MOVEMENT OF ALLELOPATHIC COMPOUND COUMARIN FROM PLANT RESIDUE OF SWEET VERNALGRASS (*Anthoxanthum odoratum* L.) TO SOIL

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ABSTRACT

This study investigated the movement of coumarin, an allelopathic compound, from the plant residue of sweet vernalgrass (*Anthoxanthum odoratum* L.) to soil, as well as the dynamics of coumarin in soil. The level of coumarin dissolved from sweet vernalgrass plant residue in both Andosols and Cambisols, which were watered every day, peaked on the 5th day after the beginning of watering, and fell gradually with each additional of day. Specifically, the coumarin content in Cambisols on the 5th day was 14.2 ppm, which is 4 times the coumarin level found in Andosols. The recovery percentage of coumarin extracted with MeOH from the soil treated coumarin water solution was about 80 % in Cambisols and 40 % in Andosols. As the days continued, the recovery rate of coumarin tended to decrease, but not in the disinfected soils. The recovery rate of coumarin in Andosols at temperatures of 25° C and 15° C rapidly decreased on the 6th and 10th days, respectively. These results indicate the coumarin of sweet vernalgrass plant residue will move into soil easily after rainfall, and that both soil type and the presence of microorganisms are related to the disappearance of coumarin in soil.

KEYWORDS

Coumarin, sweet vernalgrass, plant residue, soil type, movement, allelopathy, allelochemicals

INTRODUCTION

Several studies have been conducted on the allelopathy of sweet vernalgrass (*Athoxanthum odoratum* L.). It was reported that the root exudate from sweet vernalgrass affected the growth and phosphorus uptake (Newman and Rovia, 1975; Newman and Miller, 1977), and Yamamoto (1995) isolated and identified coumarin as an allelopathic compound produced by sweet vernalgrass. Coumarin is known to be contained in many kinds of plant species (Putnam, 1988). It is possible that coumarin in the germinated seeds of *Myroxylon peruiferum* could play an important role as an allelopathic factor (Oniki and Valio, 1992). Coumarin inhibited root growth in timothy (Avers and Goodwin, 1956) and barley (Hu, 1985), and germination of alfalfa, Italian ryegrass and velvetleaf (Dornbos and Spencer, 1990). As the most important allelopathic compound of sweet vernalgrass, how is coumarin emitted from sweet vernalgrass plants? Evidence indicated that allelopathic compounds come from plants by volatilization, exudation from roots, leaching from plants by rain, or decomposition of residues (Rice, 1984). It has been reported that coumarin is present in all parts of sweet vernalgrass, and that its concentration varied with the season and between individual plants (Yamamoto, 1995). In particular, coumarin was highly concentrated in the leaves, accounting for more than 2.5% of dry leaf weight in June. The purpose of this experiment is to investigate the movement of coumarin from plant residues of sweet vernalgrass to soil, as well as the dynamics of coumarin in soil.

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MATERIALS AND METHODS

10 g of dried sweet vernalgrass (*Anthoxanthum odoratum* L.) shoots which had been cut to 2-3 cm in length were placed in 500 ml of Andosols containing a large amount of organic matter, and in 500 ml of Cambisols (weathering granite) containing no organic matter, in 15 cm-high X 11 cm-diameter plastic pots. Then they were placed in a green house. Each day, 50 ml of distilled water was added to each pot. The daily watering of 50 ml was estimated from the mean yearly rainfall of about 1000 mm/year. The content of coumarin in soil and the shoots of sweet vernalgrass were measured in each pot on the 5th, 10th and 20th day after the start of adding distilled water. Part of the Andosols and Cambisols were disinfected by adding 5 ml of chloropicrin solution to 500 ml of soil. In a 14 cm-high X 10 cm-diameter glass beaker, 50 ml of 1000 ppm coumarin water solution was added to 500 ml of each of 4 types of soils: disinfected or normal Andosols and Cambisols. These beakers were covered with wrap film and placed in a laboratory. The content of coumarin in soil was measured in each pot on the 1st, 3rd and 7th day. In each of two glass beakers of the same size, 500 ml of Andosols was added to 50 ml of 1000 ppm coumarin water solution; the beakers were protected with wrap film, and incubated at temperatures of 15° C and 25° C. The content of coumarin in the soil was measured in each pot on the 1st, 3rd, 6th, 10th and 20th day. Coumarin in the soil of each pot and beaker was extracted with 500 ml MeOH for 12 hours at 25° C. Coumarin in sweet vernalgrass plant residues in each pot was extracted with 250 ml distilled water for 12 hours at 25°C. These extracts were immediately filtered. The concentration of coumarin in these extracts was measured using HPLC (Nippon-Bunko 880-PU). The analytical conditions were as follows: column-Sphersorb ODS-2-5; mobile phase-80 % MeOH(1.0 ml/min); column temperature-25° C; UV detection-275 nm.

RESULTS AND DISCUSSION

Movement of coumarin from sweet vernalgrass plant residue to soil. With daily watering (50 ml/pot/day), the content of coumarin in both Andosols and Cambisols peaked on the 5th day after the beginning of watering, and fell gradually as the days continued (Table 1). Specifically, the content of coumarin in Cambisols on the 5th day was 14.2 ppm, which was 4 times as great as that found in Andosols. The content of coumarin in aqueous extract of sweet vernalgrass shoot plant at the start time was 175.4 ppm, but that fell to 48.8 ppm on the 5th day and tended to fall rapidly as the days continued (Table 1). Decomposition of residues is one of the important routes by which allelopathic compounds move out of the plants. Determining whether inhibitors are released from a decaying plant is very difficult, if not impossible, because there is always the possibility that microorganisms change non-toxic compounds to toxic ones (Rice, 1984). Many investigations have reported that allelopathic compounds is identified from plant residues (Chou and Lin, 1976; Chou and Patrics, 1976; Tang and Waiss, 1978; AlSaadawi *et al.*, 1983), but there are few reports on the movement of allelopathic compounds from plant residue to soil. Therefore, these experiments investigate both the movement of the allelopathic compound, coumarin of sweet vernalgrass, from plant residue to soil, and the dynamics of coumarin in soil by measuring the soil content of coumarin and sweet vernalgrass plant residue. Coumarin exist in all parts of sweet vernalgrass, with a particularly high concentration in the leaves (Yamamoto, 1995). Also, in this experiment, a high concentration of coumarin was found in aqueous solutions of sweet vernalgrass plant residues. It is clear that coumarin from sweet vernalgrass plant residue can easily move to soil when it is watered (Table 1). Water-soluble inhibitors like coumarin should easily leach out of plant residues after plant death, when the various membranes lose their differential permeability.

Dynamics of coumarin in soil. When coumarin water solution was added to both types of soil, the recovery percentage of coumarin extracted from the soil with MeOH was about 80% in Cambisols and 40% in Andosols (Figure 1). As the days continued, the recovery percentage of coumarin tended to decrease, and on the 7th day recovery in Cambisols and Andosols were 50% and 24%, respectively. However, the recovery percentage in soils disinfected with chloropicrin solution did not decrease. At 25° C, the recovery percentage of coumarin in Andosols decreased rapidly on the 6th day, but at 15° C it decreased rapidly at the 10th day (Table 2). The recovery percentage of coumarin in Andosols tended to be maintained at the lower temperature. The coumarin concentration from plant residue is higher in Cambisols than in Andosols since Andosols contain a large amount of organic matter that could absorb coumarin. This explains why the recovery rate of coumarin in Andosols was lower than in Cambisols (Figure 1). The dynamics of coumarin in soil varied depending on whether the soils were disinfected or not, and the recovery rate of coumarin in the disinfected soil was maintained at a constant level. Consequently, it was supposed that the microorganisms are involved in the disappearance of coumarin in soil. The delay of the rapid decrease in the coumarin recovery rate at 15 ° C compared to the decrease of the rate 25° C also indicates that the microorganisms in the soil were related to the disappearance of coumarin (Table 2). Recently, it has been observed that sweet vernalgrass has invaded *Zoysia*-grassland used for grazing and recreational area in Japan. In the case of grazing, sweet vernalgrass plants are trampled by cattle; and sweet vernalgrass plants are sometimes cut together with *Zoysia japonica* in recreational areas. If the plant residue of sweet vernalgrass contains high concentrations of coumarin, rain may release coumarin, which inhibit germination or growth of other plant species like *Z. japonica*. Takahashi *et al.* (1994) reported that the seedling growth of lettuce seeds was inhibited in soils without organic matter that contained 100 ppm coumarin solution.

Allelopathy is well known to play a significant role in the dominance of grassland communities (Rice, 1984; Heisey and Delwiche, 1985; Chou, 1987; Chou and Lee, 1991). It could not be determined that the allelopathy of sweet vernalgrass in *Zoysia*-grassland was caused by the decomposition of its plant residues, because high concentrations of coumarin in soil due to decomposition of plant residues are temporary, and other pathways of coumarin movement from sweet vernalgrass plants were not studied. But it is clear that high coumarin concentrations in soil has the potential to disturb the plant growth surrounding sweet vernalgrass in *Zoysia*-grassland. The magnitude of this is variable, depending on soil type and the microorganism activity.

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Table 1

Changes in the content of coumarin in soil and Anthoxanthum. odoratum plant residue

Period	Coumarin content (ppm/500 ml extract solution ± SD)		
(days)	Andsols	Cambisols	Plant residue
0	0.00 ± 0.00	0.00 ± 0.00	175.44 ± 4.63
5	3.35 ± 2.15	14.23 ± 3.76	48.79 ± 6.18
10	0.31 ± 0.14	0.44 ± 0.09	4.15 ± 1.44
20	0.06 ± 0.03	0.07 ± 0.01	0.11 ± 0.01

Table 2

Changes in the recovery rate of coumarin in Andosols.

Period	Recovery rate of coumarin (% ± SD)	
(days)	25° C	15° C
0	46.90 ± 4.0	46.90 ± 4.0
1	39.80 ± 2.4	40.70 ± 2.0
3	26.50 ± 1.7	32.50 ± 1.9
6	2.6 ± 0.2	25.70 ± 1.8
10	0.6 ± 0.0	6.1 ± 1.2
20	0.2 ± 0.0	0.5 ± 0.1

(N = 3)

Figure 1

Dynamics of coumarin in Andosols or Cambisols, both disinfected and regular samples. Dynamics of coumarin were demonstrated by the coumarin recovery rate in MeOH extractions. ↙ ; Disinfected Andosols,↘; Andosols, ⇔; Disinfected Cambisols, ▼; Cambisols.