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# Use of Nematodes as Biomonitors of Nonfumigant Nematicide Movement through Field Soil[1]

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*Abstract:* Three field experiments were established in a loamy sand soil in the Coastal Plain of North Carolina to determine downward movement of aldicarb and fenamiphos with a nematode bioassay. Penetration of bioassay plant roots by *Meloidogyne incognita* was measured at 1, 3, 7, 14, 21, and 28 days after treatment in the greenhouse as a means of determining nematicide effectiveness. Chemical movement was similar in planted and fallow soil. Nematicidal activity was greater in soil collected from the 0 to 10 cm depth than from the 10 to 20 cm depth. Fenamiphos suppressed host penetration by the nematode more than aldicarb under the high rainfall (19 cm) and low soil temperatures that occurred soon after application in the spring. During the summer, which had 13 cm precipitation and warmer soil temperatures, both chemicals performed equally well at the 0 to 10 cm depth. At the lower soil level (10 to 20 cm), aldicarb limited nematode penetration of host roots more quickly than fenamiphos. Both of these chemicals moved readily in the sandy soil in concentrations sufficient to control *M. incognita*. Although some variability was encountered in similar experiments, nematodes such as *M. incognita* have considerable potential as biomonitors of nematicide movement in soil.

*Key words:* aldicarb, chemical movement, fenamiphos, *Glycine max*, *Meloidogyne incognita*, nematicide, nematode, root-knot nematode, soybean.

A nonfumigant organophosphate, fenamiphos, and a carbamoyl oxime, aldicarb, are among the current options for managing nematodes on numerous crops in North Carolina, especially in the Coastal Plain (20). The ease and timing of application, an adequate degree of nematode control, and the loss of effective fumigants were factors that led to a steady increase in the use of nonfumigant nematicides (9). Application of these nematicides, however, has declined recently, partially because of increasing costs of production and low prices of many crops.

The physical properties of aldicarb result in downward movement through soils mainly by mass flow (10). Fenamiphos has low volatility ($1 \times 10^{-6}$ mm Hg at 25 C) and is moderately soluble in water (400 µg/ml at 25 C) (13). Aldicarb has moderate to high volatility ($1 \times 10^{-4}$ mm Hg at 25 C) (12), is highly soluble in water (6,000 µg/ml at 10 C and 9,000 µg/ml at 30 C) (13), and moves readily in soil water (9).

Both chemicals rapidly oxidize in soil to sulfoxide and then to sulfone (2,10). Fenamiphos sulfone is further oxidized to the des-isopropyl form, which is not toxic to nematodes (2). Some aldicarb degradation products (such as its sulfoxide and sulfone) retain the *N*-methylcarbamate group and are toxic to vertebrates and invertebrates. Aldicarb sulfoxide is thought to be the main toxic moiety in soils and plants (2,10). Loss of the carbamate group by hydrolysis or by other processes inactives aldicarb and its toxic metabolites (4). Half-life under field conditions for aldicarb, fenamiphos, and their toxic degradation products is approximately 1 month (2,15).

Soil physical and chemical characteristics, method of application, time of application, amount of rainfall and (or) irrigation, properties of nematicides, and activity of associated microflora affect movement and persistance of these materials under field conditions (10,18,19). For example, furrow or sprinkler irrigation produces a distribution pattern different than that found with natural rainfall (9). Fenamiphos and aldicarb impair nematode neuromuscular activity, thereby limiting nematode movement, invasion, feed-

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ing, rate of development, and reproduction (21). The nematicidal activity of organophosphates and carbamoyl oximes is largely due to their inhibition of acetylcholinesterase and cholinesterase (8,16, 18).

Information on the relative amount and vertical distribution of biologically active nematicides and their degredation products in field soils could lead to a more efficient use of these chemicals. Timing the availability of maximum nematicide concentrations should coincide with the onset of feeding by nematodes. The objective of this study was to investigate the feasibility of monitoring vertical movement of active nematicidal materials in field soil using root-knot nematode penetration as a bioassay.

## Materials and Methods

Three experiments were conducted in a field located at the Central Crops Research Station near Clayton, North Carolina. This site was selected because it was free of major pathogenic nematodes. Soil texture was 91% sand, 7% silt, and 2% clay in the 0 to 10 cm soil zone; and 90% sand, 7% silt, and 3% clay in the 10 to 20 cm soil zone. Humic matter was 0.3% and pH was 5.2 at both levels. Plot size was one row 7.6 m long with 1-row buffers and rows spaced 1.1 m apart.

Soil was prepared by conventional tillage. Nematicide applicators were mounted in a commercial planter and consisted of jars with openings in the lids to allow the desired flow rate at a ground speed of 4.8 km/hour. Fenamiphos and aldicarb were applied onto the soil surface in an 18-cm-wide band between the seed furrow opener and a press wheel at planting. Within 24 hours after chemical application, irrigation was applied with overhead sprinklers. Thereafter, rainfall was the only source of water. Total precipitation was recorded with a rain gauge. Soil samples for the 0 to 10 cm depth nematode assay were collected in rows with a 7.6-cm-d soil bucket auger. Soil samples for the 10–20 cm depth were extracted from the same core hole, but with a 5.0-cm-d soil bucket auger to avoid contamination. Three cores were collected at 2.4-m intervals within the treated band at 0–10-cm and 10–20-cm depths and were composited for each plot one day before, at planting, and at 1, 3, 7, 14, 21, and 28 days after planting. Untreated soil samples were collected at each sampling interval for the controls. A 3-day sample was not collected for experiment 1. Samples were taken to the laboratory immediately after each sampling and frozen at −18 C. When sampling was completed, nematode inoculum for biosassay was prepared.

All soil was defrosted and air dried in the greenhouse for 48 hours (10). *Meloidogyne incognita* (Kofoid & White) Chitwood was cultured on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') for inoculum. Eggs were extracted from infected tomato roots with a modified sodium hypochlorite extraction technique (11) and were hatched on screens with 25.5 μm pores. Juveniles (J2) that hatched during the first 24 hours were discarded. Nematodes that hatched within the next 24 hours were suspended in water (100 J2/ml).

For bioassay plants, seeds of 'Lee 68' soybean (*Glycine max*) were soaked for 5 minutes in tap water and germinated in a container of moist sterile vermiculite enclosed in a plastic bag at 27 C. Three hundred grams of soil from each soil sample were placed in 9-cm-d styrofoam containers and infested with the freshly hatched J2. The 42-hour-old seedlings with 3-cm long radicals were positioned in funnel-shaped depressions created by removing 100 grams of soil. Aliquants of 500 *M. incognita* J2 in 10 ml tap water were placed on the seedling roots. Roots and inoculum were covered immediately with the previously removed soil. Water was added to each container to bring the soil to field capacity (−0.03 MPa). Twenty grams of perlite were placed over the exposed soil surface to retard evaporation. The containers were then placed in the greenhouse (with ambient air temperatures ranging from 25 to 31 C) for 48 hours.

The soybean roots were harvested at 48 hours after inoculation and the amount of J2 penetration was determined (6). Roots were placed in glycerine in inverted plastic petri dishes, flattened with slight pressure, and examined with a stereomicroscope. Nematode counts were converted to percentages of juveniles penetrating roots of plants in pesticide-treated soil versus those in untreated soil.

*Experiment 1:* This study was designed to evaluate the influence of the plant on chemical movement. The field was divided into plots that were left fallow and plots that were planted with soybeans. Aldicarb 15 G and fenamiphos 15 G were applied at 0 and 0.67 g a.i./$m^2$, and 'Delta Pine 105' soybean was planted on 23 May 1988. On the following day, 1.1 cm of water was applied via irrigation followed by 4.5 cm of natural rainfall within the next 24 hours.

*Experiment 2:* This study was designed to further characterize chemical behavior of aldicarb and fenamiphos in soils warmer than that at the typical soybean planting time of May, as was done in experiment 1. Aldicarb and fenamiphos were applied at 0, 0.67, and 1.34 g a.i./$m^2$ of soil on 18 July 1988 at planting of Delta Pine 105 soybean. Approximately 1.7 cm of water was applied the following day through sprinkler irrigation.

*Experiment 3:* The dosage rates of aldicarb were expanded in order to determine a dosage response. Aldicarb was applied at 0, 0.34, 0.67, and 1.34 g a.i./$m^2$ of soil on 23 September 1988. Later in the day, 1.3 cm of water was applied. Soil samples from the 0.67 g a.i./$m^2$ treatment were analyzed at Rhône-Poulenc (Research Triangle Park, North Carolina) for aldicarb and its sulfoxide and sulfone; the detection limit was 1 ng/ml of product. The aldicarb residue concentrations in soil were measured with a Tracor gas chromatograph fitted with a glass column (1.0 mm × 180 cm) containing 15% OV-1 on 80–100 mesh Gas Chrom Q. Nitrogen (80 ml/minute) was the carrier gas, and the injector, column, and detector temperatures were 200, 180, and 200 C, respectively. Soil samples analyzed for actual chemical content were those collected at 0, 1, 3, 7, 14, 21, and 28 days after treatment in the 0–10-cm depth and at 7 and 28 days after treatment in the 10–20-cm level.

*Statistics:* Each experiment was conducted in a randomized complete block design with four replicates. Data were analyzed by analysis of variance for a factorial arrangement of treatments. Means were compared with the Waller–Duncan Bayesian k-ratio *t*-test (k = 100).

## Results

*Experiment 1:* Nematicidal activity was similar ($P = 0.05$) between soybean planted and fallow plots at the 0–10-cm and 10–20-cm depths; thus, only data for planted plots are given (Table 1). Fenamiphos was more effective than aldicarb at all dates at the 0–10-cm soil section based on J2 penetration of host roots. At the lower soil depth, aldicarb had little effect on suppressing host penetration, except at 28 days after treatment. Fenamiphos did affect a reduction in the relative number of nematodes penetrating the bioassay roots on most dates, but it was not nearly as effective as in the upper soil level. The total precipitation was 18.8 cm (Table 2), and the average soil temperature at 10 cm was 24.2 C (Fig. 1) during the course of this experiment.

*Experiment 2:* Aldicarb and fenamiphos were similar in their influence on limiting penetration of bioassay soybean roots by *M. incognita* J2 at the 0–10-cm depth (Table 3). The average penetration for the 28-day posttreatment period ranged from <1 to 2%. Greater J2 penetration of roots occurred in soil collected from the 0–20-cm depth than that from the shallower soil depth. The nematicidal activity at the lower soil depth in the aldicarb treatment was greater than with fenamiphos. Relative penetration of roots by J2 in the 1.34 g a.i./$m^2$ soil in the aldicarb treatment averaged 38% of the control, which was less ($P = 0.05$) than fenamiphos at 0.67 and 1.34 g a.i./$m^2$ soil, with 101 and 67% relative

TABLE 1. Detection of aldicarb and fenamiphos applied at 0.67 g a.i./m$^2$ in planted field soil, with penetration of Lee 68 soybean (*Glycine max*) by second-stage juveniles of *Meloidogyne incognita* as a bioassay (experiment 1).

| Days after treatment | Percent host penetration compared to control† | | | |
|---|---|---|---|---|
| | (0–10 cm deep) | | (10–20 cm deep) | |
| | Aldicarb | Fenamiphos | Aldicarb | Fenamiphos |
| Pre‡ | 146 a | 198 a | 69 a | 69 a |
| 0 | <1 c | <1 c | 99 a | 52 b |
| 1 | 20 b | 6 b | 168 a | 177 a |
| 7 | 15 b | 5 b | 100 a | 87 a |
| 14 | 11 c | 4 d | 92 a | 61 b |
| 21 | 21 c | <1 d | 132 a | 70 b |
| 28 | 32 b | 0 c | 36 b | 49 a |
| Mean | 17 cd | 3 d | 105 a | 83 b |

† Percentage of host penetration by juveniles was computed from a paired untreated control in which an average of 8% of the applied juveniles penetrated roots.

‡ The pretreatment was not included in the mean for each chemical treatment.

Numbers followed by same letter in rows (within date) are not different ($P$ = 0.05) according to the Waller–Duncan Bayesian k-ratio $t$-test (k = 100). Data are means of four replications.

TABLE 2. Precipitation for period of 24 May to 21 June 1988 (experiment 1), 18 July to 16 August 1988 (experiment 2), and 23 September to 28 October 1988 (experiment 3).

| Days after treatment | Precipitation (cm) | | |
|---|---|---|---|
| | Experiment 1 | Experiment 2 | Experiment 3 |
| 0 | 5.6 | 1.7 | 1.4 |
| 1 | | | 1.7 |
| 2 | | 3.2 | |
| 3 | | | |
| 4 | | 0.7 | |
| 5 | | 1.1 | |
| 6 | | | |
| 7 | | 0.6 | |
| 8 | | | |
| 9 | 0.8 | | |
| 10 | | | |
| 11 | 0.1 | 1.9 | 1.2 |
| 12 | | | 0.1 |
| 13 | | | |
| 14 | | | |
| 15 | | 1.3 | |
| 16 | 3.1 | | |
| 17 | | | |
| 18 | | | |
| 19 | | 0.5 | |
| 20 | | | |
| 21 | | | |
| 22 | | | |
| 23 | | | |
| 24 | 9.2 | | |
| 25 | | | |
| 26 | | 1.9 | 3.0 |
| 27 | | | 0.2 |
| 28 | | | |

penetration, respectively. By the third day, some nematicidal activity in the aldicarb-treated plots occurred in the 10–20-cm soil zone, resulting in reduced penetration of the bioassay plants from that date to the end of the experiment. The most effective suppression of root penetration at this depth by J2 with fenamiphos was at 21 days at the highest concentration tested (Table 3). Cumulative precipitation was 12.9 cm (Table 2); the average soil temperature during this experiment was 29.8 C (Fig. 1).

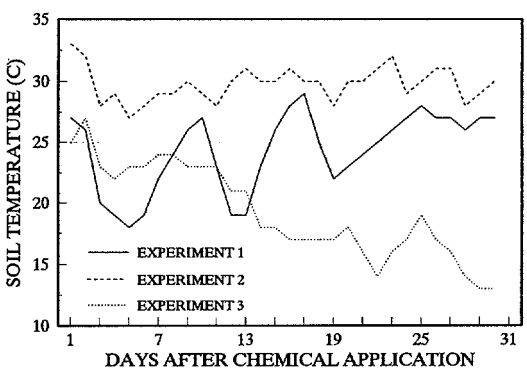


FIG. 1. Average soil temperature at 10 cm at the Central Crops Research Station near Clayton, North Carolina, for periods 24 May to 21 June 1988 (experiment 1), 18 July to 16 August 1988 (experiment 2), and 23 September to 23 October 1988 (experiment 3).

TABLE 3. Bioassay for aldicarb and fenamiphos in field soil, with penetration of Lee 68 soybean (*Glycine max*) by second-stage juveniles of *Meloidogyne incognita* (experiment 2).

| Days after treatment | Percentage of host penetration compared to control† | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (0–10 cm deep) | | | | (10–20 cm deep) | | | |
| | Aldicarb | | Fenamiphos | | Aldicarb | | Fenamiphos | |
| | 0.67 g | 1.37 g | 0.67 g | 1.37 g | 0.67 g | 1.37 g | 0.67 g | 1.37 g |
| Pre‡ | 65 a | 57 a | 85 a | 93 a | 107 a | 127 a | 155 c | 105 a |
| 0 | 0 b | 0 b | 1 b | 0 b | 86 a | 52 a | 85 a | 58 a |
| 1 | 0 c | 0 c | 1 c | 0 c | 122 ab | 157 a | 129 ab | 120 ab |
| 3 | 5 c | 4 c | 3 c | 0 c | 45 b | 33 bc | 117 a | 103 a |
| 7 | 4 b | 0 b | 0 b | 1 b | 8 b | 3 b | 68 a | 91 a |
| 14 | 1 b | 0 b | 2 b | 0 b | 28 b | 6 b | 113 a | 29 a |
| 21 | 3 c | 1 c | 0 c | 0 c | 18 b | 5 c | 64 a | 11 c |
| 28 | 3 d | 1 d | 0 d | 0 d | 17 cd | 9 cd | 131 a | 58 bc |
| Mean | 2 e | 1 e | 1 e | <1 e | 46 cd | 38 d | 101 a | 67 c |

† Percentage of host penetration by juveniles was computed from a paired untreated control in which 10% of the applied juveniles penetrated roots. Chemical rates are expressed as g a.i./$m^2$ of soil.

‡ The pretreatment was not included in the mean for each chemical treatment.

Numbers followed by the same letter in rows (within date) are not different ($P = 0.05$) according to the Waller–Duncan Bayesian k-ratio *t*-test (k = 100). Data are means of four replications.

*Experiment 3:* All rates of aldicarb treatment resulted in the effective suppression of relative penetration by J2 of bioassay plants in soil samples from the upper soil profile (Table 4). At 0.348 g a.i./$m^2$, the average penetration of all posttreatment time periods was 7% of the control. The two highest rates of aldicarb were very effective (<2% relative penetration compared to the control). At the lowest rate (0.348 g a.i./$m^2$), the activity decreased by day 7, whereas the chemical was highly effective through 28 days at 1.34 g a.i./$m^2$.

At the 10–20-cm depth from day 14 to day 28, penetration of roots by J2 was higher following the 0.34 g a.i./$m^2$ treatment than after the 0.67 or 1.34 g a.i./$m^2$ treatments (Table 4). Penetration of roots by J2 was higher at the 10–20-cm depth than at the shallower depth in all sampling times except at day 28. At 0.34 g a.i./$m^2$, the lowest root penetration (31% of the

TABLE 4. Penetration of Lee 68 soybean (*Glycine max*) by second-stage juveniles of *Meloidogyne incognita* as a bioassay for aldicarb activity (experiment 3).

| Days after treatment | Percentage of host penetration as compared to control† | | | | | |
|---|---|---|---|---|---|---|
| | (0–10 cm deep) | | | (10–20 cm deep) | | |
| | 0.34 g | 0.67 g | 1.34 g | 0.34 g | 0.67 g | 1.34 g |
| Pre‡ | 126 a | 80 a | 176 a | 69 a | 89 a | 126 a |
| 0 | 0 a | 0 a | 0 a | | | |
| 1 | 1 b | 3 b | 1 b | 76 a | 93 a | 86 a |
| 3 | 1 c | 1 c | 0 c | 134 a | 60 bc | 54 bc |
| 7 | 5 b | <1 b | 0 b | 31 ab | 33 ab | 35 a |
| 14 | 13 b | 1 b | 0 b | 101 a | 23 ab | 18 b |
| 21 | 9 c | 1 c | 0 c | 145 a | 31 b | 15 b |
| 28 | 14 b | 4 b | <1 b | 92 a | 4 b | 8 b |
| Mean | 7 c | 2 c | <1 c | 96 a | 42 b | 36 b |

† Percentage of host penetration by juveniles was computed from a paired untreated control in which 9% of the applied juveniles penetrated roots. Aldicarb rates are expressed as g a.i./$m^2$ soil.

‡ The pretreatment was not included in the mean for each chemical treatment.

Numbers followed by same letter in rows (within date) are not different ($P = 0.05$) according to the Waller–Duncan Bayesian k-ratio *t*-test (k = 100). Data are means of four replications.

control) by J2 occurred at day 7, and there was little or no nematicidal activity observed thereafter. The lowest amount of J2 root penetration associated with higher nematicide concentrations occurred in soil collected at day 28.

The concentration of aldicarb, aldicarb sulfoxide, and aldicarb sulfone in the 0–10-cm depth ranged from 1,400 ng/g of soil at the time of chemical application to 1,150 ng/g of soil at 28 days after treatment (Table 5). Concentrations of aldicarb, aldicarb sulfoxide, and aldicarb sulfone greater than 1,000 ng/g inhibited penetration of roots by J2 to less than 5% of the control. At the 10–20-cm depth, the concentration of aldicarb plus its sulfoxide and sulfone at day 7 (23 ng/g of soil) allowed a 33% relative penetration frequency. The chemical concentration increased to 179 ng/g of soil by day 28, which restricted penetration to 4% of the control. Total precipitation recorded during the course of this experiment was 7.4 cm (Table 2), and average soil temperature was 19 C (Fig. 1).

## Discussion

Host penetration of roots in aldicarb- and fenamiphos-treated soils differed among the field experiments. Key environmental factors most likely influencing the behavior of these chemicals were moisture, temperature, and their influence on soil microorganisms (19). The fairly consistent rainfall during the first 14 days of experiment 2 may account in part for the slightly greater reduction in root penetration of the bioassay plants at the lower depth than in either experiment 1 or 3. However, soil temperatures are also warmer during this midsummer period. Thus, to evaluate the impact of these two important environmental factors, experiments must be designed to measure their influence precisely. However, to assure activity, the manufacturer of fenamiphos recommends that band or broadcast applications be incorporated into soil to facilitate its downward movement (2) and to minimize its exposure to nontarget organisms.

The poorer performance and general lack of movement into the lower soil profile of aldicarb in experiment 1 is difficult to explain. Rapid leaching does not explain the lack of activity in the 10–20-cm soil depth because some activity remained in the upper soil level. Lateral movement of the chemical out of the sampling area is also not a probable cause of loss of aldicarb because there is little evidence of aldicarb moving laterally in soil (3). Cool soil temperatures would have limited volatilization (19). Microbial degradation (1,18,19) is a possibility, but neither the organisms involved nor their ecological requirements were known. Low humic matter (0.3%) in these soils would allow very little chemical binding to occur (3), allowing both nematicides to move to the 10–20-cm depth readily. Longevity of chemical as measured in half-life depends on a number of environmental conditions. Under conditions observed in these three field tests, both chemicals would have half-lives of at least 30 days. Aldicarb and fenamiphos remained biologically active in the 0–10-cm soil depth in two of the three field studies for up to 28 days. In experiment 3, measured concentrations of aldicarb and its

Table 5. Relationship of aldicarb concentration and suppression of *Meloidogyne incognita* juvenile penetration into Lee 68 soybean seedling roots.

| Days after treatment | Soil depth | Percentage penetration† | Concentration (ng/g)‡ |
|---|---|---|---|
| 0 | 0–10 cm | 0 a | 1,400 |
| 1 | 0–10 cm | 3 a | 1,450 |
| 3 | 0–10 cm | 1 a | 1,550 |
| 7 | 0–10 cm | 1 a | 1,900 |
| 14 | 0–10 cm | 1 a | 1,400 |
| 21 | 0–10 cm | 1 a | 1,100 |
| 28 | 0–10 cm | 4 a | 1,150 |
| 7 | 10–20 cm | 33 b | 23 |
| 28 | 10–20 cm | 4 a | 179 |

† Percentage host penetration by juveniles was computed from a paired untreated control that averaged 9% root penetration by the juveniles inoculated into the soil.

‡ Total of aldicarb, aldicarb sulfoxide, and aldicarb sulfone.

Numbers followed by the same letter are not different ($P = 0.05$) by the Waller–Duncan Bayesian k-ratio $t$-test (k = 100). Data are means for four replications. Aldicarb was applied at 0.67 g a.i./m$^2$.

sulfone and sulfoxide at 0–10-cm at 25 days after treatment were about 80% of that originally applied.

The fate of pesticides depends on environmental conditions, especially in Coastal Plain sandy soils. Split application of nematicide may be necessary to keep the chemical concentration high enough to achieve nematode control. This practice under some situations is effective for peanut (7). Split applications with lower rates could possibly reduce chemical contamination in soil below the root zone. Better management and use of presently registered nematicides should extend their period of availability.

Certain microbivorous nematodes have been described as excellent indicator organisms for detection of toxicants in aquatic, marine, and terrestrial habitats (14). Results described here indicate that plant-parasitic nematodes such as *Meloidogyne incognita* have the potential for being used as biomonitors of presence and movement of nematicides in soil.

Nematicidal persistence may be affected by a number of factors, including temperature and soil pH. As soil temperatures increase, the reactivity of aldicarb increases (5). This aspect was evident in experiments 1 and 2, in which both aldicarb activity and soil temperatures were lower in experiment 1 than in experiment 2. Soil pH affects ionization and, therefore, degradation. Because the pH of the soil in these three experiments was 5.2, this ionization-dependent degradation would not have influenced the pesticide's longevity. At this pH, fenamiphos requires over 20 days to hydrolyze (18), during which time the desired biological effect should have been achieved. Aldicarb degradation is accelerated with a basic pH (19); thus, rapid degradation was not likely to be a major problem as was noted in the long-term activity of this chemical in the 0–10-cm soil zone.

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