers

Inoculation	Fungicide formulation[a]	Rate (μg/ml)[a]	Mean dead plants[b] (no. per five-plant replicate)
Yes	D269	38	2.8
Yes	D269	75	2.0
Yes	D269	150	0.0
Yes	D269	300	0.0
Yes	D269D	38	9.2[c]
Yes	D269D	75	3.2
Yes	D269D	150	1.2
Yes	D269D	300	2.0
Yes	Ter E	125	2.4
Yes	Ter E	250	2.0
Yes	Control	None	13.2
No	Control	None	0.4
No	Ter E	250	0.0
No	D269D	300	0.0

[a] D269 (95% EC of Dowco 269); D269D (7.2% EC soil drench); and Ter E (25% EC ethazole). All drenches (500 ml/plant in 8-liter containers) were applied 3 days before inoculation with *Phytophthora cinnamomi*.

[b] Means of four reps of five plants each, determined after 10 weeks.

[c] Not significantly different ($P = 0.05$) from inoculated control.

TABLE 3. Number of Corsoy soybean plants killed and root rot rating of surviving seedlings in *Phytophthora megasperma* var. *sojae* race 1-infested soil treated with ethazole (Ter W and G) and Dowco 269 (D269 and D269D) furrow and drench treatments

Inoculation	Fungicide formulation[a]	Rate	Mean dead plants/row[b] (no.)	Mean root rot rating of survivors[c]
Yes	Water	none	9.5	3.3
Yes	Ter W	100 μg/ml	8.0	2.4
Yes	Ter W	200 μg/ml	2.3	2.0
Yes	D269D	100 μg/ml	0.5	1.1
Yes	D269	100 μg/ml	0.0	1.1
Yes	Clay	none	6.5	4.0
Yes	Ter G	1.12 kg/ha	0.0	1.9
Yes	Ter G	2.24 kg/ha	0.3	1.8
Yes	D269C 10%	1.12 kg/ha	0.0	1.3
Yes	D269C 10%	2.24 kg/ha	0.3	1.3
No	...	...	0.5	1.3

[a] D269 (95% EC of Dowco 269); D269D (7.2% EC soil drench); D269C (Dowco 269, dry powder/Barden clay); Ter G (5% granular ethazole); and Ter W (30% WP ethazole). Ter W, D269, and D269D drenches (200 ml/pot) applied at seeding; Ter G and D269C were spread over seed in a furrow before covering.

[b] 20 seed planted per one 30-cm row. LSD ($P = 0.05$) = 1.8.

[c] Rating system: 1 = healthy roots; 2 = up to 20% root reduction; 3 = 20-50% root reduction; 4 = 50-90% root reduction, and 5 = over 90% root reduction. LSD($P = 0.05$) =0.6.

untreated corresponding inoculated root portions. Root growth on drenched as well as nondrenched root portions was equal, indicating that the treatments were not phytotoxic.

Topical sprays with Dowco 269 (D269S) applied 7 or 2 days before inoculation (300, 600, 1,200, and 2,400 μg/ml) did not reduce the number of dead plants per treatment compared to controls. All inoculated and sprayed plants died. However, inoculated nonsprayed plants died between 14 and 20 days after inoculation, but inoculated plants sprayed with 600, 1,200, and 2,400 μg/ml died between 20 and 60 days after inoculation.

Soybean root rot.—Significantly fewer plants were killed or had symptoms of Phytophthora root rot in soil drenched with 20, 50, or 100 μg/ml Dowco 269 than in soil drenched with Dexon (150 μg/ml), ethazole (125 μg/ml), water, or a lower concentration of Dowco 269. Drenching 2 days before inoculation or 2 days after was more effective than 7 days after inoculation. Dowco 269 at 100 μg/ml was significantly better than ethazole (125 μg/ml). No plants were killed in a Dexon (150 μg/ml), ethazole (125 μg/ml), and Dowco 269 (100 μg/ml) uninoculated treatments. No phytotoxicity was observed with any of the fungicides.

Seed treatment with 2.5, 5.0, 10, and 20% Dowco 269 (D269C) increased emergence slightly in Pms-1 infested soil, but did not control postemergence root rot.

Dowco 269 applied as furrow treatments or drenches significantly improved emergence of Harosoy soybeans in Pms-1 infested soil (82-98%), as compared to the Barden clay-furrow or water drenched controls (46-50%). Significantly more ($P = 0.05$) seedlings survived in the Dowco 269 furrow treatments (88-98%) than in the drench treatments (68-72%). Few (0-4%) survived in the Barden clay-furrow or water drench treatments. Dowco 269 drench treatments applied 1 week after planting or foliar sprays applied to the unifoliolates resulted in significantly fewer surviving plants (14-24%) than drenching at planting time (68-72%). Dowco 269 drenches applied 2 weeks after planting did not control root rot. Rates of 0.67, 1.34, and 2.68 kg/ha in the furrow were equally effective.

Furrow and drench treatments of Dowco 269 and ethazole were compared using Corsoy soybean in Pms-1-infested soil (Table 3). Drenches with both the D269 and D269D formulations of Dowco 269 were more effective than ethazole drenches. Both Dowco 269 and ethazole controlled root rot at rates of 1.12 or 2.24 kg/ha in furrow treatments. However, ethazole at 2.24 kg/ha furrow stunted plants significantly, caused considerable bleaching on cotyledonary margins, and resulted in distorted white-streaked leaves. Ethazole at 1.12 kg/ha caused some streaking and distortion, but little stunting. Dowco 269 at 2.24 kg/ha caused slight stunting, but no other foliar symptoms.

DISCUSSION.—Dexon is not effective for control of rhododendron root rot if applied as a drench, which confirms earlier reports (2, 7). Better control might be obtained if Dexon were mixed with soil. However, mixing techniques used in the woody ornamentals industry generally are not sufficiently uniform to distribute a fungicide evenly.

Ethazole effectively controlled rhododendron root rot in greenhouse tests, if drenched (375 μg/ml) before inoculation. Lower rates were not effective. In a field test, some control was obtained with a 250 μg/ml drench. These rates are several-fold higher than the presently recommended rate of application for ethazole (Truban 30% WP, or Truban 25% EC). The comparative effectiveness of Dexon and ethazole for control of *P. cinnamomi* on rhododendron does not agree with field soil data on avocado, where Dexon was better than ethazole (9, 10). It is possible that Dexon leached out of containers rapidly and therefore that activity was lost.

Dowco 269 effectively controlled rhododendron root rot on greenhouse plants if applied as a 300 μg/ml drench

before inoculation. In a field trial a single 150 μg/ml drench also was effective. However, the D269D formulation was less effective. Apparently this formulation leaches out since plant losses began after 6 weeks. Dowco 269, apparently, is not redistributed from treated rhododendron roots through the crown into untreated roots since untreated inoculated root portions in split-root studies were not protected by several drenches of Dowco 269 applied to the corresponding uninoculated root portion. Dowco 269, however, does appear to be taken up by rhododendron foliage and translocated through the stem into roots, since one spray applied before inoculation delayed onset of root rot symptoms by several weeks. Possibly several topical sprays, applied over a period before and after inoculation, could control rhododendron root rot effectively.

Dexon (150-μg/ml drench) had no effect on Phytophthora root rot of soybean. Higher drench levels were not attempted. Furrow treatments with granular Dexon were not attempted because in previous work Dexon inhibited soybean seed germination (Schmitthenner, *unpublished*). Similar toxicity observations have been made on peas (5).

Ethazole drenches provided some control of soybean Phytophthora root rot, but considerable disease occurred even at the 200 μg/ml rate. Both the D269 and D269D drench formulations (100 μg/ml) of Dowco 269 control soybean Phytophthora root rot. However, seed treatment with Dowco 269 was not effective. Foliar sprays resulted in some control, but were not as effective as drenches, possibly because many seedlings were infected before adequate foliage for absorption was present (10-day-old plants).

Furrow treatments with both ethazole and Dowco 269 controlled soybean Phytophthora root rot. Ethazole was phytotoxic and Dowco 269 caused some stunting at the 2.24 kg/ha rate. These effective rates are similar to furrow treatments of insecticides for corn. Dowco 269 and possibly ethazole furrow treatments, therefore, could be applied with existing equipment. Field experiments are needed to determine the effectiveness of these materials where soybean roots grow extensively beyond the row.

In various experiments on soybean and rhododendron, it was observed that Dowco 269 does not eradicate the *Phytophthora* spp. from infected plants. Furthermore, Dowco 269 does not kill *Phytophthora* spp. in vitro. The fungistatic effect of Dowco 269 on *Phytophthora* spp. in soil may be similar to ethazole and Dexon, which also do not readily eradicate *Phytophthora* or *Pythium* spp. (8). More information is needed to substantiate this.

Dowco 269 is the most active fungicide developed for control of *Phytophthora* spp. Further, it has the unique properties of providing control as a soil drench, soil furrow treatment, and possibly as a foliar spray. It is active in vitro against 10 *Phytophthora* spp. in addition to *P. cinnamomi* or *P. megasperma* var. *sojae* used in these tests, and should be effective against many *Phytophthora*-caused diseases. However, leaf spot, dieback, and stem rot of azalea (*P. nicotiana* var. *parasitica*) was not controlled by foliar sprays of Dowco 269 (Hoitink and Schmitthenner, *unpublished*). Possibly, Dowco 269 is not active for foliage diseases or is translocated to roots out of upper plant parts too rapidly to be effective. The efficacy of Dowco 269 possibly may be restricted to root- and crown-invading *Phytophthora* spp. The volatile properties of Dowco 269 also may reduce its effectiveness as a foliar spray.

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