Fertilization and Wheat Refuse Effects on Fusarium Species Associated with Wheat Roots in Minnesota

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ABSTRACT

Isolations from roots, soil, rhizosphere, and crop residues of wheat (*Triticum aestivum* 'Chris') in field plots yielded six species of *Fusarium: F. oxysporum, F. solani, F. roseum, F. tricinctum, F. moniliforme,* and *F. episphaeria. Fusarium oxysporum* was dominant, followed by *F. solani* and *F. roseum*. Seedling blight caused by *Fusarium* spp. averaged 1-2% when fertilizer and residues were present, and 11-14% when both were absent. Fertilizer alone gave a lower blight incidence, lower root disease indices, and fewer *Fusarium*-infected roots than did residue alone. In a year when seedling blight was severe, *Fusarium roseum* comprised 90% of the *Fusarium* isolates from roots, followed by *F. oxysporum* and *F. solani*. Of the *F. roseum* cultivars, Graminearum comprised 70%, followed by Avenaceum and Culmorum with 15% each. The removal of wheat residue (mainly surface) resulted in greater survival of *F. oxysporum* and less survival of *F. roseum* and *F. solani* in soil.

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Additional key words: Helminthosporium sativum, seedling blight.

Fusarium species are relatively abundant in soil, and frequently are associated with *Helminthosporium sativum* Pam., King & Bakke in causing common root rot of wheat (7, 8). Many species of *Fusarium* can survive actively in soil in or on cover crop residues (17, 18), on root surfaces (1, 16), or in wounded tissues of root (11). Some species can survive passively as chlamydospores or as thickened hyphae in debris (13, 15). More clonal types and species may be present in cultivated than in noncultivated soils (9, 20). Changes in number and kinds of soil microorganisms have been associated with crop residues or amendments incorporated into soil (16, 18, 19).

Many factors have been reported to affect survival of *Fusarium* spp. in soil (1, 4, 7, 13, 18, 19), including organic matter, fertilizer, moisture, microflora, soil fauna, and kinds and duration of crops grown.

Experiments were made to evaluate the role of crop residue and fertilizer on (i) population and species of *Fusarium* in wheat plots cropped annually and exclusively to wheat for a decade; and (ii) survival of *Fusarium* spp. in soil and infection of wheat roots. With such information, it may be possible to develop methods for reduction of the incidence of root diseases in wheat by alteration of the cropping practices.

MATERIALS AND METHODS.—At Rosemount, Minn., wheat (*Triticum aestivum* L. 'Chris') was grown each season for a decade, during which time the crop residues (stubble and threshed straw) were either retained or removed, with and without application of fertilizer (5:20:20, N P K at 243 kg/hectare). N was NH_4NO_3, P was P_2O_5, and K was K_2O. This arrangement gave four treatments: no fertilizer or residues; no fertilizer with residues; fertilizer but no residues; and fertilizer with residues.

We evaluated seedlings for blight at 34, 46, and 60 days after sowing by examining shoots and roots, three replicates each time, 50 seedlings/replicate. A scale of 0 to 4 was used (0 = no seedling blight; 1 = trace or initial infection on culm or root; 2 = light infection on root or culm; 3 = moderate infection; and 4 = heavy infection or plant dead). We calculated disease index using the formula of Sherwood & Hagedorn (14) where 0 means all plants healthy and 100 all plants severely infected or dead.

We took soil samples by selecting three random samples (3-15 cm deep) per treatment. Soil was passed through a 2-mm sieve, and 5.0 g were successively diluted in water to give a final dilution of 1:10,000. Final soil dilutions were mixed in a shaker for 30 sec; 1-ml aliquots were pipetted into a petri dish to which was added 15 ml of cool PCNB (pentachloronitrobenzene)-peptone agar adjusted to pH 5.2 (12).

For root surface flora, root samples (each 5.0 g) were shaken in water on a rotary shaker for 20 min to remove rhizosphere soil from roots. Roots were surface-treated for 2 min in 70% ethanol, kept 2 min in 5% NaOCl, and placed on PCNB-peptone agar.

Unless stated otherwise, we removed residues by raking them from soil. However, since some residues would remain in soil by this method, we checked to see how much was left after the raking procedure by screening 2.8×10^4 cc of soil over a 2-cm screen. Residues so obtained were oven-dried for 24 hr at 110 C and weighed. We found that only 87% of the residues had been removed by the raking in plots designated as "residues removed"; and 94%, in plots designated as "residues removed but fertilizer applied". On the other hand, in plots where residues had not been removed but fertilizer had been applied, there was only 43% of the initial residue present, apparently because of accelerated decomposition.

Organic matter of soil samples was determined at 5-, 15-, 25-, and 35-cm depths; 30- to 40-g samples were then oven-dried (24 hr at 110 C), distributed evenly in 9-cm crucibles, and exposed to flames for 30 min.

RESULTS.—*Seedling blight and root rot index.*—Seedling blight symptoms and root rot severity increased early in the season, but both were arrested by 60 days after planting. Blighted seedlings averaged 1-2% of the total when fertilizer and residue were present, and 11-14% when both were absent (Fig. 1). When the results are based on a disease index (scale of 0-100) of roots, for the three sampling dates the presence of fertilizer and residue gave indices of 30-31, but the absence of both fertilizer and residue gave indices of 52-66.

In 1968, seedling blight did not occur and grain yield was nearly double that in 1969, when seedling blight was severe. An exception was in the plot with residue plus fertilizer where blight was reduced and yields were only about 50% higher than in 1969 (Table 1). Thus, residue and fertilizer, in a year when blight was severe, reduced blight, increased yield, and gave the least root infection (Table 1 versus Fig. 1).

The percentage of soil organic matter was higher in treatments where residues were retained than where they were removed. The soil organic matter content was about 5.8% where residues were removed (by raking), with or without fertilizer. However, when residues were retained, the organic matter content was 6.6% without fertilizer and 7.7% with fertilizer.

Root infection.—Immediately after blight was evaluated, we made isolations from root samples by placing 1-cm sections on water agar or potato-dextrose agar. *Helminthosporium sativum* was isolated from roots and culms more frequently than *Fusarium* spp. in all soil treatments (Fig. 2). Nevertheless, the same general trend was seen for both fungi. Often a single plant or lesion yielded both *Fusarium roseum* and *H. sativum*.

Blight caused by *H. sativum* and *Fusarium roseum* was less where fertilizer was applied, but plants infected with *Fusarium roseum* were fewer than those infected with *H. sativum*. *Fusarium* spp. were isolated from 12 to 14% of roots when both fertilizer and residue were present, and from 19 to 24% when absent. Thus, fertilizer or residue suppressed infection by either species.

Of *Fusarium* spp. isolated from lesions on roots and culms of wheat in 1969, when blight was severe,

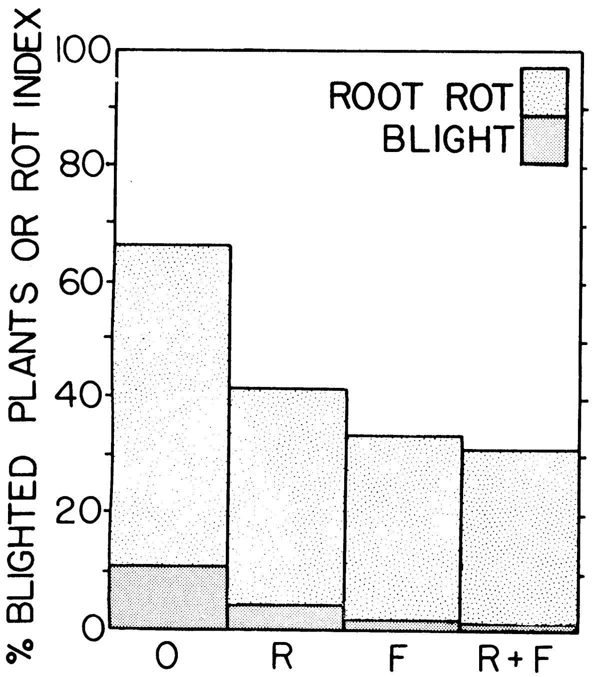

Fig. 1. The incidence of blighted seedlings and the root rot index after 60 days for wheat sown in continuous wheat plots (10 years) in which crop residues were removed and fertilizer withheld (O), residues retained but no fertilizer (R), fertilizer applied (NPK 5:20:20 at 243 kg/hectare) but residues removed (F), and residues retained and fertilizer applied (R+F).

TABLE 1. Yield of grain for 2 different years in field plots cropped to wheat and in which crop residues and fertilizer were either applied or withheld for the previous 10 years

Residue	Fertilizer[a]	Grain yield[b] 1968	Grain yield[b] 1969
		kg/hectare	
None	None	1,599	853
Present	None	1,290	712
None	Present	1,788	968
Present	Present	1,532	1,075

[a]NPK (5:20:20) at 243 kg/hectare.
[b]Average of two plots of three replicate, square-meter samples/plot.

90% were *F. roseum* (Lk.) Snyd. & Hans. f. sp. *cerealis*, and the remainder were *F. oxysporum* (Schlecht.) Snyd. & Hans. and *F. solani* (Mart.) Snyd. & Hans. The *F. roseum* cultivars included Graminearum (70%), Avenaceum (15%), and Culmorum (15%). Graminearum was most abundant when fertilizer and residue were present. *F. roseum* f. sp. *cerealis* was assumed because all isolates tested were pathogenic to some degree on wheat, but not all isolates from lesions were tested for pathogenicity.

Species in soil.—Five species of *Fusarium* could be distinguished among the 750 isolations made from soil by dilution plating on PCNB agar. Data in Fig. 3 are averages of four treatments in 1968-69.

For the 2-year study, *F. oxysporum* comprised 53% and *F. solani* and *F. roseum* about 20% each of the total population of *Fusarium* spp. *Fusarium tricinctum* (Cda.) Snyd. & Hans. and *F. episphaeria* (Tode) Snyd. & Hans. comprised only about 4% each.

The population of total *Fusarium* spp. in soil remained fairly stable, and showed little variation among treatments and years. The total count ranged from 15,000 colonies/g soil in May and up to 50,000/g by the end of August. The population was not markedly different in seasons differing in incidence of blight; nor did it seem to be affected by residues or fertilizer.

Species in host tissue.—We isolated *Fusarium* spp. from wheat residue during the fall and the following spring by placing 1-cm sections, surface-treated with NaOCl, on a PCNB medium. Each treatment was replicated twice, 50 sections/replicate. The total number (147) of *Fusarium* colonies isolated was greater on residue in the spring than the number recorded for the previous fall (99). Where fertilizer, residue, or both were present, the percentage of *F. oxysporum* colonies isolated during both periods was about

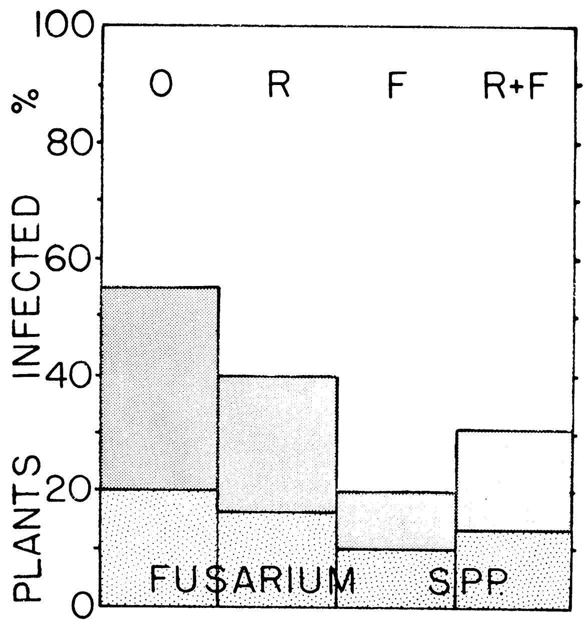

Fig. 2. The incidence of wheat plants infected with *Helminthosporium sativum* (dark shaded portion) and *Fusarium* spp. in 1969 (a severe blight year) in field plots sown continuously to wheat and in which crop residues were removed (0) or retained (R); or in which residues were removed and fertilizer applied (F), or both residue retained and fertilizer applied (R+F). Values are based on three replicates of 30 plants/replicate.

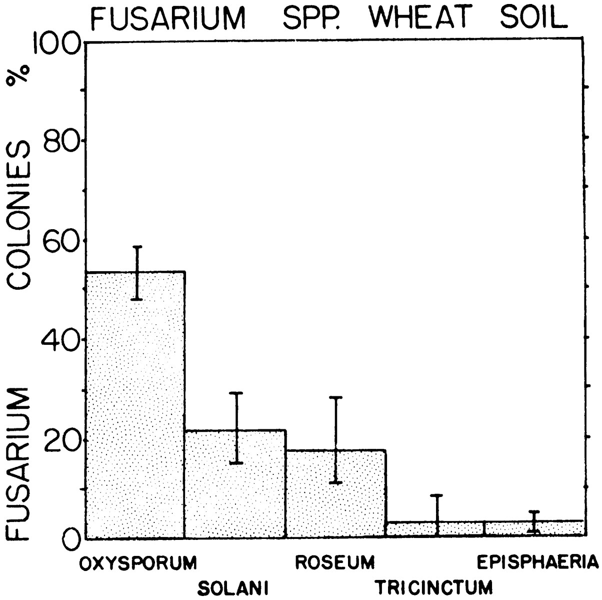

Fig. 3. The incidence of *Fusarium* species isolated from soil from continuous wheat plots in the field: bars represent averages of 2 years for four treatments in which crop residues were retained or removed, with and without fertilizer application (vertical lines represent the variation among the 2 years and four treatments).

equal, but in the absence of both residues and fertilizers, the percentage of *F. oxysporum* increased markedly during the spring (Fig. 4).

In a similar trial, when the residue was washed in running water for 30 min and plated out, the total number (260) of *Fusarium* colonies was lower in the spring than the number (309) in the fall.

Of the recognized *F. roseum* cultivars pathogenic to wheat, Avenaceum accounted for 40% of the population in the fall and 60% in the spring. Graminearum decreased by half from fall to spring (40-24%). The population of Culmorum remained fairly stable during both sampling periods.

Species in rhizosphere.–Five *Fusarium* spp. were isolated from rhizosphere soil. *Fusarium oxysporum* was the most abundant, followed by *F. solani, F. roseum, F. tricinctum,* and *F. moniliforme.* Figure 5 shows the rhizosphere to soil (R:S) ratios obtained in 1969 (blight year). Similar results were obtained in 1968.

The effect of fertilizer on the R:S ratio was clearly reflected in the slightly lower R:S ratio for *F. oxysporum* compared to the markedly higher R:S ratio for *F. roseum* (Fig. 5). The R:S ratio for *F. roseum* was nearly 5:1 when fertilizer was added, but for *F. oxysporum* the ratio was less than 1:1. The R:S ratio for *F. solani* was not changed appreciably by fertilizer or residue.

DISCUSSION.–*Fusarium roseum* is the major root-infecting *Fusarium* spp. of cereals when compared to *F. solani* and *F. oxysporum,* but it was less

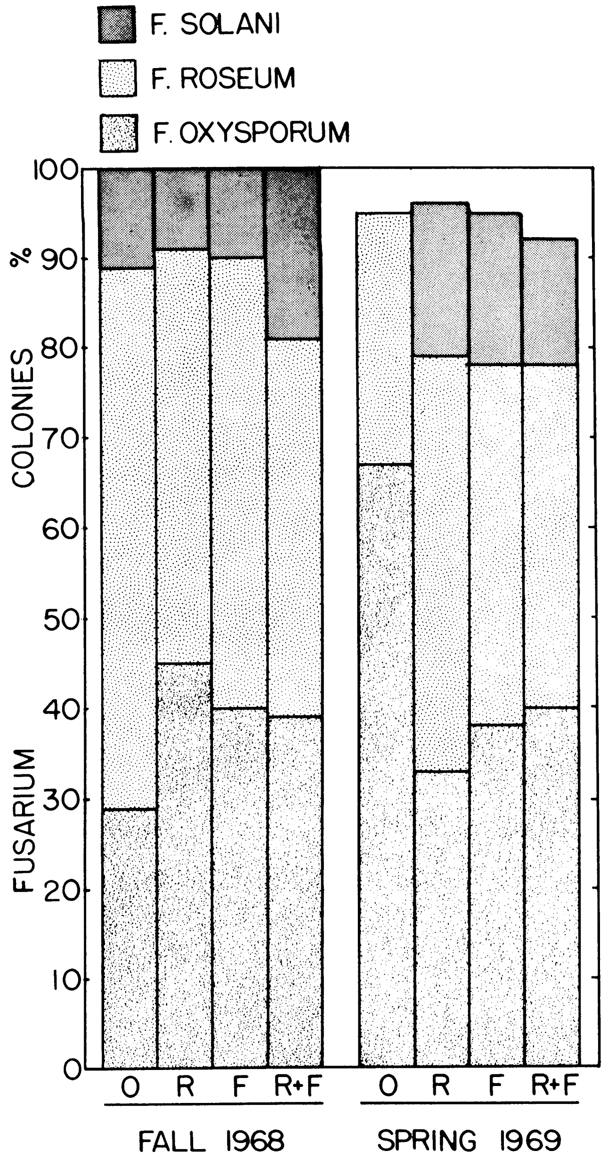

Fig. 4. The incidence of colonies of three *Fusarium* species isolated from straw residue (represented as a percentage of the total *Fusarium* colonies) in the fall of 1968 and spring of 1969 in field plots of wheat in which crop residue was removed (0) or retained (R), or in which residue was removed but fertilizer applied (F), or in which both residue was retained and fertilizer applied (R+F). The three species in 1969 did not total 100% as other species appeared in low frequency and were not recorded.

abundant than *F. oxysporum* and about equal to *F. solani* on roots, in soil, rhizosphere, and residue of wheat. Other workers have reported similar results (5, 6, 9). However, Snyder & Nash (15) found that *F. roseum* was the dominant species in the monoculture wheat plot in Rothamsted, and the number of *F. oxysporum* and *F. solani* isolates was lower.

That *F. roseum* persists in wheat residue is well known. This ability to persist in host residue is important to the survival of the pathogen, particularly where the residue does not decompose readily or where monoculture is practiced. Few studies have emphasized the ability of *Fusarium* spp. to survive in host tissue parasitized in the field. Our results show that removal of residue reduced the number of propagules of *F. roseum* that survived in Minnesota, and the retention of residue or addition of fertilizer, or both, favored survival of this species. Removal of residue could have removed inoculum of *F. roseum*; whereas retention of residue would have allowed *F. roseum* to grow and increase its inoculum potential in the residue if antagonists were absent or low in number. When residue was removed, with or without fertilizer, the population of *F. roseum* was higher in the fall than spring. This may be due to its poor ability to colonize exposed tissue in competition with other microorganisms. Cook & Bruehl (4) found that most straw that was occupied by Culmorum appeared colonized through parasitism, and that straw exposed to soil highly infested with Culmorum still yielded Culmorum primarily in the basal portions. Nyvall & Kommedahl (10) reported similar results. Thus, survival of *F. roseum* is better when residue is colonized through parasitism, and saprophytic colonization may not be significant in survival.

Isolations from roots and residue indicate that Graminearum is probably the major *F. roseum* cultivar to infect wheat in Minnesota, which is in agreement with previous experience. Avenaceum and Culmorum were also involved. Overwintering of residue decreased the proportion of wheat straws occupied by Graminearum from 40 to 24%. Thus, saprophytic colonization by Graminearum may be of minor importance in survival or multiplication of the fungus in the field. Graminearum constituted 70% of the cultivars on diseased wheat plants during a seedling blight epidemic. Thus, parasitism appears more significant than saprophytism in accounting for its occupancy of residue in the field. Chlamydospores of Graminearum are not abundant or universally obtained in the field (9, 15); therefore, survival of Graminearum may depend on alternate survival structures (hyphae and perithecia). Once colonized host tissue decomposes, population of Graminearum decreases.

Culmorum survives mainly as chlamydospores in the field (3). No perithecia have been recorded for the pathogen (15). Cook & Bruehl (4) found that straw occupied by Culmorum appeared colonized through parasitism. Also, culms exposed in the field up to 12 months to soil highly infested with Culmorum yielded Culmorum primarily from basal parts – the probable results of parasitism. This interpretation helps to explain our results where the population of Culmorum was stable during both sampling periods in the field, and is consistent with the results of Nyvall & Kommedahl (10) for these same plots. Apparently, only that part of the plant already parasitized yielded Culmorum.

Although Avenaceum does not survive as chlamydospores in the field (2), we found an increase

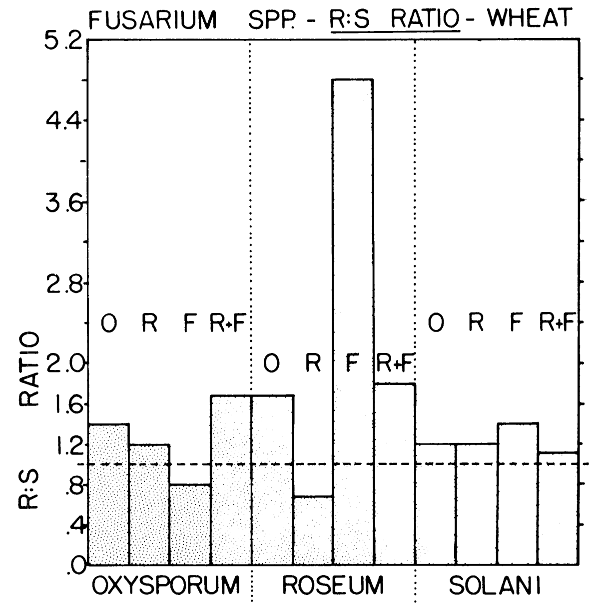

Fig. 5. The rhizosphere to soil ratio (R:S) of three *Fusarium* species in field plots of wheat in which crop residue was removed (0) or retained (R), or in which residue was removed but fertilizer applied (F), or in which both residue was retained and fertilizer applied (R+F). The broken line indicates unity where the number of colonies in the rhizosphere equaled the number in the soil.

in population (20-40%) on residue from fall to spring. Overwintering of perithecia and mycelia in residue are the primary means of survival over winter. Since ascospores are the primary inoculum infecting wheat the next growing season (2, 15), the higher population found on residue in the spring could have been attributed to ascospores.

Applications of fertilizer effected more rapid decomposition of residue and thereby reduced the population of *F. roseum* in the soil, but when the residue was retained, less decomposition and thereby less antagonism occurred so the population was greater. *Fusarium roseum*, a root inhabitant and poor colonizer of residue, survived in soil when residue was present since it occupied that substrate as a pathogen. However, *F. oxysporum*, a soil inhabitant and aggressive pioneer colonizer of tissues, remains in soil when residue was removed because it colonizes other host tissues or survives as chlamydospores. Thus, the removal of residues favors survival of *F. oxysporum* over *F. roseum*.

Of the six *Fusarium* spp. isolated from soil, rhizosphere, roots, and crop residue of wheat, *F. oxysporum* accounted for 53% of the 3,038 isolates. Two other species, *F. solani* and *F. roseum*, constituted 20% each. These are listed as the three species most frequently isolated from soil (6) and from rhizosphere and roots of cereals (16). Gordon (6) reported that these species accounted for 90% of the *Fusarium* spp. isolated from cereal-field soil in Canada, with *F. oxysporum* making up ca. 67%; *F.*

roseum, 13%; and *F. solani*, 10% of this population. Palmer & Kommedahl (11) reported that of the four species of *Fusarium* isolated from rootworm-infested roots of corn, *F. oxysporum* constituted 95% of the *Fusarium* isolates.

Fusarium oxysporum is not a primary pathogen of wheat. It is probably of minor importance in the disease complex of wheat, and then only as a wound parasite. Snyder & Nash (15), in a survey for populations of *Fusarium* spp. in soil of various fields at the Rothamsted Experiment Station, found that frequent cropping with cereals does not favor multiplication of *F. oxysporum*. However, a field cropped continuously with root crops favored survival of *F. oxysporum*. The abundance of *F. oxysporum* in different habitats is apparently due to the ability of this species to be an early colonizer of fresh organic matter; to penetrate host tissues deeply (13); to grow actively on nonhost plants (1); and to grow saprophytically on crop residues (13).

Since soil populations of *Fusarium* spp. remained fairly stable, the mechanism of action affecting population changes may be on the root surface or rhizosphere. Altering the rhizosphere or root surface microbial population by applications of fertilizer or incorporation of organic matter may aid in suppression of root rot and seedling blight of wheat. It might be concluded that incorporation of residue, addition of fertilizer, or both are more effective in reducing wheat seedling blight and incidence of root rot than the removal of the residue.

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