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Decomposition of Plant Debris by the Nematophagous Fungus ARF[1]

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Abstract: In the study of the biological control of plant-parasitic nematodes, knowledge of the saprophytic ability of a nematophagous fungus is necessary to understand its establishment and survival in the soil. The objectives of this study were (i) to determine if the nematophagous fungus ARF (Arkansas Fungus) shows differential use of plant residues; and (ii) to determine if ARF still existed in the soil of a field in which ARF was found originally and in which the population level of *Heterodera glycines* had remained very low, despite 15 years of continuous, susceptible soybean. Laboratory studies of the decomposition of wheat straw or soybean root by ARF were conducted in two separate experiments, using a CO_2 collection apparatus, where CO_2-free air was passed through sterilized cotton to remove the microorganisms in the air and then was passed over the samples, and evolved CO_2 was trapped by KOH. Milligrams of C as CO_2 was used to calculate the percentage decomposition of the plant debris by ARF. Data indicated ARF decomposed 11.7% of total organic carbon of the wheat straw and 20.1% of the soybean roots in 6 weeks. In the field soil study, 21 soil samples were taken randomly from the field. Only 3 months after the infestation of the soil with *H. glycines,* the percentage of parasitized eggs of *H. glycines* reached 64 ± 19%, and ARF was isolated from most parasitized eggs of *H. glycines.* Research results indicated ARF could use plant residues to survive.

Key words: ARF, decompose, *Heterodera glycines,* nematophagous fungus, plant debris, soybean cyst nematode.

Biological control of plant-parasitic nematodes is an attractive research topic because of the great losses caused by nematodes in the world (about $9 billion annually) and the environmental hazards of nematicide use (Stirling, 1992). Much research in this area has been conducted in laboratory and in greenhouse studies, and the results have been promising; however, the promise has rarely been fulfilled in subsequent field trials (Stirling, 1992). When a nematophagous fungus is delivered into soil to control plant-parasitic nematodes, degree of success is affected by many factors. An organism is not easily established in an unmodified field soil in which it faces competition, antibiosis, and hyperparasitism from the native soil microbial community. Some nematophagous fungi can parasitize and destroy the nematodes under certain conditions, but the initial development of the nematophagous fungi in the soil and the biocontrol efficacy depends not only on their parasitic ability but also on the ability of the nematophagous fungi to compete as saprophytes in soil. Usually strong saprophytic ability of the nematophagous fungi can help them become established in the soil. A better understanding of the saprophytic ability of the nematophagous fungi in soil can increase the probability of success in controlling the nematodes.

The sterile nematophagous fungus, ARF, was isolated from soybean cyst nematode *Heterodera glycines* Ichinohe, and has showed potential for the biocontrol of soybean cyst nematode in lab and greenhouse studies (Kim and Riggs, 1995; Kim et al., 1998; Timper and Riggs, 1998; and Timper et al., 1999). Because this fungus does not sporulate, it cannot be identified; therefore, it was designated as the ARF (Arkansas fungus). The fungus is an ascomycete as indicated by Woronin bodies in the septa (Alexopoulos et al., 1996).

ARF formed mycelial mats in nonsterilized soil without the presence of its host nematodes (Timper et al., 1999), which indicated that the ARF fungus grew saprophytically in soil. However, what ARF used as nutrient sources in soil in the absence of the nematodes was unknown. One possibility was that ARF could use plant debris as a substrate for growth. The saprophytic ability of a soil fungus is related to its ability to decompose organic materials and to use soluble substrates released from their decomposition. The decomposition process results in the release of carbon dioxide through metabolic activities. Therefore, the measurement of CO_2 evolution can be used to indicate the level of microbial activity (Anderson, 1982; Paul and Clark, 1989; Zibilske, 1994). The objectives of this study were to determine if ARF shows differential use of plant residues and if it still existed in the soil of a field in which ARF was found originally and the population level of *Heterodera glycines* had remained very low for about 15 years, despite the planting of a susceptible soybean cultivar continuously.

Materials and Methods

Laboratory studies: The ARF isolate used in this study was maintained on Emerson's yeast phosphate soluble starch (YPSS) medium at the Nematology Laboratory, University of Arkansas. The fungus was cultured in 500-ml flasks containing 250 ml pea juice medium (100 ml canned sweet pea juice plus 150 ml distilled water) in shaking culture (60 rpm) for 15 days at 25 °C. Mycelium was harvested from the liquid cultures with a Buchner funnel under vacuum, rinsed with sterilized distilled water, and homogenized in sterilized water in a blender.

Laboratory studies of the decomposition of wheat straw or soybean root by ARF were conducted in two separate experiments using a CO_2 collection apparatus. Wheat straw or soybean root was macerated using a mill

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to a size range of 0.5 mm to 2.0 mm and was autoclaved. The percentages of total C, N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, and Cu of the wheat straw and soybean roots were determined by The Agricultural Diagnostic Laboratory, University of Arkansas. One gram wet mycelium of ARF and 0.5 g wheat straw or soybean roots were added into 100 g (dry weight equivalent) sieved and sterilized silt loam soil. After the soil was mixed under aseptic conditions, moisture was adjusted to 15% by adding sterile distilled water. The soil was then transferred into a 500-ml sterilized incubation flask that was connected to the CO_2 collection apparatus. Treatments were: (i) soil amended with plant residue and ARF mycelium; (ii) control, soil amended only with ARF mycelium; and (iii) blank, no soil, ARF, or plant residue amended. Each treatment was replicated four times in the experiment.

The CO_2 collection apparatus was composed of prescrubber tubes and absorption tubes. Forty ml KOH with 4 M concentration in prescrubber tube was used to remove CO_2 in the air. Thirty-five ml KOH with 1 M concentration in absorption tube was used to trap evolved CO_2 from respiration. The 500-ml sterilized incubation flasks were connected with the CO_2 collection apparatus in which CO_2-free air was passed through sterilized cotton to remove the microorganisms in the air and was then passed over the samples, and evolved CO_2 was trapped in KOH. Base traps were assayed at weekly intervals for CO_2 by titration with 0.5 M HCl after addition of 1 M $BaCl_2$. The mixture was incubated for 6 weeks at room temperature (about 22 °C to 25 °C).

The formula used to calculate the carbon level was: mg C as CO_2 = (B-V) NED, where V = volume (ml) of acid to titrate sample, B = volume (ml) of acid to titrate blank, N = normality of acid, E = equivalent weight of carbon (12/2 = 6), and D = dilution factor (35 ml KOH in absorption tube/5 ml KOH titrated) (Anderson, 1982; Zibiliske, 1994). The formula used to calculate the decomposition was: % decomposition = $(X - Y)/Z \times 100$, where X = mg C as CO_2 evolved from treatment #1 (soil amended with plant residue and ARF mycelium), Y = mg C as CO_2 evolved from control (soil amended only with ARF mycelium), and Z = total of mg C of the wheat straw or the soybean roots that were amended in the soil (Anderson, 1982; Zibiliske, 1994).

Mathematical description of decomposition kinetics and statistical analysis: Mathematical models have been used to describe the kinetics of the decomposition of plant debris (Gilmour et al., 1998; Molina et al., 1983). Plant debris contains two decomposition fractions: (i) the rapid fraction composed of sugars and amino acids, and (ii) the slow fraction composed of polysaccharides (cellulose and hemicellulose) and lignin (Gilmour et al., 1998; Molina et al., 1983). The kinetics of the decomposition usually are described by a sequential model or a simultaneous model (Ajwa and Tabatabai, 1994; Gilmour et al., 1985). For the sequential model, the rapid and slow fractions of the plant debris are thought to be decomposed in sequence, and it is described by the following equation: $\ln(\%\ C_{remaining}) = -kt + b$, where $\%\ C_{remaining}$ is the percentage of plant debris C remaining after a period of decomposition at time *t,* the constant *k* is the first-order decomposition rate, and the constant *b* is the intercept of this equation (Gilmour et al., 1998). In this study, the experiment was conducted for only 6 weeks, so the CO_2 evolved was mainly from the decomposition of the rapid fractions of the debris. The sequential model used to describe the kinetics of the decomposition of the rapid fractions of the debris was: $\%\ C_{decomposed} = \%\ \text{Rapid} - e^{-kt+b}$. The parameters k, % Rapid, and b were calculated using the nonlinear regression of JMP Version 5.2 (SAS Institute, Cary, NC). Linear regression also was used to compare the decomposition predicted by the model with the observed values.

Field soil study: In June 2000, soil samples were taken from the field (located in St. Francis County, AR) from which the ARF fungus was isolated originally. In this field, the population density of *H. glycines* has remained very low (less than 4 cysts/kg soil in 2000) for about 15 years, despite the planting of a susceptible soybean cultivar continuously for 14 years.

Twenty-one soil samples were taken randomly from this field, placed in 10-cm-diam. clay pots in a greenhouse with temperature averaging from 26 °C to 30 °C, and seedlings of the soybean cultivar Lee 74, susceptible to *H. glycines,* were transplanted into soil in the pots. Eggs (3,000/pot) of *H. glycines* were added to the soil. Three months after planting, cysts in the soil were extracted from each pot by the wet-sieving and centrifugal sugar flotation method (Southey, 1986). Percentage of parasitized eggs was determined and egg-parasitic fungi were isolated from the parasitized eggs as described previously (Wang, 2003).

Results

The nematophagous fungus ARF decomposed 11.7% of total organic C of wheat straw and 20.1% of total organic C of soybean roots in 6 weeks (Fig. 1). ARF decomposed the soybean roots more readily than the wheat straw. Regression models for the decomposition of the wheat straw and the soybean roots were similar (Figs. 2,3). Rapid fraction decomposition rates were estimated by nonlinear regression as 0.0348 d^{-1} for the soybean roots and 0.0134 d^{-1} for the wheat straw. The percentage of the plant debris organic C in the rapid fraction was estimated by nonlinear regression as 28.39% for the soybean roots and 24.72% for the wheat straw. The decomposition rate (k) of the wheat straw by ARF was similar to values reported for wheat straw decomposition by other microbes in earlier studies (Table 1). However, the soybean root decomposition rate by

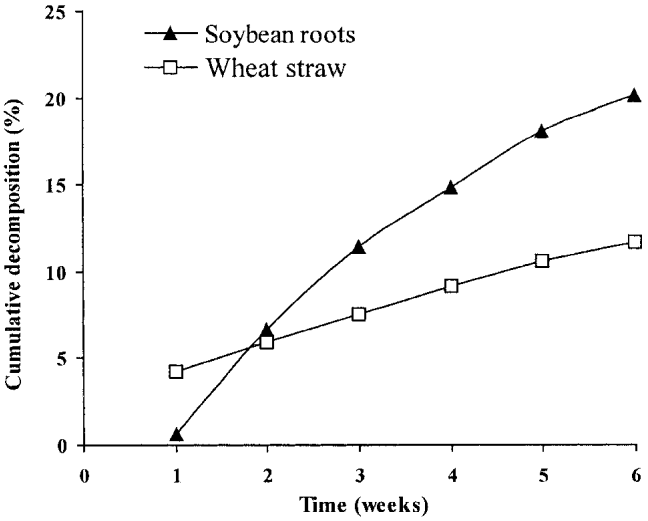

FIG. 1. Cumulative decomposition (%) of macerated wheat straw and soybean root by the nematophagous fungus ARF. The decomposition experiments of wheat straw and soybean root by ARF were conducted separately at two different times.

ARF was higher than the value reported for soybean root decomposition by other microbes in earlier studies (Table 1).

In the greenhouse study with field soil, 3 months after infestation of the soil with soybean cyst nematodes the percentage of parasitized *H. glycines* eggs reached 64 ± 19%, and ARF was isolated from most of the parasitized eggs.

DISCUSSION

Decomposition and respiration are processes that result in the release of carbon dioxide as a product of microbial activity. Wagner and Wolf (1998) defined decomposition as chemical breakdown of a compound into simpler compounds, often accomplished by microbial metabolism. Zibiliske (1994) defined soil respiration as "the sum of all soil metabolic activities that produce CO_2 or that result in the uptake of O_2 or release of CO_2 by metabolically active soil organisms."

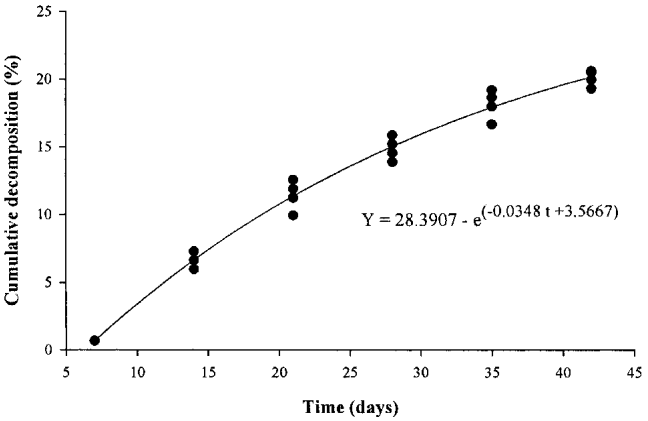

FIG. 2. Nonlinear regression model fitted for the decomposition of soybean roots vs. time (days).

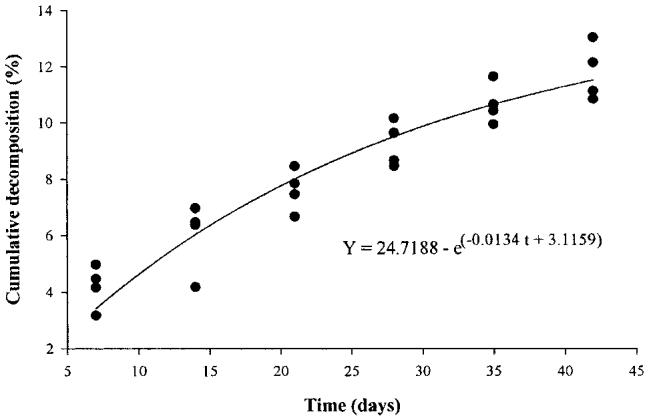

FIG. 3. Nonlinear regression model fitted for the decomposition of wheat straw vs. time (days).

Simple carbohydrates such as glucose can be transported across fungal membranes, metabolized, and enter the tricarboxylic acid (TCA) cycle and produce ATP and CO_2. Soil fungi must secrete extracellular enzymes to decompose complex polysaccharides, such as hemicellulose, cellulose, and lignin, and fungi vary in their ability to do that. Usually, the fast-growing Phycomycetes such as *Mucor* sp. are dependent upon soluble sugars and are called "sugar fungi." None of the sugar fungi can use lignin and few can use cellulose, but their rapid assimilation of the soluble sugars allows them to grow rapidly and out-compete other fungi. Cellulose and lignin can be degraded by groups of fungi that belong to the Ascomycetes, Basidiomycetes, and Deuteromycetes. The common soil saprophytes such as *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp. usually can decompose 12% to 25% of the carbon of wheat straw, based on CO_2 evolution in 10 weeks (Harper and Lynch, 1985). Compared to those fungi, the ARF fungus decomposed 11.7% of the carbon of the wheat straw in 6 weeks. The nonlinear regression model in this study predicted that about 15.8% of the carbon of the wheat straw could be decomposed after 10 weeks by the ARF fungus. Therefore, compared with the common soil saprophytes, the nematophagous fungus ARF has a similar ability to use the plant debris in soil. The decomposition rates of the wheat straw and the soybean

TABLE 1. Kinetics for selected decomposition of plant debris conducted in the laboratory.

Crop residue	Duration (day)	k (d^{-1})	Rapid fraction (%)	Source
Soybean	30	0.013	>33	Ajwa and Tabatabai (1994)
Wheat	35	0.015	11	Reinertsen et al. (1984)
Wheat	35	0.012	14	Reinertsen et al. (1984)
Wheat	203	0.0081	51	Nommik (1961)
Wheat	203	0.017	55	Nommik (1961)

roots by the ARF fungus in this study also supported this conclusion.

In a complex soil environment, usually a slow-growing and weakly saprophytic fungus could not compete with other soil microorganisms and, in the end, the fungus would be suppressed or would disappear. However, ARF is a common fungus in soybean fields and has been found in many areas of the United States (Kim et al., 1998). This also indicates that the ARF fungus should be a good saprophyte in the soil.

In these tests, ARF decomposed soybean roots more readily than wheat straw (20.1% vs. 11.7%). Possible explanations are that: (i) soybean roots may contain more simple nutrients such as soluble sugar than wheat straw; (ii) the ARF fungus may be more specific to soybean roots than wheat straw due to some biotic or abiotic factors in soybean roots that stimulate the secretion of extracellular enzymes of ARF to decompose the cellulose of soybean roots; (iii) because of its parasitism of *H. glycines,* ARF may have become more adapted to decomposition of soybean roots than wheat straw; or (iv) the C:N ratio of soybean roots (20:1) is lower than the C:N ratio of wheat straw (92:1), which means soybean roots contain more organic N than wheat straw (2.02% vs. 0.46%). Gilmour et al. (1985, 1998) reported the C:N ratio was related to decomposition rate and that the decomposition rate of alfalfa and clover, which usually contain high levels of organic N, was greater than bermudagrass and ryegrass. Janzen and Kucey (1988) also reported that differences in decomposition rates were positively correlated with crop N content.

The field soil study further corroborated the conclusion obtained from the laboratory study. ARF fungus apparently used plant residues in the soil to survive for an extended period of time, even when nematodes were not available as nutrients in the soil.

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