Effect of Chlorophenoxy Herbicides on Free Amino Acids in Sequentially Senescent Leaves of *Poa pratensis* and on Pathogenesis by *Bipolaris sorokiniana*

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ABSTRACT

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The herbicides 2-(2-methyl-4-chlorophenoxy) propionic acid (MCPP) and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) were evaluated for effect on the free amino acid content of four sequentially aged leaves of herbicide-tolerant *Poa pratensis* and on subsequent leaf spot severity after infection by *Bipolaris sorokiniana*. The content of free amino acids in uninoculated leaves of herbicide-untreated control plants generally declined from the youngest to oldest leaf. The herbicides had no influence on total amino acids in leaves of any age. Most leaves of plants treated with MCPP contained less Pro, His, Lys, Arg, Ala, and Phe than did the control. Most leaves of plants treated with 2,4,5-TP contained less Pro and Lys than did the control. Uninoculated MCPP-treated plants contained more Ser and Glu in older leaves only, whereas uninoculated 2,4,5-TP-treated plants contained more Arg, Thr, Ala, and Val in younger leaves only. Infected leaves of plants treated with either herbicide generally were more severely diseased than leaves of herbicide-untreated control plants, but only MCPP-treated plants had increased leaf spot on the youngest leaf. The results suggest that changes in free amino acid levels in leaves after treatment of *P. pratensis* with chlorophenoxy herbicides may be a component of physiological changes that are similar to changes during senescence. Changes in amino acid content induced by chlorophenoxy herbicides may promote leaf senescence in *P. pratensis* and the subsequent enhancement of leaf spot. However, changes in amino acid content independent of other metabolic changes occurring during senescence probably have limited direct influence on leaf spot severity.

Additional key words: *Drechslera sorokiniana*, *Helminthosporium sativum*, Kentucky bluegrass, mecoprop, silvex.

Severity of leaf spot caused by *Bipolaris sorokiniana* (Sacc.) Subram. (1) (= *Helminthosporium sativum* P. K. and B.) on *Poa pratensis* L. is directly related to the age of the leaves of *P. pratensis* (9–11,16). Shoots of *P. pratensis* generally maintain three to four visible leaves under mowing during the growing season. Leaf spot is minimal on the two youngest leaves and is most severe on the two oldest leaves (9,16). Symptoms on older leaves are large necrotic lesions with chlorotic halos and some chlorotic streaking (10). A hypothesis that the promotion of leaf senescence in *P. pratensis* enhances leaf spot severity was proposed on the basis of similar results of studies on the effects of chlorophenoxy herbicides and of light quality and photoperiod on leaf spot (10). Auxinlike chlorophenoxy herbicides generally stimulate leaf spot of *P.*

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pratensis, especially on older leaves (9,16). Leaf spot symptoms of herbicide-treated *P. pratensis* are necrotic lesions surrounded by large chlorotic areas, which, in older leaves, may extend throughout the whole leaf (9). Similar symptoms occur under blue + far-red light spectra and short photoperiods (19,20). Both chlorophenoxy herbicides and light conditions may promote conditions in older leaves typical of leaf senescence that result in enhanced leaf spot severity.

Hydrolysis of protein is typical of senescing leaves (28). Free amino acids are utilized as respiratory substrate (28) or are translocated out of leaves to other plant parts (28), resulting in a net loss of amino acids in senescing leaves. Application of chlorophenoxy herbicides to herbicide-sensitive plants typically results in decreased amino acid levels in leaves and increased levels in stems and roots (3,7,18,24). Application of chlorophenoxy herbicides to herbicide-tolerant *P. pratensis* influences individual free amino acid content in leaves on a whole-plant basis (15).

Pathogenesis by *B. sorokiniana* and amino acid levels in infected leaves may be related. Preinfection (uninoculated) levels of amino acids are not significantly correlated with subsequent leaf spot severity on a whole-shoot basis after infection (15). Amino acid levels in leaves generally increase after infection by *B. sorokiniana* (15). Infection sites may act as a sink, resulting in an influx of amino acids to infected leaves (12,23,29), and some amino acids may be utilized by the pathogen as substrate (2,29).

A chlorophenoxy herbicide, amino acid, senescence relationship may exist. The purpose of this investigation was to determine how chlorophenoxy herbicides influence free amino acids in sequentially older leaves of *P. pratensis* and how the subsequent effects influence leaf spot severity.

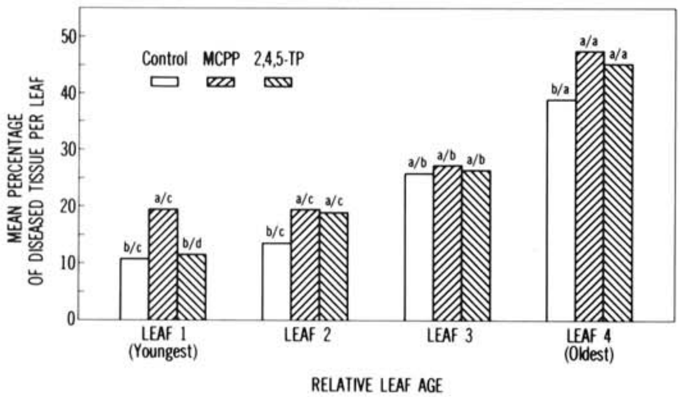

Fig. 1. Mean percentage of diseased leaf area of four sequentially aged leaves of control and herbicide-treated *Poa pratensis* inoculated with *Bipolaris sorokiniana*. Values with the same letter among control and herbicide treatments within relative leaf age (across a/) or among relative leaf age within control and herbicide treatments (across /a) are not significantly different according to Duncan's multiple range test ($P = 0.05$). NOTE: The data in this histogram were previously presented (16). The previous paper and the present paper were component parts of the same study and were referenced to the same disease response data of this histogram.

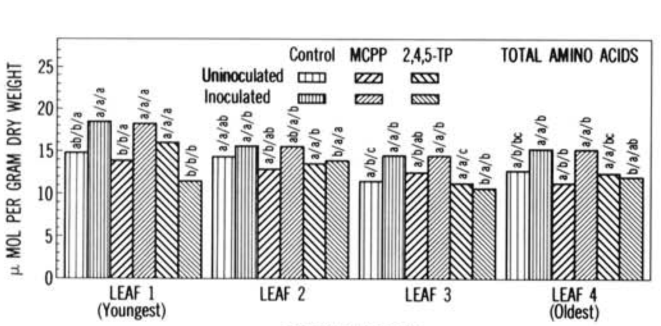

Fig. 2. Mean content of the total of 15 individual free amino acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. Values with the same letter among control and herbicide treatments within inoculation status and relative leaf age (across a/ /), between uninoculated and inoculated plants within control and herbicide treatments and relative leaf age (across /a/) or among relative leaf ages within control and herbicide treatments and inoculation status (across / /a) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

MATERIALS AND METHODS

Poa pratensis L. 'Newport' was vegetatively propagated and grown in the greenhouse as described previously (15). Cultures of *Bipolaris sorokiniana* (Sacc.) Subram. were grown and maintained by a method described previously (15).

Herbicide treatments consisted of 40 ml (20 ml each, 4 and 2 days before inoculation) of 10^{-4}M 2-(2-methyl-4 chlorophenoxy) propionic acid (MCPP) or 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) or with distilled water (control) applied to the soil. The four youngest visible leaves (leaf 1, youngest; leaf 4, oldest) of one shoot were inoculated with 5–10 conidia suspended in 0.02 ml of sterile distilled water at five positions, 1 cm apart, along a 10-cm section of the leaf ~ 5 cm from the leaf tip in a special inoculation apparatus (25). Uninoculated plants received 0.02 ml of sterile distilled water as a control. The concentration of conidial suspensions was determined with a particle counter (High Accuracy Products Corp., Montclair, CA). Each treatment consisted of 17 individual shoots and was replicated three times. Plants were incubated for 6 days at 24 C under continuous fluorescent light (80–90 $\mu E \cdot m^{-2} \cdot sec^{-1}$) and then evaluated for disease severity (15).

Leaf tissue was prepared for free amino acid content determination on a leaf-age basis by a method described previously (15). Data were analyzed as a $3 \times 2 \times 4$ factorial design for the mean percentage of diseased leaf tissue (15) and for individual and total free amino acids of the four sequentially aged leaves of shoots of each treatment. Individual amino acids were grouped according to chemical structure (17).

RESULTS

Disease severity. The percentage of diseased tissue of the two youngest inoculated leaves (leaves 1 and 2) of herbicide untreated control plants was not different (Fig. 1). However, the percentage of diseased tissue increased from leaf 2 to leaf 3 to leaf 4 of control plants (Fig. 1). Soil applications of 10^{-4}M MCPP enhanced leaf spot on all but the third leaf, whereas applications of 2,4,5-TP stimulated disease of leaves 2 and 4 only.

Changes in amino acid content. Application of either MCPP or 2,4,5-TP had little effect on the content of total free amino acids of

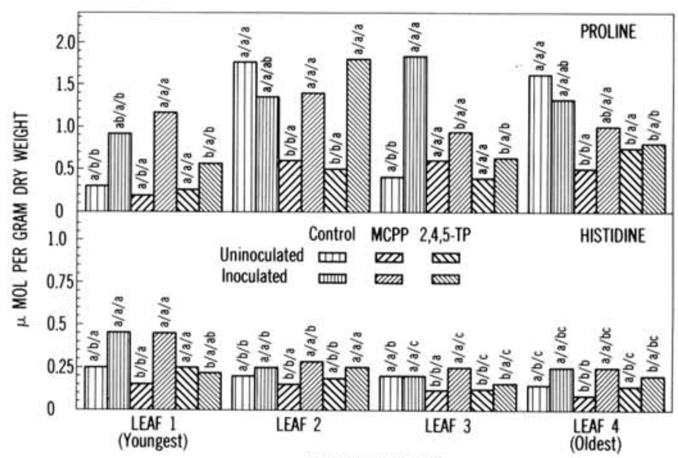

Fig. 3. Mean content of free heterocyclic amino acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. See Fig. 2 for explanation of statistical relationships.

uninoculated leaves of *P. pratensis* (Fig. 2). No differences in total amino acids in leaves of any age existed between the uninoculated untreated control and uninoculated plants treated with either herbicide. Total amino acid content of leaves of plants treated with either herbicide declined from the youngest to oldest leaves.

Changes in specific individual amino acids occurred after herbicide treatment. Application of MCPP to uninoculated plants resulted in decreased Pro in leaves 2 and 4 (Fig. 3), His in all leaves (Fig. 3), Lys in all leaves (Fig. 4), Arg in leaves 1, 2, and 4 (Fig. 4), Ala in leaves 2, 3, and 4 (Fig. 5), and Phe in leaves 2 and 4 (Fig. 6). Application of 2,4,5-TP to uninoculated plants resulted in decreased Pro in leaves 2 and 4 (Fig. 3) and Lys in all leaves (Fig. 4), as well as His (Fig. 3), Leu (Fig. 5), and Tyr (Fig. 6) in the third leaf only.

Content of some individual amino acids increased in uninoculated leaves of plants treated with either herbicide. MCPP resulted in increased Ser in leaf 3 (Fig. 5), Asp in leaf 2 (Fig. 7), and Glu in leaves 3 and 4 (Fig. 7). Application of 2,4,5-TP resulted in increased Arg in leaf 1 (Fig. 4), Thr and Ala in leaf 1 (Fig. 5), Val in leaves 1 and 2 (Fig. 5), Ile and Glu in leaf 4 (Figs. 5 and 7).

Inoculation of leaves of both untreated and herbicide-treated plants resulted in changes in total and individual amino acids. Inoculated leaves of any age of untreated and MCPP-treated plants contained a greater amount of total amino acids than did uninoculated leaves (Fig. 2). Content of most individual amino acids generally increased after inoculation of untreated and MCPP-treated plants, except for Lys which decreased in all inoculated leaves of untreated plants (Fig. 4). Inoculation of leaves of plants treated with 2,4,5-TP resulted in decreased total amino acid content in the youngest leaf and no changes in other leaves compared with uninoculated 2,4,5-TP-treated leaves (Fig. 2). This relationship was generally the same for individual amino acids in inoculated leaves of 2,4,5-TP-treated plants.

DISCUSSION

No close association between amino acids and leaf spot severity was evident in this study. Correlation coefficients were determined for the comparison of free amino acid content of leaves of untreated controls with leaf spot severity. No significant correlation existed at $P = 0.05$. A similar absence of correlations between nitrogen fertilization, free amino acid content, and leaf spot severity also has been observed (26).

Amino acid content is influenced by treatment with chlorophenoxy herbicides in a manner that is consistent with changes occurring during leaf senescence. The senescence process involves proteolysis, decreased photosynthesis, increased respiration, a decline in RNA synthesis, and the loss of chlorophyll (28). The auxinlike postemergence herbicides, MCPP and 2,4,5-TP, initially increase the free amino acid content of leaves of herbicide sensitive plants as a result of proteolysis. Free amio acids are then translocated to the stem and growing point (3,7,24). Application of MCPP to herbicide-tolerant *P. pratensis* decreased Pro, His, Lys, Arg, Ala, and Phe in two or more leaves and increased Glu in two leaves and Ser and Asp in a single leaf (Figs. 3–7). Application of 2,4,5-TP decreased Pro and Lys in two or more leaves and His, Leu, and Tyr in the third leaf only (Figs. 3, 5, and 6). The 2,4,5-TP treatment resulted in increased Arg, Thr, Ala,

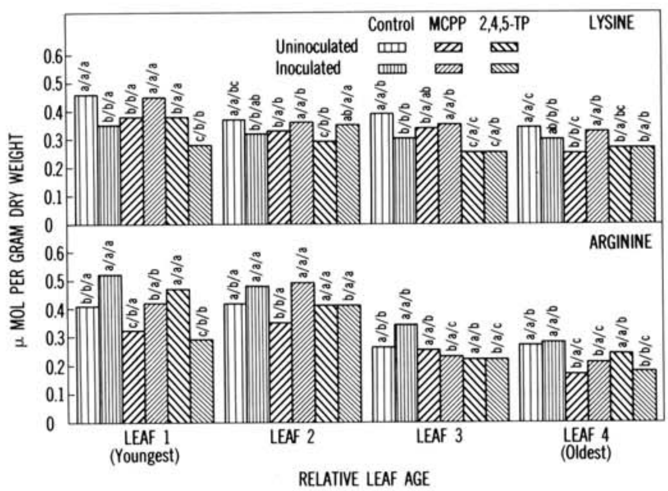

Fig. 4. Mean content of free diamino-monocarboxylic acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. See Fig. 2 for explanation of statistical relationships.

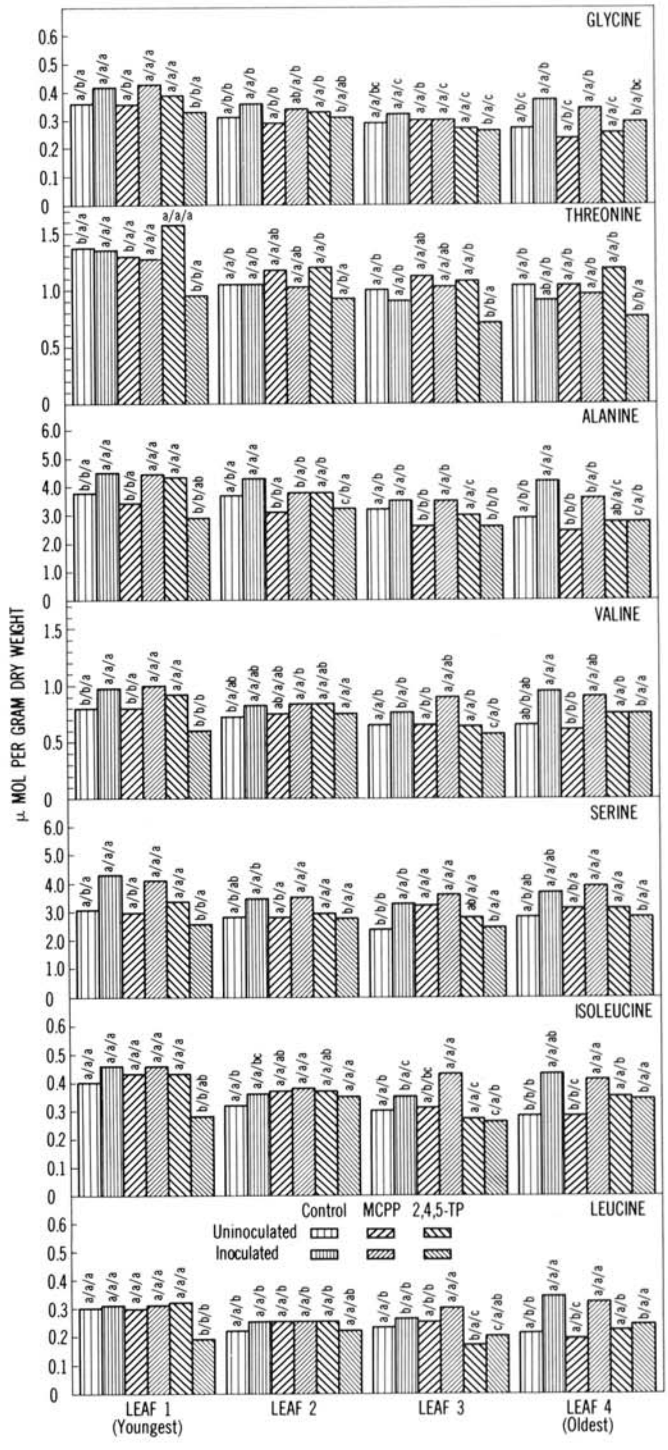

Fig. 5. Mean content of free monoamino-monocarboxylic acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. See Fig. 2 for explanation of statistical relationships.

Val, Ile, and Glu in at least one leaf, usually the youngest (Figs. 4, 5, and 7). These changes may be analogous to increased proteolysis and translocation of free amino acids during leaf senescence.

Changes in amino acid content after treatment with chlorophenoxy herbicides independent of other changes may have limited direct influence on leaf spot severity. The influence of amino acids on preinfection characteristics of *B. sorokiniana* (26), however, suggests that herbicide-induced changes in amino acids may be one component of a complex of physiological changes, similar to those occurring during leaf senescence, which seem to increase leaf spot severity. Changes in soluble sugars also are part of this complex system. Decreased sucrose and total sugar content are typical of leaf senescence and are associated with increased leaf spot severity on MCPP-treated plants (16). Infection of leaves by *B. sorokiniana* may, in itself, promote senescence. The content of most amino acids increased in infected leaves of herbicide-untreated control plants (Figs. 2–7). Infection sites may act as sinks for amino acids from proteolysis (12,23,29) and may enhance senescence of the plant and facilitate subsequent development of *B. sorokiniana* (6,21).

The results suggest that the physiological activity of MCPP and 2,4,5-TP in *P. pratensis* is different. The effect of MCPP on amino acid content of uninoculated leaves generally was evident in all four leaves of the shoot; the effect of 2,4,5-TP was predominantly in the younger leaves (Figs. 2–7). Inoculation of plants treated with MCPP caused most amino acids to increase in all four leaves of the shoot compared with uninoculated MCPP-treated plants (Figs. 2–7). Inoculation of plants treated with 2,4,5-TP had little influence on amino acids in the three oldest leaves of a shoot, but generally decreased amino acids in the youngest leaf (Figs. 2–7).

Chlorophenoxy herbicides inhibit root elongation and stimulate cell division in some grasses (30). Monochlorinated MCPP is absorbed by roots and translocated to leaves of herbicide-sensitive plants more readily than is 2,4,5-TP (13,22,27). The less-mobile 2,4,5-TP accumulates in root tips with limited translocation to the shoot in plants sensitive to the herbicide (4,14) and may be injurious to grass roots (5). Leaf spot severity on *P. pratensis* decreases in response to 2,4,5-TP as the treatment concentration is increased (8). In the present study, the percentage of diseased leaf tissue on the youngest leaf was less on 2,4,5-TP-treated plants than on those treated with MCPP (Fig. 1). MCPP may be more mobile than 2,4,5-TP in *P. pratensis* and function to decrease the amino acid content in leaves of all ages and contribute to enhanced leaf spot. 2,4,5-TP may be less mobile in *P. pratensis* and subsequently have a minimal effect on amino acids and leaf spot severity.

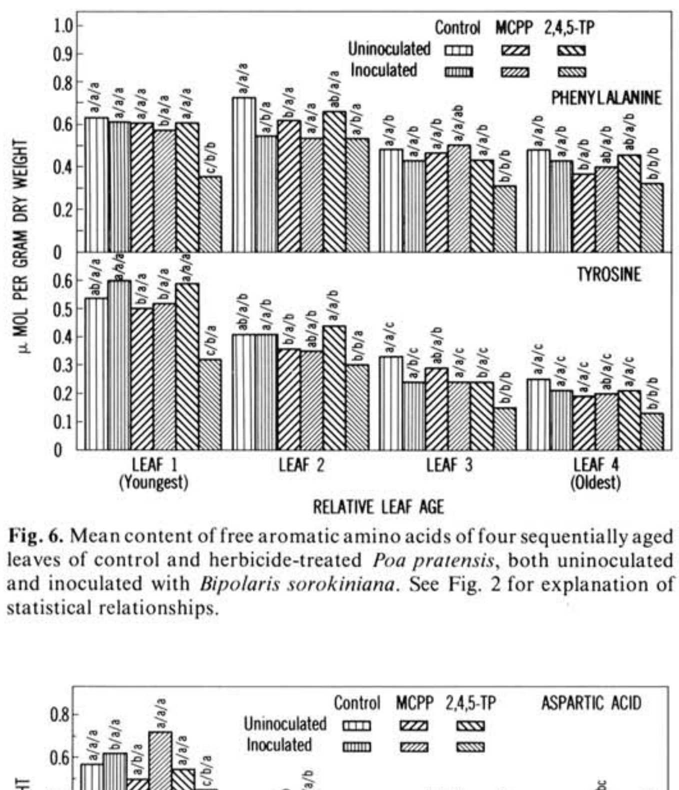

Fig. 6. Mean content of free aromatic amino acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. See Fig. 2 for explanation of statistical relationships.

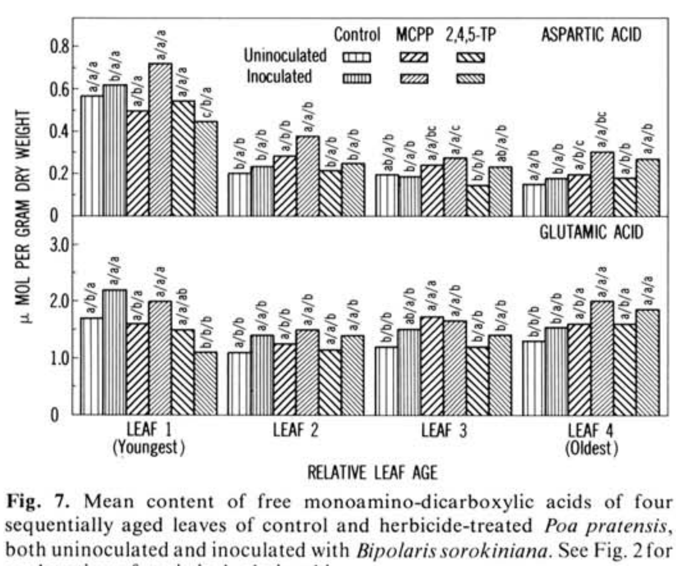

Fig. 7. Mean content of free monoamino-dicarboxylic acids of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Bipolaris sorokiniana*. See Fig. 2 for explanation of statistical relationships.

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