南九州地域の黒ボク上充填小型枠におけるソルガム，マリーゴールド，エゴマ栽培がアーバスキュラー菌根菌胞子数に及ぼす影響

Effect of Cultivating Sorghum, Marigold, and Egoma (Perilla) on the Spore Population of Arbuscular Mycorrhizal Fungi in Small Plots Filled with Andosol in the Southern Kyushu Region (Japan)

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Summary

A small-plot (2 m × 2 m) experiment was conducted by filling the plots with Andosol (volcanic ash soil, previously uncultivated). Crops of sorghum, marigold, and egoma (perilla) were cultivated continuously for two to four years in the plots. After the cultivation of the crops or before the cultivation of the succeeding crops, intra-row soil or soil mixed by tillage was sampled, and the spore population of arbuscular mycorrhizal fungi (AM fungi) was counted by the sieving method. Sorghum cultivation induced an increase of AM fungal spore population in the cultivated soil to a high level exceeding 40 spores per 10g dry soil. Marigold cultivation leaded to the same level of the AM fungal spore population with the fallow control soil, or increased the spore population two- to three-fold, compared with the fallow control soil. Egoma cultivation increased the spore population in the cultivated soil, compared with the fallow control soil, to approx. 20 to 30 spores per 10g dry soil. Shoot dry matter production of sorghum was much higher than that of marigold and egoma, and the cultivation duration of sorghum (six months) was longer than those of marigold (three months) and egoma (four months). In the sorghum-cultivated plots, sorghum growth disorder caused by continuous cropping was observed, especially in the third- or fourth-year croppings. After the low shoot production of sorghum by continuous cropping, the spore population of mycorrhizal fungi also decreased, compared with that before the cultivation. These results suggest that sorghum cultivation without growth disorder caused by continuous cropping may induce an increase of AM fungal spore population in the cultivated soil to a high level compared with marigold and egoma cultivations. These findings may lead to the development of techniques to increase AM fungal spore population, enhance their activity in the soil, and utilize their functions in crop rotation systems in the southern Kyushu region of Japan.

Keywords: arbuscular mycorrhizal fungi, egoma (perilla), marigold, shoot production, sorghum, spore population.

Ⅰ. Introduction

In agroecosystems, close interactions between crop and microorganisms cause various biological functions in relation to symbiosis, growth promotion, and pathogenesis. The typical interaction sites are the rhizosphere, rhizoplane, and phylloplane. In addition, certain endophytic microorganisms live inside the crop plants without causing pathogenisity. Endomycorrhizal fungi (arbuscular mycorrhizal fungi, AM fungi) infect the insides of crop roots, and it is suggested that mycorrhizal infection influences crop growth. Tawaraya[14] reviewed the arbuscular mycorrhizal dependency of different plant species and cultivars. Studies on ecology and utilization of AM fungi in crop rotation systems and on clarifying the mechanism of AM fungal functions have been conducted mainly in central and northeastern areas of Japan. However, relatively few studies have been conducted in the Kyushu region, which is in the southwestern area. Thus, information about the ecology and biological functions of AM fungi in the area is limited, although interactions actually occur between crops and AM fungi even under relatively high temperatures and humidity conditions in the area.

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In this study, we examined how the cultivation of crops（sorghum, marigold, and egoma）in the Kyushu region affects the population of AM fungal spores in the soil, compared with that in fallow soil. Sorghum（*Sorghum bicolor* L. Moench）is one of the major forage crops in the Kyushu region, although sorghum growth disorder due to continuous cropping is a problem for stable sorghum production there.[1,5,6,13] Marigold（*Tagetes erecta* L.）is one of the green manure crops, and one of the famous nematode-suppressive plants.[2,4] Marigold is often utilized as host plants in AM fungal studies.[3] Egoma（*Perilla frutescens* var. *japonica*）is a traditional oil plant for squeezed oil of egoma seeds. Egoma oil is considered to have health benefits because of its high content of α-linolenic acid. Studies on allelopathic compounds of marigold and egoma against phytopathogenic fungi have been reported.[7,8] Also, Sugawara[12] reported the effective control of weeds by planting egoma; hence, marigold and egoma may have promising potential for green manure or industrial crops.

The objectives of this study are:（1）to determine the effect of cultivating crops（sorghum, marigold, and egoma）on the AM fungal population, and（2）to utilize effectively the biological functions of AM fungi in crop rotation systems in the Kyushu region of southwestern Japan.

Ⅱ. Materials and methods

1. Crop cultivation in two to four years of continuous cropping

Small 4 m^2（2 m×2 m）plots were filled to a depth of 70cm with Andosol（volcanic ash soil, Loam）taken from a previously uncultivated experiment field（latitude 31°45' N, longitude 131°01' E）of the National Agricultural Research Center for the Kyushu Okinawa Region, Miyakonojo Research Station, Miyakonojo, Miyazaki Japan. Ten plots were filled with the soil in 1999; six were filled in 2000; and eight were filled 2001（a total of 22 plots）. Three crops, sorghum（*Sorghum bicolor* L. Moench, cultivar Sanjakusorugo）, marigold（*Tagetes erecta* L., african-tall type）, and egoma（the Japanese name for *Perilla frutescens* var. *japonica*, traditional cultivar Abukuma, black seed）, were cultivated in the plots. Sorghum seeds were sown in the middle to the end of May, with a 30cm intra-row and a 65cm inter-row spacing（three rows in a plot）. In each sowing point, 12 seeds were sown in a 10cm length along the row. The sorghum shoots were harvested in late August to early September, re-germinated from the stubbles, and harvested a second time in early December. Marigold and egoma were sown in early May in the nursery filled with Andosol taken from the same previously uncultivated experimental field described above, and young plants were transplanted in the plots with a 30cm intra-row spacing and a 65cm inter-row spacing in early to mid June. Marigold shoots were harvested in late August to early September. Egoma shoots were harvested in early October. Eleven cultivation treatments were designed in duplicate; chemical fertilizer, lime, and farmyard manure were applied（Table 1）. Chemical fertilizer and lime were applied every year just before cultivating the crops. All plots（including fallow plots）were treated with lime at a dose of 500kg ha^{-1}（50g m^{-2}）. Chemical fertilizer（N, P_2O_5, and K_2O）was applied at a rate of 100kg ha^{-1}（10g m^{-2}）（100kg ha^{-1} of N, 100kg ha^{-1} of P_2O_5, and 100kg ha^{-1} of K_2O）for sorghum cultivation and at a rate of 50kg ha^{-1}（5g m^{-2}）for marigold and egoma cultivation. Only in 1999, half amount of chemical fertilizer（50kg ha^{-1} of N, P_2O_5, and K_2O）was applied in T1 plot for sorghum. Several plots were treated with farmyard manure prepared from monocotyledonous crop（maize, barley, and sorghum）residues and cow dung at a rate of 10t ha^{-1}（1kg m^{-2}）（for T5, T8, T10, and T11; Table1）. The farmyard manure treated in April 2001（water content 67.3%）contained N 3.1%, P_2O_5 3.9%, and K_2O 4.6% on dry matter base, the manure in April 2002（water content 51.4%）contained N 2.8%, P_2O_5 3.3%, and K_2O 4.2% on dry matter base, and the manure in May 2003（water content 53.3%）contained N 2.7%, P_2O_5 3.4%, and K_2O 4.4% on dry matter base.

2. Soil sampling

After the second harvest of sorghum in December, intra-row soil was sampled at depths of 0 to 15cm. Three soil cores were taken in each plot and mixed to make a

Table 1. Design of eleven cultivation treatments in continuous cropping experiment using small plots filled with Andosol

Treatment No.	Year when plots were filled with Andosol (Onset year)	Crop, continuously cultivated	Cultivation duration[a]	Farmyard manure application[b]	Years of continuous cropping (or fallow)
T1	1999	Sorghum	6 months	-	4 y (1999-2002)
T2	1999	Fallow	-	-	5 y (1999-2003)
T3	1999	Marigold	3 months	-	4 y (1999-2002)
T4	1999	Egoma	4 months	-	4 y (1999-2003)
T5	2000	Egoma	4 months	+	3 y (2000-2002)
T6	2000	Fallow	-	-	4 y (2000-2003)
T7	2000	Sorghum	6 months	-	3 y (2000-2002)
T8	2001	Egoma	4 months	+	2 y (2001-2002)
T9	2001	Fallow	-	-	3 y (2001-2003)
T10	2001	Marigold	3 months	+	3 y (2001-2003)
T11	2001	Sorghum	6 months	+	3 y (2001-2003)

a. Cultivation durations are shown by approximate months from sowing or transplanting to harvest.
b. Farmyard manure was applied at a dose of 10t ha^{-1} before cultivation every year for T5, T8, T10, and T11.

representative sample. The plot soils were tilled by using small mechanical tillers in March or April of the next year, before the succeeding cultivation. Alternative soil samplings were also conducted in March or April by similar procedures of three cores sampling from depths of 0 to 15cm in each plot. Hence, the soil samples for mycorrhizal spore counting were intra-row soil or soil mixed by tillage.

3. Counting mycorrhizal fungal spores

Mycorrhizal fungal spores were collected by adopting wet-sieving procedures[9, 10, 11] with minor modification as follows, and counted under a stereo microscope. The sampled soils were passed through a 2mm sieve, and 10g, 20g, or 25g of the sieved soil were taken for the following wet-sieving procedure. The soil was suspended in 1L of tap water. The soil suspension was poured into a fine sieve (106μm mesh) through a coarse sieve (500μm mesh). The materials on the 500μm sieve were manually washed with tap water. The materials on the 106μm sieve were then transferred into a tall beaker and treated with ultra-sonication (Ultra Sonic Automatic Washer, US-2, 120W, 38kHz, Iuchi) for 5 min. The ultra-sonicated materials were again poured into a 106μm sieve and gently washed with tap water. The materials on the 106μm sieve were transferred into centrifuge tubes and centrifuged at 590×*g* for 5 min. The supernatant phases were poured into a 106μm sieve by decantation, and the materials on the sieve were transferred into Petri dish #1. The precipitated materials in the centrifuge tubes were again dispersed in 55% sucrose solution and then centrifuged at 410×*g* for 30 sec. The supernatant phases were poured onto a 106μm sieve and transferred into Petri dish #2. This process using 55% sucrose solution was repeated twice to collect the materials in dish #2. The materials in Petri dishes #1 and #2 were examined under a stereo microscope (Stereo Microscope, SZH10, Olympus) to count arbuscular mycorrhizal fungal spores. The total spore number in Petri dishes #1 and #2 was counted, and the number was converted to the number per 10g dry soil. Small spores that passed through the 106μm sieve were not examined.

4. Succession to crop rotation systems from continuous cropping

After two to four years of continuous cropping of sorghum, marigold, and egoma, the crop rotation systems presented in Table 2 were designed and implemented from 2003. Cultivation variables (sowing, transplanting, liming, and application of chemical fertilizer) for each

Table 2. Design of crop rotation systems starting from 2003 after the continuous cropping experiment

Treatment No.	Crop cultivated in			Farmyard manure application[a]
	2002		2003	
T1	Sorghum	→	Marigold	-
T3	Marigold	→	Sorghum	-
T4	Egoma	→	Sorghum	-
T5	Egoma	→	Sorghum	+
T7	Sorghum	→	Fallow	-
T8	Egoma	→	Sorghum	+

a. Farmyard manure was applied at a dose of 10t ha^{-1} before cultivation for T5（2000-2003, continuously applied for four years）, and for T8（2001-2003, continuously applied for three years）.

crop were the same as those in the preceding continuous croppings. Farmyard manure was applied continuously at a dose of 10t ha^{-1} to the plots of T5, T8, T10, and T11. Shoot dry matter production and AM fungal spore population were measured with soil sampling in April（or early May）before the succeeding cropping.

Ⅲ. Results and Discussion

The fluctuations of shoot dry matter production of sorghum, marigold, and egoma in the continuous cropping experiment are depicted in Fig. 1. The shoot production of sorghum（Fig. 1A）is expressed as the sum of the first（September）and second（November）shoot harvests. The sorghum shoot productions ranged from 794 to 1594g m^{-2} in the first to third croppings in T1, and in the first and second croppings in T7 and T11. However, the shoot production was drastically decreased due to growth disorder by the continuous cropping in the fourth cropping in T1, and decreased in the third cropping in T7 and T11. The phenomena of sorghum initial growth disorder due to continuous cropping in Andosol in the southern Kyushu region was observed and reported several decades ago.[5, 6, 13] The cause of this initial growth disorder is likely related to a soil biological change caused by sorghum continuous cropping,[5, 6] but it has not yet been fully determined.

The shoot productions of marigold（Fig. 1B）ranged from 74 to 248g m^{-2}, whereas those of egoma（Fig. 1C）ranged from 111 to 549g m^{-2}. In both marigold and egoma cultivations, the application of farmyard manure at doses of 10t ha^{-1}（1kg m^{-2}）increased shoot dry matter production.

The fluctuations of AM fungal spore populations in the intra-row soil after the proceeding cultivation or the soil mixed by tillage before the succeeding cultivation in the continuous cropping experiment are depicted in Fig. 2. In November 2000 and November 2001, when the soil samples were taken from intra-row sites, AM fungal spore populations in sorghum plots（T1, T7, and T11; Fig. 2A）ranged from 64 ± 26（average ± SD）to 126 ± 74 spores per 10g dry soil with high standard deviation（SD）values between the two replications. The soil sampling site（intra-row）may have caused the high SD variation between the replications because spore clusters were sometimes observed in the materials in the Petri dishes prepared from the soil samples taken from sorghum intra-row sites, and they might have increased the spore-count variation. In March 2003 and April 2004, when the soil samples were taken from the soil mixed by tillage before the succeeding cultivation, spore populations in sorghum plots（T1, T7, and T11; Fig. 2A）ranged from 21 ± 1 to 75 ± 29 spores per 10g dry soil, with rather lower SDs than in the results in November 2000 and November 2001. Soil mixing by tillage may have affected the SD variations in the later results in March 2003 and April 2004. In the plots of T11 in 2003, sorghum dry matter production was drastically decreased by continuous cropping, and spore population after cultivation was also clearly decreased.

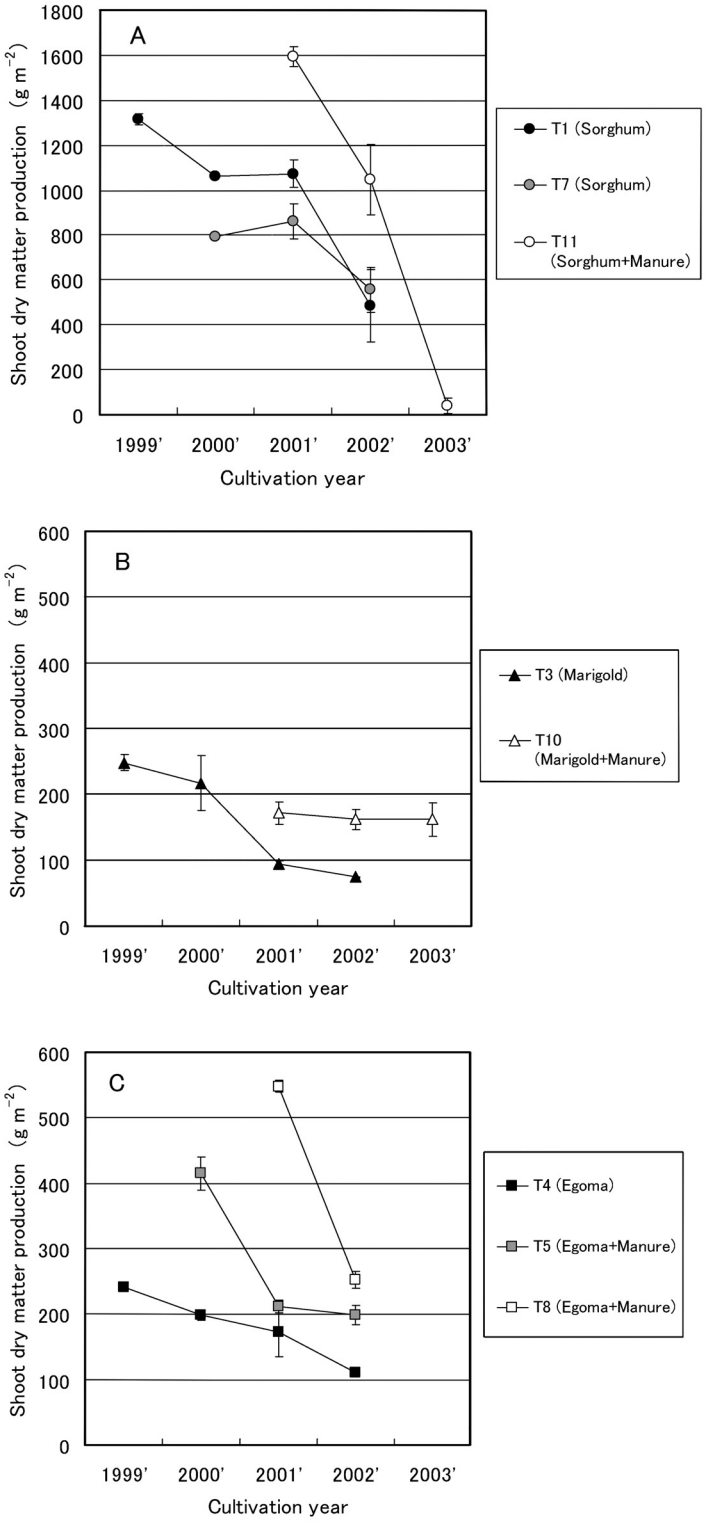

Fig.1. Fluctuations of shoot dry matter production of sorghum (A), marigold (B), and egoma (C) in the continuous cropping experiment

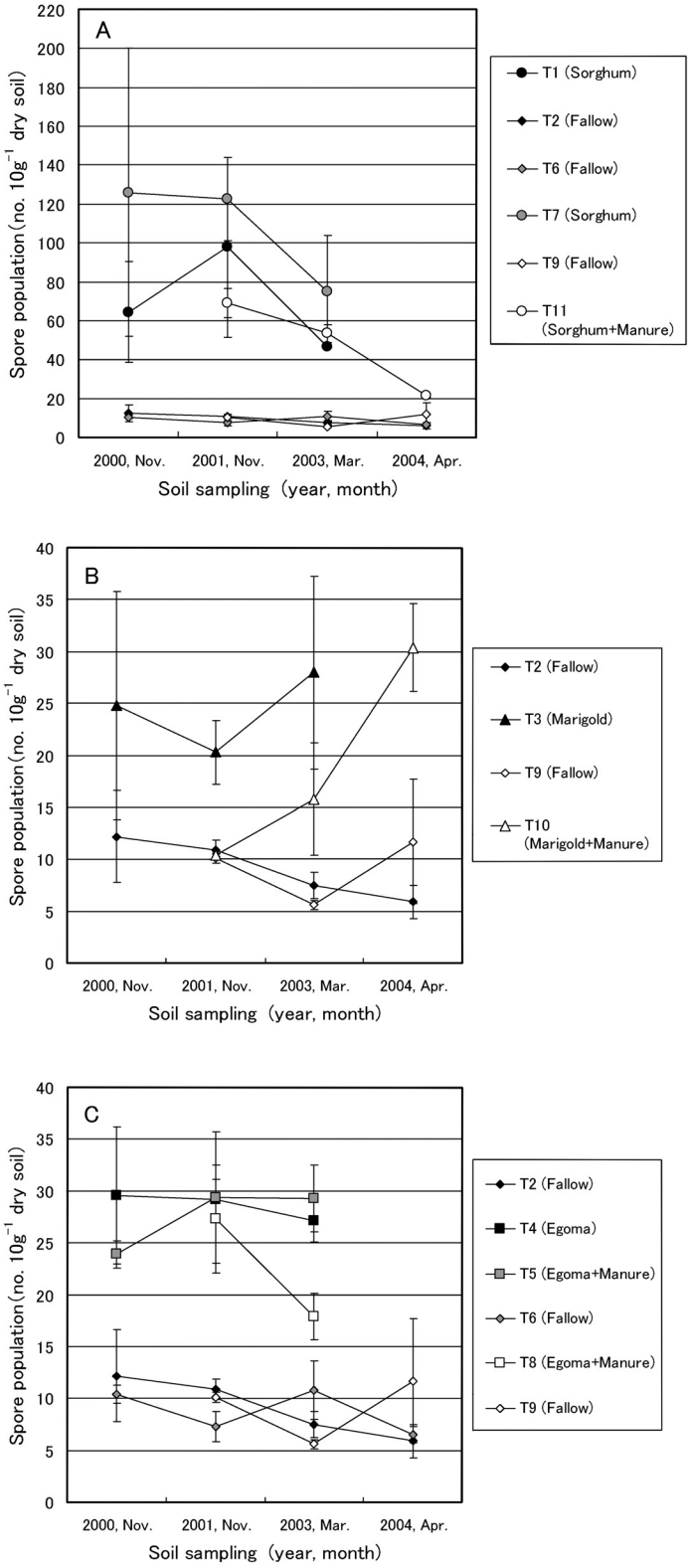

Fig. 2.　Fluctuations of AM fungal spore populations in sorghum-cultivated plots and fallow control plots（A）, marigold-cultivated plots and fallow plots（B）, and egoma-cultivated plots and fallow pots（C）, by analyzing the intra-row soil after cultivation or the soil mixed by tillage before the succeeding cultivation, in the continuous cropping experiment

The intra-row soil was analyzed after the proceeding cultivation in the November 2000 and November 2001 soil samples; in the March 2003 and April 2004 soil samples, the soil mixed by tillage before the succeeding cultivation was analyzed to determine if it was affected by the proceeding cultivation in the previous year.

In the marigold plots (Fig. 2B), spore populations ranged from 10 ± 0 to 30 ± 4 spores per 10g dry soil during November 2000 to April 2004; in the egoma plots (Fig. 2C), they ranged from 18 ± 2 to 30 ± 7 spores per 10g dry soil during November 2000 to March 2003. In contrast, spore populations in continuously fallow plots (T2, T6, and T9) ranged from 6 ± 0 to 12 ± 4 spores per 10g dry soil during November 2000 to April 2004.

The former-half results in November 2000 and November 2001 in Fig. 2A confirmed that sorghum cultivation drastically increased the spore population five- to twelve-fold, compared with the continuously fallow control soil. The latter-half results in March 2003 and April 2004 in Fig. 2A revealed that sorghum cultivation increased the spore population two- to seven-fold, compared with the continuously fallow control soil. In this term, sorghum growth disorder due to continuous cropping was observed; in relation to this growth reduction, the spore population also tended to decrease after cultivation, compared with that before the cultivation (T11 in April 2004).

Marigold cultivation (Fig.2B) leaded to the same level of the AM fungal population with the fallow control soil, or increased the spore population two- to three-fold, compared with the fallow control soil. On the other hand, egoma cultivation (Fig. 2C) increased the spore population, compared with the fallow control soil, to approx. 20 to 30 spores per 10g dry soil.

After two to four years of continuous cropping of sorghum, marigold, and egoma, crop rotation systems were employed from 2003. Shoot dry matter production and AM fungal spore population, after cultivation in the rotation systems were implemented, are presented in Table 3. In the transition from continuous marigold cropping to sorghum cropping (T3), the AM fungal spore population increased from 28 ± 9 to 40 ± 17 spores per 10g dry soil. In the transition from continuous egoma cropping to sorghum cropping (T4, T5, and T8), the spore populations increased from 27 ± 2 to 46 ± 6 spores per 10g in T4, from 29 ± 3 to 55 ± 9 spores per 10g in T5, and from 18 ± 2 to 47 ± 9 spores per 10g in T8. However, in the transition from continuous

Table 3. Shoot dry matter production and AM fungal spore population after cultivation (in the soil mixed by tillage before the succeeding cultivation) in the rotation cropping systems

Treatment No.		Cultivated in 2002 Crop		Cultivated in 2003 Crop	
T1	Shoot production[a]	Sorghum	485 ± 162	Marigold	240 ± 16
	Spore population[b]		47 ± 1		18 ± 6
T3	Shoot production	Marigold	74 ± 1	Sorghum	734 ± 180
	Spore population		28 ± 9		40 ± 17
T4	Shoot production	Egoma	111 ± 2	Sorghum	1142 ± 86
	Spore population		27 ± 2		46 ± 6
T5	Shoot production	Egoma	199 ± 15	Sorghum	1441 ± 50
	Spore population		29 ± 3		55 ± 9
T7	Shoot production	Sorghum	556 ± 101	Fallow	- (0)
	Spore population		75 ± 29		18 ± 5
T8	Shoot production	Egoma	253 ± 13	Sorghum	1545 ± 36
	Spore population		18 ± 2		47 ± 9

a. Shoot production, shoot dry matter production, g m^{-2}.

b. Spore population, AM fungal spore population after cultivation (in the soil mixed by tillage before the succeeding cropping), spores $10g^{-1}$ dry soil.

AM fungal spore population in the soil which was taken from the same previously uncultivated experiment field in April 2002, with the same manner as done in the onset years (1999, 2000, and 2001) when the plots were filled, was 6 ± 1 spores per 10g dry soil.

sorghum cropping to marigold cropping（T1），the spore population decreased from 47 ± 1 to 18 ± 6 spores per 10g dry soil，whereas in the transition from continuous sorghum cropping to fallow（T7），the spore population decreased drastically from 75 ± 29 to 18 ± 5 spores per 10g dry soil. Summarizing these results, in the transition from continuous marigold and egoma croppings to sorghum cropping（T3，T4，T5，and T8）increased the AM fungal spore population in the soil to the high level exceeding 40 spores per 10g dry soil, and adversely in the transition from sorghum croppings to marigold cropping and fallow（T1 and T7）decreased the spore population to the level falling below 40 spores per 10g dry soil. These results in the rotation cropping systems clearly indicate that succession of crop cultivation influences AM fungal spore population in the soil.

Ⅳ. Conclusions

The results of both continuous cropping and rotation cropping experiments indicated the following.

1. Sorghum cultivation produced much higher shoot biomass than did cultivation of marigold and egoma, and cultivation duration of sorghum（six months）was longer than that of marigold（three months）and egoma（four months）.
2. The sorghum cultivation increased the AM fungal spore population in the cultivated soil to a high level exceeding 40 spores per 10g dry soil in this small-plot experiment.
3. In the marigold cultivated plots，AM fungal populations ranged from 10 to 30 spores per 10g dry soil. In contrast，spore populations in continuously fallow plots ranged from 6 to 12 spores per 10g dry soil. Cultivating marigold leaded to the same level of the AM fungal population with the fallow control soil, or increased the spore population two- to three-fold, compared with fallow soil.
4. Cultivating egoma increased the spore population in the cultivated soil，compared with the fallow control soil，to approx. 20 to 30 spores per 10g dry soil.
5. These findings may lead to the development of techniques to increase AM fungal spore population, enhance their activity in the soil，and utilize their functions in crop rotation systems in the southern Kyushu region of Japan.

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南九州地域の黒ボク土充填小型枠におけるソルガム, マリーゴールド, エゴマ栽培がアーバスキュラー菌根菌胞子数に及ぼす影響

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要　　旨

南九州地域の黒ボク土充填小型枠におけるソルガム, マリーゴールド, エゴマ栽培が土壌中のアーバスキュラー菌根菌胞子数に及ぼす影響を調査した。供試作物をそれぞれ 2 年～ 4 年連作し，その後一部の処理区では輪作試験へ移行した。栽培後の晩秋または翌年春に株間土壌または耕うん後土壌を採取し，一部改変したふるい分け＋ショ糖遠心法により菌根菌胞子を計数した。作物栽培による菌根菌胞子数の推移を調べた。ソルガム栽培により菌根菌胞子数は，40 胞子 10 g^{-1} 乾土以上の高いレベルとなった。マリーゴールド栽培により菌根菌胞子数は 10 ～ 30 胞子 10g^{-1} 乾土の範囲になり，対照区（土壌充填後休閑区，菌根菌胞子数 6 ～ 12 胞子 10g^{-1} 乾土の範囲）と比べて同等または増加した。エゴマ栽培により菌根菌胞子数は対照区と比べて増加し，約 20 ～ 30 胞子 10 g^{-1} 乾土となった。ソルガムの地上部生産量は, マリーゴールドやエゴマの地上部生産量と比べてはるかに大きかった。ただし，ソルガムを 3 年あるいは 4 年連作した区では，連作障害（初期生育障害）が発生し，地上部生産量が低下した。これに伴って，菌根菌胞子数が栽培前と比較して減少する傾向を示した。マリーゴールド・エゴマ連作からソルガム栽培へ移行すると，菌根菌胞子数は増加した。ソルガム連作からマリーゴールド栽培あるいは休閑へ移行すると，胞子数は減少した。以上の結果から，ソルガム栽培により土壌中の菌根菌胞子数が高いレベルに増加することが示唆された。こうした結果を，九州地域における菌根菌活性を高める輪作体系へつなげることが今後の課題である。

キーワード：アーバスキュラー菌根菌，エゴマ，ソルガム，地上部生産量，胞子数，マリーゴールド。

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