Use of Fluid-Drilling Gels to Deliver Biological Control Agents to Soil

KENNETH E. CONWAY, Associate Professor of Plant Pathology, Oklahoma State University, Stillwater 74078

ABSTRACT

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Use of fluid-drilling systems to deliver the biological control agents *Trichoderma harzianum* and *Laetisaria arvalis* with germinated vegetable seeds and as a slurry on apple seedlings was evaluated in field microplots. Delivery of sclerotia of *L. arvalis* with pepper seeds in gel increased stand counts in microplots containing fumigated soil infested with *Rhizoctonia solani* and significantly reduced the rate of preemergence and postemergence damping-off compared with unamended gel controls. In each of 2 yr, *L. arvalis* significantly reduced the rate of development of southern blight (*Sclerotium rolfsii*) on apple seedlings in infested plots compared with *T. harzianum* (first year) and unamended gel controls. During the first year, chlamydospores of *T. harzianum* added to gel provided complete control of southern blight for 86 days after planting, but disease subsequently progressed at a rate greater than in the unamended gel control. There were no significant differences between the biological control agents during the second year.

Fluid drilling has been used in England (4,8) and the United States (9,10,17,19,23) to deliver several types of small-seeded vegetables into soil, and several formulations of gels are commercially available. Advantages of using fluid-drilled germinated seeds include earlier and more uniform emergence and reduction of overseeding and costly thinning operations (4,17). Fungicides have been incorporated into gels to control white rot of onion caused by *Sclerotium cepivorum* Berk. (8) and damping-off of peppers (3) and tomatoes (19) caused by *Rhizoctonia solani* Kühn and *Pythium aphanidermatum* (Edson) Fitzp., respectively. Fluid-drilling techniques also provide a method for incorporating biological control organisms into soil.

Incorporation of biological control organisms into field soil has involved uneconomic bulk carriers (1,15,18,22,24), seed treatments (12,13,22), and direct addition of propagules without carriers to soil (16,18). Wells et al (24) incorporated *Trichoderma harzianum* Rifai into soil, using annual ryegrass seeds as an organic carrier. Backman and Rodriguez-Kabana (1) developed a more economical carrier system for *T. harzianum*, using a diatomaceous earth granule impregnated with 10% molasses solution. Granules were incorporated at a rate of 112–140 kg/ha and were applied twice during the growing season to control *S. rolfsii* Sacc. in peanut. Although this system had advantages over others, it still required a large amount of carrier and additional field applications. Harman et al (12,13) used *T. hamatum* (Bon.) Bain suspended in Methocel (Dow Chemical Co., Midland, MI) as a seed treatment to control damping-off of radish and pea caused by *R. solani* and *Pythium* spp., respectively, in the greenhouse. Ruppel et al (22) reported that seed treatment with *T. harzianum* (= *T. hamatum* of Harman et al [12,13]) was effective in New York when applied to snap bean, field corn, pea, soybean, and squash but not in Colorado when used on sugar beet seeds. Seed treatment of sugar beet seeds was less effective in reducing root rot caused by *R. solani* than in-row incorporation of *T. harzianum* on either a clay granule or wheat bran.

Odvody et al (18) reported that *Laetisaria arvalis* Burdsall was more effective in controlling *R. solani* when added to soil in a sugar beet pulp mixture than when added to soil as sclerotia alone. Martin et al (16) made similar conclusions but questioned whether large amendments of *L. arvalis* would be economically feasible for a single season or crop.

This paper reports the use of fluid-drilling gels to incorporate *T. harzianum* and *L. arvalis* into soil. It also reports the efficacy of biocontrol agents applied in fluid-drilling gels to control damping-off of Bahamian hot peppers caused by *R. solani* and southern blight of apple seedlings caused by *S. rolfsii*.

MATERIALS AND METHODS

***Laetisaria*/pepper.** Experiments were conducted in 1983 at Stillwater, OK, in 16 raised microplots (2.1 × 2.1 m) filled to a depth of 20 cm with sandy loam soil (pH

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5.7–6.3). NPK and Fe contents were 25, 60, 180, and 11 μg/g, respectively. Microplots were fumigated with methyl bromide (Dowfume MC-2) at 147.8 g/m^2 before use. Plastic coverings were removed from microplots 5 days before planting.

L. arvalis (Zh-1), obtained from the Forest Products Laboratory, Madison, WI, was grown at 25 C for 4 wk on autoclaved oat seeds. The culture was dried at room temperature, and sclerotia were removed from seeds by sieving through a 1-mm screen onto a 125-μm screen. Sclerotia (≥300 μm) were added to Polysurf-C gel (1.5%) (hydrophobically modified hydroxyethyl cellulose; Hercules, Inc., Wilmington, DE) at a density of 100 sclerotia per milliliter of gel.

Eight microplots were infested with a cornmeal-sand culture of *R. solani* (AG-4) 1 day before planting. This culture was incorporated into soil to a depth of 7.5 cm at a rate equivalent to a 2% (v/v) mixture. Treatments consisted of germinated pepper seeds (*Capsicum annuum* L.) in: gel amended with *L. arvalis* and incorporated into *R. solani*-infested soil, gel amended with *L. arvalis* and added to noninfested soil, unamended gel added to *R. solani*-infested soil, and unamended gel added to noninfested soil (control). Forty milliliters of gel containing 66 seeds was extruded through a small hole in a plastic bag into the 2-m furrow and covered loosely with soil. Four rows of peppers were planted in each microplot. Two outer rows were untreated guard rows, as were several plants at each end of the inner rows. The two inner treatment rows were 2 m long. Experimental design was a 4 × 4 Latin square. Stand count for each row was recorded during the first month. Soil samples were removed within a 2.5-cm radius of plants from each inner row 16, 30, and 44 days after planting and assayed for sclerotial density of *L. arvalis* and *R. solani* with a soil-pellet sampler (14) and selective media of Papavizas et al (21) and Doornick (5), respectively.

Stand count for each row was recorded on a 2- to 3-day schedule for 33 days after planting, and data from treatments were statistically analyzed using Duncan's multiple comparison on means and their rank transformations (2). Fractions (x) of plants infected were determined using the formula $1 - A/B$, where A = number of emergent plants per row and $B = 66$ seeds planted per row. Fractions were transformed to $\log_e 1/1 - x$ to adjust for multiple infections (11) and were plotted against time. Slopes of disease progress, as determined by linear regression analysis, were statistically analyzed using a "dummy" variable multiple regression model (6).

***Trichoderma* and *Laetisaria*/apple seedlings.** Efficacy of *T. harzianum* and *L. arvalis* to suppress southern blight, caused by *S. rolfsii*, in apple seedlings was evaluated during 1984 and 1985 in raised microplots (as described). A strain of the original isolate of *T. harzianum* from Wells et al (24) was obtained from Abbott Laboratories, Chicago, IL. Chlamydospores of *T. harzianum* were prepared as a molasses-wheat bran fermentation product (20) and suspended in the gel at 10^6 propagules per milliliter of gel. Sclerotia of *L. arvalis* produced on potato-dextrose broth were washed with tap water on a 250-μm screen and air-dried. Sclerotia were suspended in gel at 1 g of air-dried sclerotia per 120 ml of gel. Dry preparations of each biocontrol agent were rehydrated in water for 30 min before they were added to Polysurf-C gel.

Soil in the microplots had been treated with metam-sodium (Vapam) on 31 October 1983 and had remained exposed to natural conditions until planting on 23 April 1984. No soil treatment (fumigation) was used during 1984 or 1985. Sclerotia of *S. rolfsii* were produced on autoclaved oat seed, air-dried, and incorporated into soil of three planting rows in each microplot at 6.4 g of dried oat seed-sclerotia mix per row 6 days before planting. A fourth row was not infested with *S. rolfsii*. Inclusion of a noninfested row was not possible in the experimental design for the 1985 evaluation.

Each biocontrol agent was prepared in 360 ml of Polysurf-C and applied as a slurry to the total length (about 20 cm) of 72 common apple rootstock seedlings (Greenleaf Nursery, Park Hill, OK). Controls consisted of seedlings treated with unamended Polysurf-C gel. Nine trees of each treatment were planted per row, and treatments were randomly arranged in each microplot. Each treatment was replicated eight times. Eight trees were planted per row in the 1985 evaluation.

Southern blight was confirmed on dead or dying seedlings by the presence of white mycelium and/or sclerotia on the crown. If necessary, seedlings were removed, washed, and incubated in moist chambers to produce these structures. Disease was monitored for 184 days. Soil samples were removed at 1-, 4-, 8-, and 12-wk intervals during 1984 from within a zone 4–5 cm from selected trees from each treatment and were analyzed for populations of *L. arvalis* and *T. harzianum*. The assay for sclerotia of *L. arvalis* was similar to that described for peppers. Soil from the *T. harzianum* treatment was bulked for each replicate. A subsample of each soil was diluted through a water blank series and plated on a *Trichoderma*-selective medium (7).

RESULTS

***Laetisaria*/pepper.** Sclerotia of *L. arvalis* delivered in a gel carrier reduced disease caused by *R. solani* and significantly increased the stand of pepper seedlings in plots infested with *R. solani* (Table 1). The use of *L. arvalis* as an amendment to gel in the absence of disease pressure had no adverse effect on stand of pepper compared with the control treatment. When *L. arvalis* was applied in the gel in the presence of *R. solani*, there was a significant decrease in preemergence and postemergence damping-off of pepper seedlings as indicated by lower intercept and slope values for regression lines compared with the unamended gel/*R. solani*-infested treatment (Fig. 1). Densities of viable sclerotia of *L. arvalis* in plots infested with *R. solani* were 3.9, 2.6, 5.3, and 0/100 g of soil at 16, 30, 44, and 126 days after planting.

The slope of disease progress in the plots infested with *R. solani* and planted with unamended gel was significantly greater than in the other treatments (Fig. 1). Even though all microplots had been fumigated, recontamination by *R. solani* in noninfested plots was confirmed in soil bioassays 16 days after planting.

***Trichoderma* and *Laetisaria*/apple seedlings.** In 1984, *S. rolfsii* was first noted in the microplots at the bases of dying trees 30 days after planting, and counts of dead trees began 34 days later. Densities of *T. harzianum* and *L. arvalis* recovered from around selected trees never exceeded 10^3 propagules and four sclerotia, respectively, per gram of soil. Densities of *L. arvalis* were greatest during the first week after planting and were still recovered at very low densities

Table 1. Stand count of pepper fluid-drilled in unamended and *Laetisaria arvalis*-amended gels and sclerotial density of *L. arvalis* in microplots containing fumigated soil or soil infested with *Rhizoctonia solani*

Treatment (gel)[w]	Soil[x]	Mean stand count/2-m row[y]	Mean rank of stand[z]	Sclerotial density of *L. arvalis*/100 g of soil[y]
Unamended	F	23.5	22.4 a	0.0
L. arvalis	F	24.8	21.7 a	5.0
L. arvalis	I	15.9	17.4 a	2.6
Unamended	I	1.4	4.5 b	0.0

[w] Unamended consisted of 1.5% Polysurf-C fluid-drilling gel. *L. arvalis* was added to gel at 100 sclerotia per milliliter of gel.
[x] F = fumigated soil and I = soil infested with *R. solani* cornmeal-sand mixture (2%, v/v).
[y] Stand count and sclerotial density determined 1 mo after planting, means of eight replicates.
[z] Mean ranks followed by different letters are significantly different as determined by Duncan's multiple comparison of rank transformations ($P = 0.05$).

of less than one sclerotium per gram of soil at the 12-wk sampling period. Percentages of mortality of apple seedlings 184 days after planting in soils amended with *L. arvalis* or *T. harzianum* or unamended were 6, 17, and 17, respectively. Percentages of mortality were transformed to adjust for multiple infections (11) and plotted against time (Fig. 2). Chlamydospores of *T. harzianum* applied in a slurry to apple seedlings controlled southern blight for 86 days after planting because no disease was recorded during this time. Efficacy of this treatment was lost after this period, and disease progressed at a rate (slope 0.0015) greater than the control treatment (slope 0.0009). Addition of *L. arvalis* to gel used as a slurry treatment on apple seedlings at planting significantly reduced the progress of southern blight compared with the control during the period 64–184 days after planting. Slopes of disease progress determined by linear regression analysis were 0.0003 and 0.0009 for the *L. arvalis* treatment and the unamended control, respectively. Slope values determined by multiple regression analysis (6) of disease progress for the three treatments were highly significantly different at $P = 0.01$.

In 1985, southern blight was first noticed on the apple seedlings 21 days after planting, and weekly counts of dead trees were begun at that time. Percentages of mortality 182 days after planting were 24.2, 18.2, and 34.8 in the *T. harzianum, L. arvalis,* and unamended gel treatments, respectively. Data transformation and analyses were the same as performed for 1984 data. Disease progress was significantly reduced for both biological treatments compared with the unamended control (Fig. 3). There was no significant difference in efficacy between the biocontrol agents for control of southern blight.

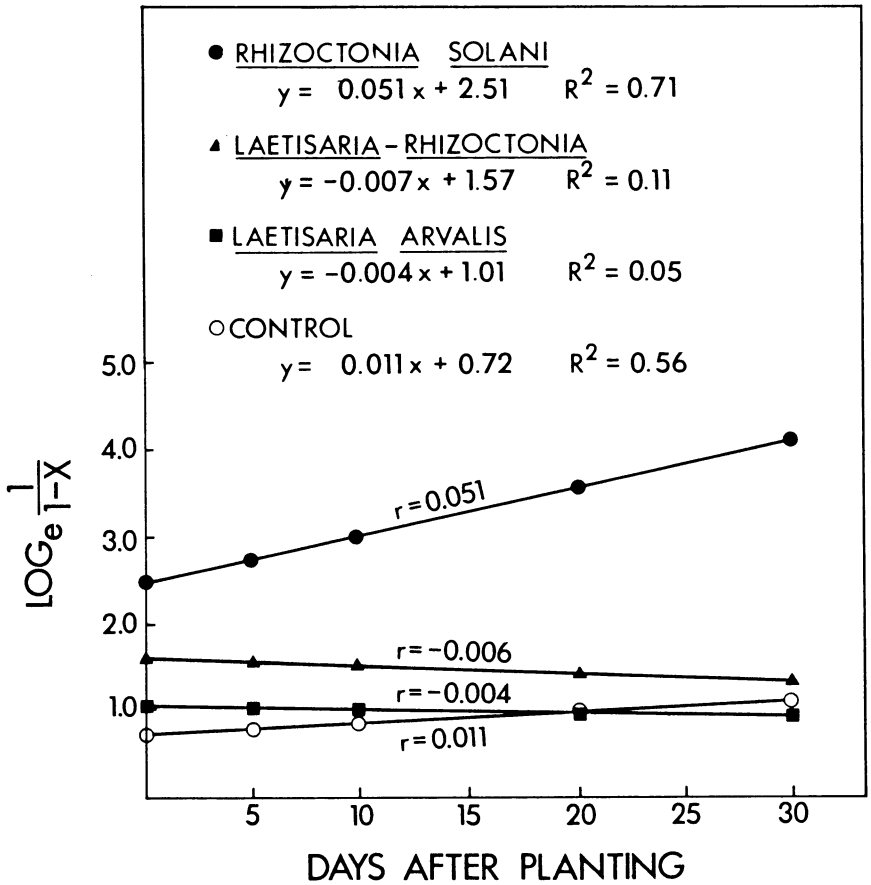

Fig. 1. Regression lines of the relationship of the biological control agent *Laetisaria arvalis* applied to soil in a fluid-drilling gel with germinated seeds of Bahamian hot pepper to the incidence of damping-off (x), adjusted for multiple infections, of *Rhizoctonia solani* from 0 to 30 days after planting. The slope value for *R. solani* is significantly greater than those for the other treatments ($P = 0.01$).

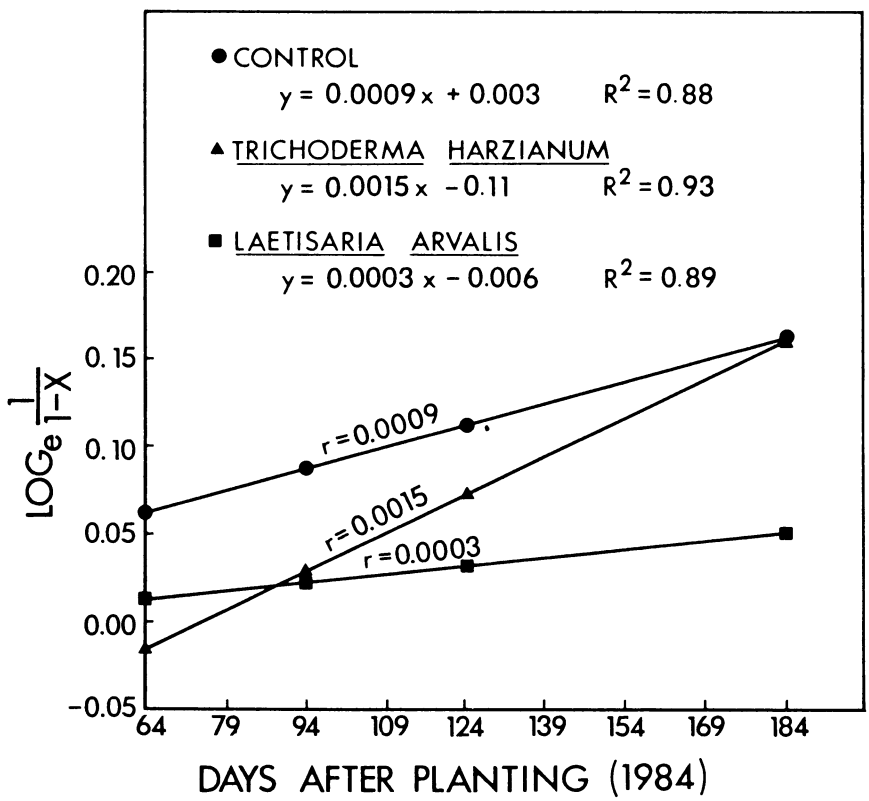

Fig. 2. Regression lines of the relationship of biological control agents *Laetisaria arvalis* and *Trichoderma harzianum*, applied to apple seedlings in a gel slurry, to the incidence of infection (x), adjusted for multiple infections, of *Sclerotium rolfsii* from 64 to 184 days after planting during 1984. All slope values are highly significantly different ($P = 0.01$).

DISCUSSION

In 1984, *T. harzianum*, prepared primarily as a chlamydosporic fermentation product, controlled southern blight of apple seedlings for up to 86 days after planting. Percent disease incidences prior to that time were 0, 1.4, and 6.9 for the *T. harzianum, L. arvalis,* and control treatments, respectively. This delay and sudden increase in disease in *T. harzianum* treatments did not occur in 1985. Addition of sclerotia of *L. arvalis* to gel provided the best disease reduction in our systems using pepper and apple seedlings.

The low coefficients of correlation of disease progress lines for *L. arvalis* and controls in pepper indicated that there was no correlation between postemergence damping-off and these treatments during the time of evaluation. The percent stand for gel seeding of 30–60% compares very favorably with 10% or less for dry seeding.

Papavizas et al (21) recovered, on their *L. arvalis*-selective medium, an unidenti-

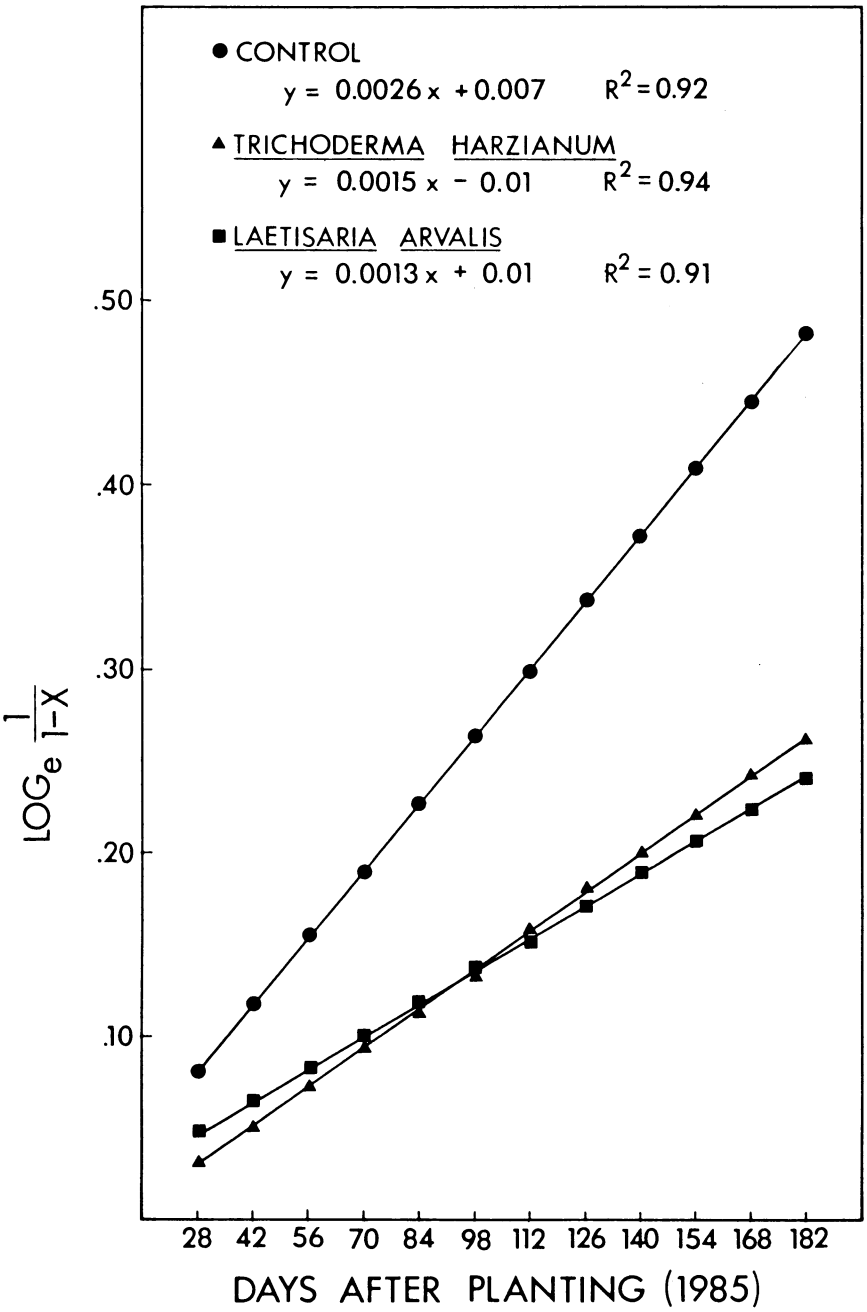

Fig. 3. Regression lines of the relationship of biological control agents *Laetisaria arvalis* and *Trichoderma harzianum*, applied to apple seedlings in a gel slurry, to the incidence of infection (x), adjusted for multiple infections, of *Sclerotium rolfsii* for 182 days after planting during 1985. Slope values for biological control treatments are significantly different from control ($P = 0.01$).

fied, nonsporulating fungus with orange sclerotia and hyphae lacking clamp connections. I have also recovered this orange sclerotial fungus and another basidiomycetous fungus with their medium and have identified them as *R. zeae* Voorhees and *Coprinus* sp., respectively. Care should be taken when enumerating *L. arvalis* on this selective medium (21), and I concur with the authors that when in doubt, transfer of colonies to potato-dextrose agar or similar growth media is a necessity to confirm the identity of *L. arvalis*.

The original objective of this research was not to compare the efficacy of these biocontrol agents but to determine whether fluid-drilling gels could be used to apply effective quantities of these organisms either with germinated seeds or to apple seedlings to control soilborne diseases. Although populations of both *T. harzianum* and *L. arvalis* could be recovered from soil within their respective treatments, their densities, as determined by our sampling procedures, generally never reached quantities that other researchers have considered necessary for biological control (13,16). Removing soil samples closer to the trees (<4 cm) in the vicinity where the biocontrol agents had been applied was avoided to prevent injury to the root systems, which may have provided for artificial entry into the trees by the pathogen. However, the reduction of disease in the biocontrol treatments indicated that effective densities of each agent were present. In each of three years, significant reduction of soilborne diseases was achieved using gels as carriers for biocontrol agents compared with unamended gel treatments.

Research is needed to identify and produce the most effective forms of these agents (such as conidia, chlamydospores, or sclerotia). The effects of organic or chemical amendments on the efficacy of biocontrol agents delivered in gels should also be investigated. As gel seeding technologies develop and improved equipment becomes available, the use of gels to deliver biological control agents concomitant with germinated vegetable seeds or as a slurry to rootstock offers an economical and efficient method of applying these agents to the field.

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