ALLELOPATHIC POTENTIAL OF *Anthoxanthum odoratum* FOR INVADING Zoysia-GRASSLAND IN JAPAN

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Abstract—The growth of *Zoysia japonica* surrounding *Anthoxanthum odoratum* on Zoysia-grassland in Japan was investigated in June 1990. The stem density of *Z. japonica* tended to decrease with short distances between two *A. odoratum* plants. This showed that the growth of *Z. japonica* was reduced. Basic, neutral, and acidic fractions extracted from *A. odoratum* plants inhibited the seedling growth of lettuce. In particular the neutral fraction showed the strongest activity among the three fractions. The main inhibitory compound obtained at *R*$_t$ 0.6–0.7, on the thin-layer chromatogram of the neutral fraction, was isolated and identified as coumarin by means of GC-MS. Coumarin solution inhibited seedling growth of *Z. japonica* in low concentrations but, conversely, promoted seedling growth of *A. odoratum*. Coumarin was contained in all parts of *A. odoratum* and its concentration varied with the season and from one individual plant to another. In particular, coumarin was highly concentrated in the leaves, accounting for more than 2.5% of dry leaf weight in June. The inhibitory effect of these aqueous extracts was correlated to the amount of coumarin in *A. odoratum* leaves and coumarin was considered to be the main inhibitory compound.

Key Words—*Anthoxanthum odoratum*, allelopathy, coumarin, *Zoysia japonica*, allelochemicals, grassland.

INTRODUCTION

*Zoysia japonica* is one of the important native grasses mainly dominant in the seminatural grassland of Japan, but recently, the author observed that *Anthoxanthum odoratum* has invaded *Zoysia*-type vegetation and formed a pure stand. Judging from the distribution pattern of *A. odoratum* in *Zoysia*-type vegetation, the author hypothesized that allelopathy contributed to its aggressiveness. *A.*
*Anthoxanthum odoratum* was one of the pasture plants introduced from Europe as horse feed about 100 years ago, but it has escaped from pasture and spread all over Japan except for the Okinawa region. Several studies have been made on allelopathy of *A. odoratum*; for example, it was reported that the root exudate from *A. odoratum* affected the growth and phosphorus uptake (Newman and Rovia, 1975; Newman and Miller, 1977). However, the effects of allelochemicals and the allelopathic role of *A. odoratum* in grassland communities have not yet been studied. Therefore, the author started this research on the allelopathy of *A. odoratum*.

In this study, the growth of *Z. japonica* surrounding *A. odoratum* was investigated, and the main allelopathic substances of *A. odoratum* were isolated and identified. In addition, the allelochemical content of *A. odoratum* was measured, and the effects of this allelochemical on the growth of *Z. japonica* and *A. odoratum* were assessed.

**METHODS AND MATERIALS**

*Field Survey of Growth of* *Zoysia japonica*. The growth of *Z. japonica* surrounding *Anthoxanthum odoratum* in *Zoysia*-type vegetation was investigated in June 1990. First, two *A. odoratum* plants were selected in *Zoysia*-type vegetation, on the condition that there was no *A. odoratum* within a circle the diameter of which was the distance between the two plants. The stem numbers of the two *A. odoratum* plants were counted (Figure 1). A quadrat (10 x 10 cm) was set between the two *A. odoratum* plants, and the stem numbers of *Z. japonica* within the quadrat were counted. This investigation was carried out on a total of 22 circles.

*Extraction of Active Substances from* *A. odoratum* *and TLC Fractionation*. The plant top of *A. odoratum* was collected in March and freeze-dried. The extraction procedure used is shown in Figure 2. Thin-layer chromatography was carried out on a 20-cm × 20-cm silica gel plate (Whatman, Silica gel 60A PK6F) 1 mm thick using benzene-MeOH-acetic acid (45:8:4 v/v/v) as a developing solvent. After the TLC plate was dried, it was divided into 10 parts from the origin to front. Each fraction was eluted with MeOH for 12 hr at 25°C, and the eluates were filtered. These fractions were bioassayed using lettuce seedlings. Thirty seeds were placed on filter paper moistened with 1.6 ml of the test solution in a 20-mm-high × 55-mm-ID glass Petri dish. Lettuce seeds were incubated for four days in a growth chamber at 25°C in darkness. The average hypocotyl length of 10 germinated plants was compared with that of controls grown in distilled water.

*Isolation and Identification of Allelochemical*. An active compound separated by TLC was isolated and identified using a Shimadzu GCMS-QP1000S