Journal of Nematology 20(1):40–46. 1988.

Behavior, Parasitism, Morphology, and Biochemistry of *Criconemella xenoplax* and *C. ornata* on Peach

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Abstract: Host–parasite relationships of *Criconemella xenoplax* and *C. ornata* on Nemaguard peach and common bermudagrass were determined in the greenhouse. *Criconemella xenoplax* reproduced on peach and reduced root volume, height, and dry stem weight after 6 months, compared with the noninfested check. Numbers of *C. ornata* did not increase on peach or influence peach growth, but they did reduce dry top weight and root volume of common bermudagrass, compared with *C. xenoplax. Criconemella xenoplax* and *C. ornata* produced the enzyme β-glucosidase and were capable of metabolizing prunasin, but only *C. xenoplax* produced β-cyanoalanine synthase to detoxify the cyanide released from prunasin. The apparent inability of *C. ornata* to detoxify cyanide is one explanation why numbers of this species did not increase on peach. *Criconemella xenoplax* and *C. ornata* can be distinguished by using stylet length, vaginal configuration, and shape of the anterior head region.

Key words: Criconemella ornata, C. xenoplax, parasitism, peach, peach tree short life, *Prunus persica,* ring nematode.

The ring nematode, *Criconemella xenoplax* (Raski) Luc & Raski, is an important soil-inhabiting parasite involved in peach (*Prunus persica* (L.) Batsch) susceptibility to peach tree short life (PTSL) (16,22). In a survey of PTSL orchards (primarily of trees on Nemaguard and Lovell rootstocks) throughout the major production areas of Georgia and South Carolina, *C. xenoplax, C. ornata* (Raski) Luc & Raski, and *C. sphaerocephala* (Taylor) Luc & Raski were detected (17). *Criconemella xenoplax* and *C. ornata* occurred in 100% of the orchards, and *C. sphaerocephala* was detected in 11% (GA) and 40% (SC) of the samples. *Criconemella xenoplax,* however, was the most abundant species in all orchards sampled. In another study (R. E. Motsinger, unpubl.), *C. ornata* was more common than *C. xenoplax* in some PTSL orchards.

Criconemella xenoplax has been associated with peach diseases (11,16,22), and *C. ornata* has been associated with reduced yield of peanut, *Arachis hypogaea* (L.) (12,15). Centipede grass, *Eremochloa ophiuroides* (Munro) Hack (21) and bermudagrasses, *Cynodon dactylon* (L.) Pers. (8) are also good hosts for *C. ornata,* whereas Lovell peach (21) is not.

Hydrogen cyanide may act as a feeding deterrent in peach leaves to the oblique-banded leafroller, *Choristoneura rosaceana* (Harris) insect (9). In injured tissue, prunasin, the primary cyanogenic glucoside in peach roots, is metabolized by β-glucosidase to benzaldehyde and cyanide (14,18). These metabolites are toxic to animals and plants (9,13). The enzyme, β-glucosidase, has been detected in such plant-parasitic nematodes as *Globodera rostochiensis* (Woll.) and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurman-Stekhoven (5).

The present study was designed to 1) determine the host–parasite relationship between *C. ornata* and Nemaguard peach, 2) determine if common bermudagrass is a host to *C. xenoplax,* 3) compare the degradation of prunasin and detoxification of cyanide by *C. xenoplax* and *C. ornata,* and 4) compare morphological characteristics of *C. xenoplax* and *C. ornata.*

Materials and Methods

Host–parasite relationships: Approximately 25 seeds of common bermudagrass or stratified (3 months) Nemaguard peach seeds were planted into 15-cm-d plastic pots containing approximately 1,500 cm^3 steam

Received for publication 15 May 1987.

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The authors thank D. Watts for technical assistance.

pasteurized loamy sand (86% sand, 10% silt, 4% clay). Seventeen days later, pots with seedlings of uniform size were selected for inoculation with *C. xenoplax* or *C. ornata*. The isolate of *C. xenoplax* was obtained from a PTSL orchard near Byron, Georgia, and was increased on Nemaguard peach grown in a sand : vermiculite (1:1 v/v) medium in the greenhouse. The isolate of *C. ornata* was also obtained from a peach orchard and increased on common bermudagrass grown in loamy sand. Nematodes were extracted from culture pots using the centrifugal-flotation method (7). Treatments were 500 *C. xenoplax* or *C. ornata* per pot of Nemaguard peach, 500 *C. xenoplax* or *C. ornata* per pot of common bermudagrass, and noninoculated Nemaguard peach check. Each inoculated pot received 50 ml aqueous nematode suspension which was poured onto the soil surface that had been previously disrupted to a depth of 1.0 cm, and additional water (ca. 100 ml) was applied to wash the nematodes into the soil. A 50-ml aliquot of nematode-free solution obtained from the extraction procedure was added in a similar manner to soil of noninoculated controls. Treatments, replicated six times, were arranged in a randomized complete block design on a bench in an air-conditioned greenhouse where the ambient temperature was maintained at 25 ± 5 C.

Peach seedlings were pruned to a height of 18 cm and the grass was trimmed to the top of the pot after 3 months. After 4 months, two soil cores (2.5 cm d × 10 cm deep) were collected from each pot, the nematodes were extracted (50 cm^3 soil) via elutriation (4) and centrifugation (7), and the population density per 100 cm^3 soil extrapolated. The study was terminated after 6 months at which time root volume, dry root and stem weight (minus leaves), height increase (as determined on new terminal shoot growth only), and the final nematode population densities per 100 cm^3 soil were determined. Root volume was determined by submerging the root system in a graduated cylinder and recording the volume of water that was displaced. Data were subjected to general linear model analysis and linear contrasts.

Morphology: Observations and measurements of the nematodes were made with the aid of a compound microscope, and the nematodes were identified to species with appropriate keys and descriptions (6,19,20). Morphological characteristics utilized to identify *C. xenoplax* and *C. ornata* included body length, tail tip shape, anterior head region shape, stylet length, annulation, and vagina shape from 20 viable heat-relaxed mature females. Comparisons were made among these characteristics to determine their utility in differentiating the two species.

Biochemistry: Nematodes from the previously mentioned populations were reared in loamy sand pot cultures and extracted as previously described (7). Nematodes were separated from roots and silt by sucrose-gradient centrifugation. Sucrose solutions of 1.0 M (20 ml), 0.5 M (6 ml), and 0.25 M (6 ml) concentration were layered in a 50-ml centrifuge tube, and 10 ml water containing *C. xenoplax* or *C. ornata* was layered on top. Tubes were centrifuged at ca. 920 *g* for 1 minute. *C. xenoplax* and *C. ornata* were pipetted from the 0.25 M and 0.5 M sucrose, rinsed free of sugar by back washing, and collected on a 25-μm-pore (500-mesh) sieve. Nematodes were counted, and ca. 68,000 viable individuals of each species were transferred to a 15-ml Corex centrifuge tube and concentrated by centrifuging at ca. 12,100 *g* for 10 minutes. The nematode pellet was resuspended in 2 ml 0.1 M sodium citrate, sodium phosphate buffer (pH 5.5), and the suspension was transferred to a Ten Broeck homogenizer. Nematodes were homogenized for 4 minutes in tubes submerged in ice. An additional 2 ml ice-cold buffer was added to each tube for a total volume of 4 ml.

The concentration of protein in each nematode homogenate was determined by the Bradford method using BSA as the protein standard (3). Two slightly modified assays were used to detect β-glucosidase activity, the enzyme responsible for metabolizing glucoside compounds, such as pru-

TABLE 1. Growth response of Nemaguard peach and common bermudagrass to inoculation with *Criconemella xenoplax* (Cx) and *C. ornata* (Co) after 6 months in the greenhouse.

Treatment	Root volume (ml)	Dry root weight (g)	Dry stem weight (g)	Height increase (cm)
1. Control-peach	1.85	4.39	5.28	69.8
2. Cx-peach	1.43	3.46	4.09	57.3
3. Co-peach	2.13	5.13	5.54	70.7
4. Cx-grass	2.68	3.32	8.61	
5. Co-grass	1.55	2.47	6.89	
Contrasts				
1 vs. 2	+	NS	**	**
1 vs. 3	NS	NS	NS	NS
4 vs. 5	**	NS	**	

Data subjected to general linear model analysis and linear contrasts. + = $P = 0.10$; ** = $P = 0.01$; NS = no significant differences.

TABLE 2. Population densities of *Criconemella xenoplax* (Cx) and *C. ornata* (Co) on Nemaguard peach and common bermudagrass after 4 and 6 months in the greenhouse.

Treatment	Number Cx or Co/100 cm³ soil†	
	4 months	6 months
Control-peach	0	0
Cx-peach	7,771	29,532
Co-peach	7	7
Cx-grass	70	564
Co-grass	10,588	72,538

† Initial population density = 500 nematodes/15-cm-d pot (1,500 cm³ soil).

nasin. The first assay detected the release of a p-nitrophenyl (p-NP) (9,23) from p-nitrophenyl-β-D-glucopyranoside, and the other detected hydrogen cyanide (HCN) (10) from prunasin. The modified reaction mixture for both assays contained 1 ml 0.1 M sodium citrate, sodium phosphate buffer, pH 5.5, 1 ml 5 mM p-nitrophenyl-β-D-glucopyranoside or prunasin (D-mandelonitrile β-D-glucoside; Sigma), and 0.5 ml crude nematode homogenate; it was reacted at 25 C. The p-NP reaction was terminated after 4 hours by adding 1 ml 1 M sodium carbonate and measured at an absorbance of 400 nm using an MR580 microelisa spectrophotometer. Concentration was calculated from a standard curve generated by the reaction to completion of measured concentrations of substrate with β-glucosidase (Sigma, type II; 4–8 units/mg protein). Control tubes included 1) lacking either enzyme (β-glucosidase or nematode homogenate) or substrate; 2) boiled enzyme or nematode homogenate; and 3) nematode extraction solution (minus nematodes prior to sucrose-gradient centrifugation).

Briefly, the presence of HCN was determined by adding 1 ml succinimide-N-chlorosuccinimide reagent, 1 ml barbituric acid-pyridine reagent, and 20 ml distilled water to 1 ml reaction solution and mixing. The absorbance was measured after 10–20 minutes at 570 nm. Concentration was calculated from a standard curve by the reaction utilizing measured concentrations of KCN. Control tube treatments were similar to those previously described above.

The β-cyanoalanine synthase (cyanide detoxification) assay was adapted from the method of Blumenthal et al. (2). The reaction mixture consisted of 0.5 ml 0.1 M Tris HCl buffer, pH 8.5; 0.5 ml 10 mM L-cysteine HCl; 0.5 ml 50 mM KCN, and 0.5 ml crude nematode extract in capped tubes for 0, 5, 15, and 30 minutes at 30 C. The reaction was terminated by the addition of 0.5 ml 0.03 M $FeCl_3$ in 1.2 N HCl and 0.02 M N,N-dimethyl-p-phenylenediamine sulfate in 7.2 N HCl. After 20 minutes, the absorbance at 630 nm was determined by the reduction of methylene blue with the liberated hydrogen sulfide. Concentration was calculated from a standard curve by the reaction utilizing measured concentrations of sodium sulfide. Control tubes included 1) lacking nematode homogenate or substrate, 2) boiled nematode homogenate, and 3) nematode extraction solution (as described).

RESULTS

Host–parasite relationships: Criconemella xenoplax caused significantly lower root volume ($P = 0.10$), dry stem weight ($P = 0.01$), and height increase ($P = 0.01$) of Nemaguard peach after 6 months when compared with the check (Table 1). *Criconemella ornata* did not affect growth of

TABLE 3. Morphological characteristics differentiating *Criconemella xenoplax* and *C. ornata*, based on 20 heat-relaxed mature females in water.

	C. xenoplax	*C. ornata*
Length (L) (μm)	712.3 ± 16.3 (404–620)	538.9 ± 33.3 (363–444)
Stylet length (μm)	78.9 ± 2.6 (71–86)	50.0 ± 2.3 (48–56)
Vagina shape	Sigmoidal	Straight
Tail shape	Bluntly rounded (lobed button)	Rounded (truncate)
Body annules	Smooth with irregular edges	Smooth with irregular edges
Anterior head, lip region shape	Offsetting (first two annules offset)	Continuous

Ranges in parentheses taken from published keys, fixed specimens (5,16,17).

peach, but it reduced root volume ($P = 0.01$) and dry stem weight ($P = 0.01$) of common bermudagrass when compared with *C. xenoplax*.

The number of *C. xenoplax* was greater than the number of *C. ornata* on peach after 4 and 6 months (Table 2). The population density of *C. xenoplax* on peach increased 3.8 times between sampling periods, whereas *C. ornata* remained below the initial inoculum level. The opposite was true on common bermudagrass; *C. ornata* reproduced at a faster rate than *C. xenoplax*. Numbers of *C. xenoplax*, however, were greater on common bermudagrass after 4 and 6 months than numbers of *C. ornata* on peach.

Morphology: Morphological characteristics were used to identify *C. xenoplax* and *C. ornata* (Table 3). The outstanding characteristics differentiating *C. xenoplax* from *C. ornata* include adult female body length, stylet length, vagina shape, and shape of the head region. Body and stylet lengths of *C. xenoplax* were greater than *C. ornata* (Fig. 1A, B). Mean body lengths for both nematode species were greater than those listed in the keys (6,19,20), but stylet lengths were not. The head region of *C. xenoplax* appeared offset compared with a more continuous nonoffset configuration for *C. ornata* (Fig. 1C, D). Body annules for both nematodes are described in the key as being smooth with irregular edges, but those of *C. xenoplax* appear to be coarser than *C. ornata*.

Tail shape was the most variable character studied, primarily because it depended on position of the nematode specimen when viewed. *C. xenoplax* has a bluntly rounded tail (lobed button), and *C. ornata* has a rounded (truncate) tail (Table 3). Vagina shape was sigmoidal for *C. xenoplax* and straight for *C. ornata* (Fig. 1E, F).

Biochemistry: Both nematode extracts reacted with p-nitrophenyl-β-D-glucopyranoside to release p-nitrophenyl (Table 4). This reaction is similar to the reaction that occurs with prunasin and indicates that both nematodes contain the enzyme, β-glucosidase. This reaction was further substantiated by the cyanide assay (Table 4) which detected cyanide release from prunasin, indicating that *C. xenoplax* and *C. ornata* were able to metabolize prunasin, thus releasing cyanide. The inactivation of enzyme by boiling occurred in both nematode homogenates and the pure β-glucosidase enzyme as indicated by the total reduction of p-NP or CN formation. These results indicate that the reactions were not artifacts. The amount of product produced by an excess of pure enzyme indicated substrate was not rate limiting. β-cyanoalanine synthase activity which combines cyanide and L-cysteine in the detoxification of cyanide only occurred with the *C. xenoplax* homogenate (Table 5). The reaction time was linear for *C. xenoplax* for 30 minutes, indicating an enzyme mediated event, and did not occur if the extracts were boiled or cysteine omitted.

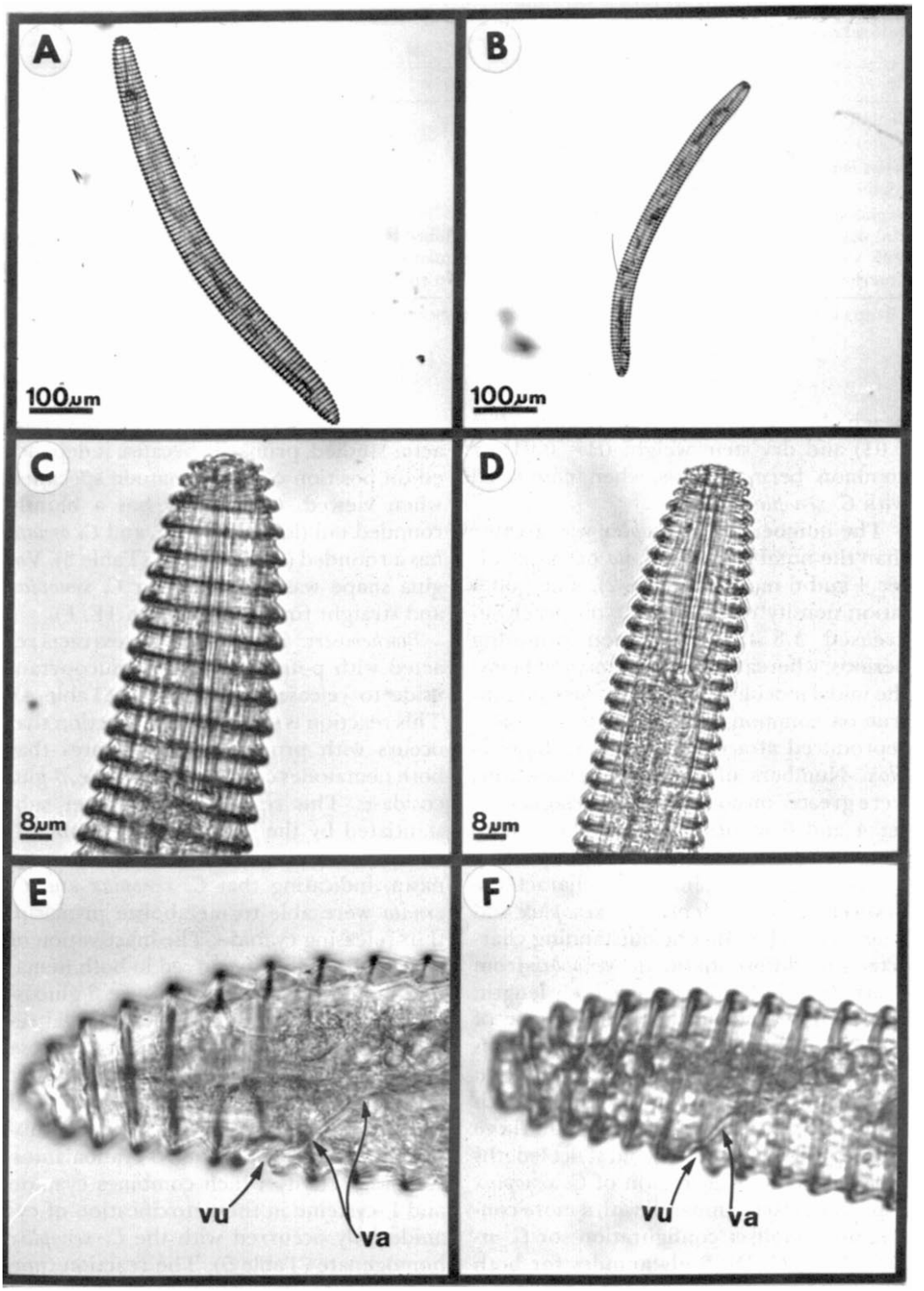

FIG. 1. Photomicrographs of *Criconemella xenoplax* (Cx) and *C. ornata* (Co). A) Mature female, Cx. B) Mature female, Co. C) Anterior region, Cx. D) Anterior region, Co. E) Posterior region, Cx. F) Posterior region, Co. Vu = vulva; Va = vagina.

TABLE 4. β-glucosidase activity of extracts from *Criconemella xenoplax* (Cx) and *C. ornata* (Co).

Treatment	Activity	
	p-NP† (μg)	CN‡ (ppm)
Cx	28.24	0.50
Cx-boiled	0.00	0.00
Co	27.35	0.43
Co-boiled	0.00	0.00
β-glucosidase§	>120.00	>10.00
β-glucosidase-boiled	0.00	0.00

No positive reaction occurred in control tubes containing the nematode extraction solution or lacking substrate or enzyme.

† p-nitrophenyl-β-D-glucopyranoside (PNPG) substrate reacts with Cx or Co homogenate or β-glucosidase in buffer to form p-nitrophenyl (p-NP μg/assay).

‡ CN = prunasin substrate reacts with Cx or Co homogenate or β-glucosidase to release cyanide (CN).

§ β-glucosidase (Sigma, type II: 4–8 units/mg protein).

TABLE 5. Detoxification of cyanide over time by β-cyanoalanine synthase from the *Criconemella xenoplax* (Cx) and *C. ornata* (Co) enzyme extracts.

Reaction time (min)	Reaction mixture†		μg-S‡	
	Nematode extract§	Cysteine	Cx	Co
0	+	+	0.00	0.00
5	+	+	0.47	0.00
15	+	+	1.42	0.00
30	+	+	2.61	0.24
30	+	−	0.00	0.00
30	−	+	0.00	0.00
30 (boiled extract)	+	+	0.00	0.00

† Reaction mixture contained buffer and KCN.

‡ S = μg sulfur liberated from cysteine reaction.

§ No positive reaction occurred in control tubes containing nematode extraction solution alone.

DISCUSSION

Nemaguard and Lovell are the primary peach rootstocks widely used in the southeastern United States, but trees on Nemaguard are more susceptible than Lovell to peach tree short life. Based on the results of the present study, Nemaguard was a poor host for *C. ornata* compared with *C. xenoplax*. These results help explain the low population levels of *C. ornata* reported in a previous survey of Georgia and South Carolina (17) and suggest that *C. ornata* is probably not a factor in PTSL. One explanation for peach being a poor host to *C. ornata* is the nematode's inability to detoxify cyanide that is released upon metabolism of prunasin. Some researchers speculate that peach is not a good host to some pests because of the release of toxic metabolites when the plant is injured (9). In contrast, *Criconemella xenoplax* reproduced well on Nemaguard peach, reduced peach growth (1,11), and detoxified cyanide. *Criconemella xenoplax* is also capable of reproducing slowly on common bermudagrass, a very common and difficult-to-control weed in peach orchards. It is not certain at this time if the life cycle of *C. xenoplax* is longer on this host, compared with peach, or if fewer females actually mature. This emphasizes, however, the importance for proper weed control before orchard establishment to eliminate alternate hosts that would support populations of *C. xenoplax*.

Common bermudagrass is a good host for *C. ornata*. Poor control of common bermudagrass in orchards may explain why Motsinger (unpubl.) found high populations of *C. ornata* around peach trees in certain PTSL sites in Georgia. Another reason to control common bermudagrass is that nematicide recommendations may be based on total numbers of ring nematode per volume soil. If common bermudagrass is not controlled, high *C. ornata* populations could result in a recommendation to treat the orchard, even if *C. xenoplax* levels are low. This results in an added cost for unnecessary nematicide application. Under such orchard conditions, ring nematode speciation would be helpful.

The most useful criteria for differentiation of *C. xenoplax* and *C. ornata* include stylet length, vaginal configuration, and the shape of the anterior region. Total body length and tail shape were less useful. Bodies of *C. xenoplax* were longer than those of *C. ornata*, as expected, but the average lengths for each nematode species were not in the range as listed in respective keys. This was probably the result of the fixing method used, since in this study heat-relaxed specimens were mounted in water

which results in less shrinkage than other methods. Tail shape was also difficult to assess because the nematode had to be positioned at the appropriate angle in order to observe its shape. Differences in body annule configuration become more noticeable as one works with these two nematode species on a regular basis.

Criconemella xenoplax and *C. ornata* are two ring nematode species that can commonly occur in PTSL sites in Georgia and South Carolina (17). Knowledge of the species present in peach orchards will enable the grower and (or) extension specialists to select the appropriate control practice.

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