Effects of Nonionic Surfactants on Ethylene and Chlorophyll Content of *Poa pratensis* Leaves Infected with *Bipolaris sorokiniana*

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ABSTRACT

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Research was initiated to determine the effects of the nonionic surfactants Aqua-Gro, Hydro-Wet, and Surf-Side 37 on the endogenous ethylene and chlorophyll content of healthy and *Bipolaris sorokiniana*-infected leaves of *Poa pratensis*. Short and long exposure of uninoculated plants to the surfactants failed to cause any changes in endogenous ethylene. After inoculation, plants from all treatments showed an increase in endogenous ethylene that peaked at 48 or 72 hr and then declined by 96 hr; few differences occurred in endogenous ethylene of leaves between inoculated plants exposed to surfactants and inoculated water-treated plants. Surfactants induced increases and decreases in leaf chlorophyll content, most of which were not significant. Inoculation of leaves of plants exposed to the surfactants resulted in a progressive loss of chlorophyll over time, but in most instances, the loss did not differ from that in inoculated leaves of water-treated plants. Inoculated leaves of plants subjected to long exposure to Aqua-Gro and Hydro-Wet were exceptions; chlorophyll loss was decreased in the Aqua-Gro treatment at 96 hr and increased in the Hydro-Wet treatment at 72 hr. The responses are discussed relative to ethylene-chlorophyll-disease interactions.

Additional key words: disease physiology, *Helminthosporium*, Kentucky bluegrass, leaf spot, wetting agents

Use of surfactants on turfgrasses to improve effectiveness of foliar-applied pesticides and to increase water infiltration and percolation through hydrophobic soils is increasing. There is evidence that besides reducing surface tension at interfaces, surfactants are capable of exerting favorable and adverse biochemical effects on living organisms (16).

Favorable or useful effects of surfactants include control or eradication of apple powdery mildew and apple scab (2–5,8,11), enhanced apple-thinning effectiveness of napthaleneacetic acid by adding Tween 20 (9), increased growth and chlorophyll content in peas by polyvinyl alcohol (6), and shoot growth stimulation of barley by Aqua-Gro (7).

Adverse effects of surfactants include phytotoxicity to apples (2), sugarcane (19), and roots of ryegrass, tall fescue, Kentucky bluegrass, and creeping bentgrass (7). Other adverse effects include increased fungal decay of Anjou pear when Ortho X-77 is added to certain fungicides (20), changes in the bacterial population of soils where surfactants are applied (10), absence of earthworms in upper soil layers where didecyl dimethyl ammonium bromide is applied (10), and induced ethylene production and necrotic lesions when some of the Triton-X series of surfactants are applied to leaves of cowpea and sour cherry (14).

The increasing use of surfactants on turf, combined with the general knowledge that surfactants may have both favorable and adverse side-effects on plants, suggests that research into the physiological effects on grasses and into potential interactions with other biotic factors is warranted. The observations that some surfactants can affect chlorophyll content (6) and ethylene production (14) are of special interest relative to leaf spot caused by *Bipolaris sorokiniana* (Sacc.) Subram. in *Poa pratensis* L. Leaf spot is the most widespread disease of *P. pratensis* and is characterized by necrotic lesions with chlorotic halos and eventual chlorosis of the entire leaf (12). The severity of these symptoms may be measured by chlorophyll loss, and recent observations suggest that ethylene may be a primary factor contributing to chlorophyll loss during pathogenesis (12). This study was initiated to determine whether selected, nonionic surfactants might influence the endogenous ethylene and chlorophyll content of healthy and *B. sorokiniana*-inoculated leaves of *P. pratensis* during pathogenesis.

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MATERIALS AND METHODS

Plant materials. *P. pratensis* 'Newport' was vegetatively propagated in a steamed loam-peat soil mix (2:1, v/v) in 7.6-cm-square plastic pots under natural light for 4 mo. Each plant was maintained as a single shoot with four leaves by cutting tillers and rhizomes to the soil surface. Plants selected for treatments were preconditioned in growth chambers for 4 days at 22 C with a 9-hr photoperiod and irradiance of 175 μE m^{-2} s^{-1}. *B. sorokiniana* was cultured on 20 ml of Bacto agar (3%, v/v) in sterile plastic petri dishes (15 × 100 mm) in an incubator for 20 ± 5 days at 21 C with a 12-hr photoperiod.

Treatments and inoculations. Plants were treated with one of three nonionic surfactants: Aqua-Gro (50% polyoxyethylene ether of alkylated phenols and 50% polyoxyethylene esters of cyclic acid, Aquatrols Corp. of America, Pennsauken, NJ), Hydro-Wet (polyoxyethylene-polypropoxypropanol, Kalo Agricultural Chemicals, Inc., Overland Park, KS), and Surf-Side 37 (chemistry unavailable, Montco Products Corporation, Ambler, PA). Surfactants were applied to *P. pratensis* as a soil drench or foliar spray at label rates after a 4-day preconditioning period in the growth chambers. All rates were expressed as amount of surfactant product in H_2O: Aqua-Gro, 8 ml L^{-1} (1 oz/gal), Hydro-Wet, 6 ml L^{-1} (0.8 oz/gal), and Surf-Side 37, 20 ml L^{-1} (2.6 oz/gal). Soil-drench applications were made with a repeating pipet at 40 ml of each surfactant solution applied to the soil surface of each 7.6-cm pot. Foliar-spray applications were made with a DeVilbiss hand-held atomizer and air compressor (561 series) at 10 ml of each surfactant solution applied to the single shoot in each pot. While leaves were sprayed, pots were held sideways and upside down to prevent the surfactants from entering the soil.

Plants receiving soil-drench and foliar-spray applications of the surfactants were divided into short- and long-exposure treatment groups. Plants within the short-exposure group were inoculated immediately after application of surfac-

tants. All leaves of all plants in the short-exposure group were analyzed for endogenous ethylene and chlorophyll content 24, 48, 72, and 96 hr after inoculation. Plants within the long-exposure group were maintained 7 days and received multiple applications of the surfactants. Foliar-spray and soil-drench applications were as follows: day 1, label rate applications; day 4, plants moved to growth chambers for preconditioning; day 5, applications at diluted rates (Aqua-Gro, 2.5 ml L^{-1}; Hydro-Wet, 1.5 ml L^{-1}; Surf-Side 37, 5.0 ml L^{-1}); and day 7, label rate application and inoculation. Controls for short- and long-exposure studies included untreated uninoculated and inoculated plants. All studies were replicated three times and analyzed as a randomized complete block.

Inoculations were accomplished by placing the four leaves of each shoot into glass inoculating tubes of a previously described apparatus (18). Cylindrical plugs (5 mm in diameter) were cut from *B. sorokiniana* cultures and placed with mycelium against the upper epidermis of the leaves of *P. pratensis* on 1-cm centers. A free-water continuum was maintained between the agar cylinder and the leaf surface to prevent drying and to ensure infection. Plants not treated with surfactants also were inoculated and served as water-treated controls.

Ethylene and chlorophyll analyses. Endogenous ethylene was determined by vacuum extraction of the internal gases of the leaves (1). The four leaf blades of each shoot were detached from the plant, taken from the inoculation tubes, and placed in the collection tubes immersed in saturated $(NH_4)_2SO_4$. Internal gasses were released and collected from leaves by subjecting them to a negative pressure of 14 kPa maintained for 3 min. A 100-μl sample of the gas was injected into a Varian 3700 gas chromatograph operated at injector and column temperatures of 150 C and a flame ionization detector (FID) temperature of 250 C. The FID signal was fed to a Cary model 401 electrometer coupled to a Spectra-Physics 4100 computing integrator. After calibration, sample data were plotted and quantified by the computing integrator and expressed as microliters of ethylene per liter.

Chlorophyll content of the four leaf blade sections (10 cm) collected from the inoculation tubes was determined by a previously described method (13). Chlorophyll concentrations were determined as micrograms per milligram dry leaf weight. The chlorophyll concentration of treated and inoculated leaves was expressed as a percentage of untreated, uninoculated water controls.

RESULTS

Endogenous ethylene. No increase in endogenous ethylene occurred among uninoculated control plants (water, foliar

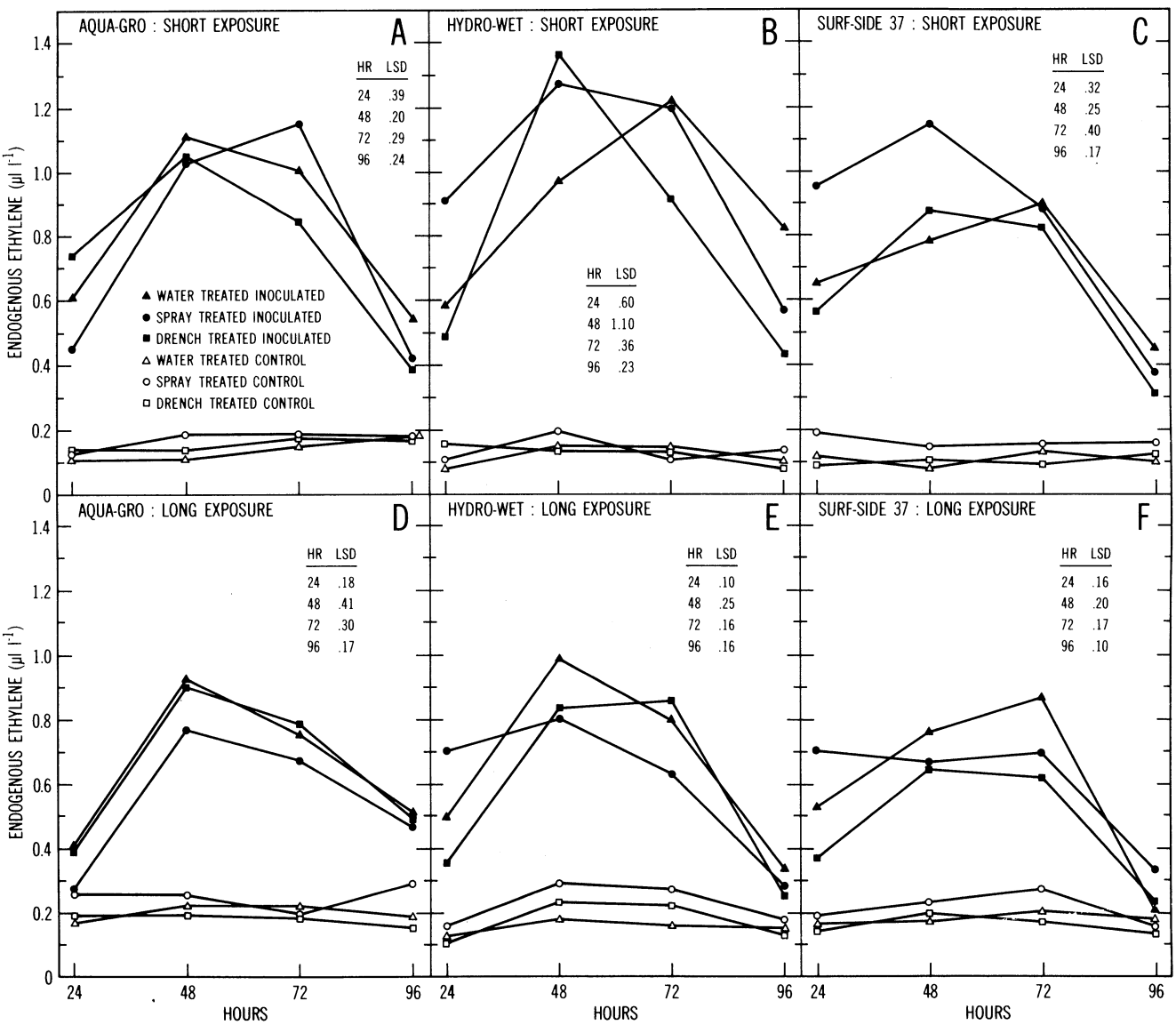

Fig. 1. Endogenous ethylene of healthy and *Bipolaris sorokiniana*-infected leaves of *Poa pratensis* on plants subjected to short and long exposures to the nonionic surfactants Aqua-Gro, Hydro-Wet, and Surf-Side 37. LSD = 0.05.

spray, or soil drench) subjected to short (Fig. 1A–C) or long (Fig. 1D–F) exposure to any of the surfactants. Endogenous ethylene of all inoculated leaves increased with or without exposure to surfactants (Fig. 1A–F). The increase in endogenous ethylene in response to infection peaked at 48 or 72 hr and then decreased.

Short or long exposure of plants to Aqua-Gro (foliar spray or soil drench) had no effect on the endogenous ethylene of leaves after infection by *B. sorokiniana* (Fig. 1A,D). Exposure of plants to Hydro-Wet and Surf-Side 37 resulted in some changes in endogenous ethylene of leaves after infection, but the changes were erratic. Endogenous ethylene of infected leaves on plants subjected to short exposure to Hydro-Wet (foliar spray and soil drench) decreased at 96 hr relative to water-treated infected controls (Fig. 1B). Long exposure to Hydro-Wet spray increased ethylene in infected leaves at 24 hr and decreased it at 72 hr; exposure of plants by soil drench decreased ethylene in infected leaves at 24 hr (Fig. 1E). Short exposure to Surf-Side 37 (foliar spray) increased ethylene in infected leaves at 24 and 48 hr (Fig. 1C); long exposure to the foliar spray increased ethylene at 24 and 96 hr and decreased it at 72 hr relative to water-treated infected controls (Fig. 1F). Soil-drench applications of Surf-Side 37 decreased ethylene in infected leaves in long-exposure studies at 24 and 72 hr (Fig. 1F).

Chlorophyll content. Plants subjected to short or long exposures to the surfactants by foliar spray or soil drench showed fluctuations in chlorophyll content of their leaves (Fig. 2A–F). Most of the increases or decreases in chlorophyll were not significant relative to the uninoculated water-treated control plants (represented by 0% chlorophyll loss line in Fig. 2). The only significant increase in chlorophyll was in response to Aqua-Gro (long exposure, foliar spray) at 24 hr (Fig. 2D). The only significant decrease in chlorophyll occurred at 72 hr in response to a short exposure to foliar spray and soil drench of Surf-Side 37 (Fig. 2C); however, long exposure to this surfactant failed to show the same response (Fig. 2F).

The chlorophyll content of inoculated leaves of plants subjected to all short- and long-exposure treatments decreased over time (Fig. 2A–F). Inoculated leaves of water-treated control plants (except in Fig. 2B) showed a significant loss of chlorophyll by 96 hr relative to the uninoculated water-treated controls (Fig. 2A–F). Inoculated leaves of plants

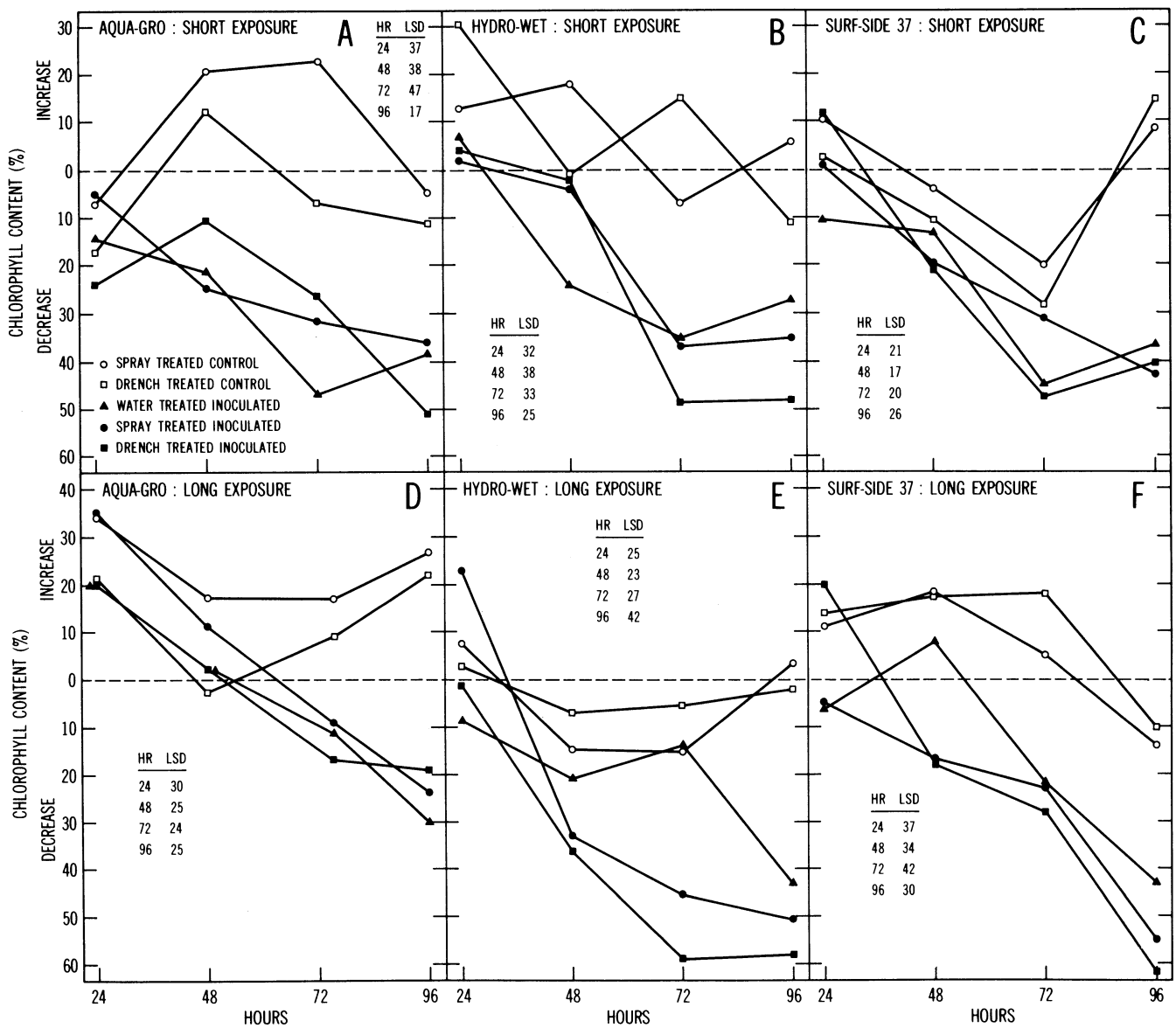

Fig. 2. Chlorophyll content of healthy and *Bipolaris sorokiniana*-infected leaves of *Poa pratensis* on plants subjected to short and long exposures to the nonionic surfactants Aqua-Gro, Hydro-Wet, and Surf-Side 37. LSD = 0.05. (The uninoculated water-treated control is represented by the 0% chlorophyll loss line to which all other treatments are referenced.)

subjected to short-exposure foliar-spray and soil-drench treatments of the surfactants did not differ in chlorophyll content from inoculated leaves of water-treated control plants (Fig. 2A–C). Chlorophyll content of most inoculated leaves of plants subjected to long-exposure foliar-spray and soil-drench treatments of the surfactants were not significantly different from that of inoculated water-treated control. Exceptions occurred with Aqua-Gro (Fig. 2D), where no significant loss of chlorophyll occurred in response to infection of leaves of treated plants, and with Hydro-Wet (Fig. 2E), where chlorophyll loss of infected foliar-spray- and soil-drench-treated plants exceeded that in infected water-treated control plants at 72 hr. No differences occurred in chlorophyll loss between inoculated plants exposed to Surf-Side 37 and inoculated water-treated control plants (Fig. 2F).

DISCUSSION

The surfactants examined in this study had no effect on endogenous ethylene of *P. pratensis* leaves (Fig. 1A–F) and a minimal effect on the chlorophyll content of the leaves (Fig. 2A–F). Leaf chlorophyll increased and decreased erratically, but few of the changes were significant and the nature of the changes failed to show a consistent positive or detrimental trend. These nonsignificant changes may be of interest, however, in that some surfactants may affect the biosynthesis of prophyrin structures (6) and that the nonionic surfactants Sterox SK, Renex 36, and Tween 20 alter chloroplast membrane ultrastructure and organization resulting in abnormal grana (22,23). Evaluation of greater or lesser amounts of the surfactants might have caused significant changes in the chlorophyll content.

Inoculation of leaves of plants with *B. sorokiniana* increases endogenous ethylene with or without exposure to surfactants (Fig. 1A–F); this response has also been observed in previous studies (12). Most significant increases in ethylene occur 48 and 72 hr after inoculation; fewer significant changes occur at 24 and 96 hr. The increases in ethylene, however, showed few differences between inoculated leaves of plants exposed and not exposed to surfactants. Even where differences occurred, there was no trend to suggest any major interaction between infection and the surfactants relative to ethylene content.

Several studies suggest that increases in ethylene may result in degradation of chlorophyll (15,17,21). Peak increases in endogenous ethylene in *P. pratensis* leaves after infection by *B. sorokiniana* precede major chlorophyll loss (12). Ethylene generally peaks in response to infection 48 to 72 hr after inoculation and then declines by 96 hr; chlorophyll loss occurs at 72 or 96 hr, directly after the ethylene peaks. These responses were present among inoculated water-treated control plants in the present study and did not vary significantly on plants exposed to the surfactants except for two treatments: 1) Inoculated leaves of plants subjected to long exposure to Aqua-Gro had an ethylene peak at 48 hr, which did not differ from the inoculated water-treated plants (Fig. 1D), but there was no significant loss of chlorophyll except in the inoculated water-treated plants at 96 hr (Fig. 2D); this response suggests that Aqua-Gro may slow the loss of chlorophyll after infection regardless of the increase in ethylene. 2) Inoculated leaves of plants subjected to long exposure to Hydro-Wet showed ethylene peaks primarily at 48 hr, with no difference between treatments (Fig. 1E), but losses of chlorophyll among leaves exposed to the surfactant were greater than those of the inoculated water-treated plants at 72 hr (Fig. 2E). This response suggests that the loss of chlorophyll may be enhanced by Hydro-Wet before the ethylene peak after infection and that factors other than ethylene are involved in the chlorophyll loss. It seems that long exposure to Aqua-Gro or Hydro-Wet influences pathogenesis; however, Surf-Side 37 seems to have no effect on pathogenesis. The effects of these surfactants on pathogenesis of *B. sorokiniana* leaf spot are essentially benign when the materials are used at label rates.

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