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REVIEW

Traits of the Genus *Crotalaria* Used as a Green Manure Legume on Sustainable Cropping Systems

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Abstract

To evaluate the potentials of growth and nitrogen (N) fixation of genus *Crotalaria* used as green manure, interspecific differences in dry matter production, root nodule formation, and N uptake among several species were investigated in field and pot experiments at Osaka, Japan. *C. juncea*, *C. spectabilis*, *C. pallida*, and *C. incana* exhibited vigorous vegetative growth, expanding leaf area and branching, and they began to flower in mid-July to early August. *C. juncea* had a high dry weight and a great N content at early growing stage probably due to faster root nodule formation. On the other hand, *C. pallida* grew slowly at the early stage due to slower nodulation, but had a high N content at the late stage. Incorporation of each species provided a large amount of N necessary for the succeeding wheat crops. Several indicators for rapid decomposition of the plants, such as total C, N and lignin contents, were investigated, and the possible increases in N supply and growth inhibition by incorporation to wheat crops were discussed. N-absorbing activity of *C. juncea* grown under the excess N condition (70 gN m^{-2}) was also evaluated. The proportion of fixed N to total N was less than 1%, and the C/N ratio was significantly lower than that in *Z. mays*, suggesting that this plant species could be also useful as a cleaning crop.

Discipline: Crop production
Additional key words: cleaning crop, crop rotation, decomposition, nitrogen fixation, rhizobia

Introduction

Establishment of efficient use of nitrogen fixing legumes in crop production systems is one of the current goals of agricultural research, in which many researchers have been aiming to accomplish the sustainability and stability of crop production. Several summer leguminous green manure plants have been utilized in sustainable agricultural systems especially in tropical and subtropical regions, and their effects on growth and nitrogen uptake in the succeeding crops have been evaluated[2,3,12,22,28].

The genus *Crotalaria* is one of the important green manure legumes. This plant genus includes annual and perennial species native to India and Africa, and it has been spread to several tropical and subtropical regions as fiber and green manure crops[2,8,14]. *Crotalaria* plants are also expected to be useful in temperate regions as plants antagonistic to parasitic nematodes, because of their nematocidal root property[13,14]. Among about 470 species described in the world, *C. juncea*, *C. spectabilis* and *C. breviflora* are used as green manure and/or antagonistic plants in Japan, and they should be further introduced to various sustainable cropping systems, especially for environmental rehabilitation in intensive crop production systems.

Prerequisites for efficient application of green manure legumes are selection of an appropriate plant species and the identification of the appropriate time of incorporation into soil for the succeeding crops. The author has conducted researches on the potentials of dry matter production and nitrogen fixation in some *Crotalaria* species, such as *C. juncea*, *C. spectabilis*, *C. pallida*, and *C. incana* also on both positive and negative effects of the green manures on the succeeding crops in regard to the supply of fixed nitrogen and allelopathic inhibition during plant decomposition. In the present paper, several traits of these *Crotalaria* species were described, and the possibility of the use of these species as green manures was discussed from the viewpoints of

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low input and sustainable crop production systems.

Interspecific differences in growth and N fixation among *Crotalaria* species

In order to apply the green manure plants efficiently under the various cropping systems, it is essential to evaluate several ecophysiological traits, such as dry matter production, earliness and nutrient-use efficiency of the plants. Especially in leguminous green manure plants, characteristics in relation to nitrogen fixation have to be investigated. We evaluated the interspecific difference in growth and root nodule formation among three *Crotalaria* species, *C. juncea*, *C. spectabilis*, and *C. pallida*[6].

The experiment was carried out on the Experiment Farm at Osaka Prefecture University in Sakai, Osaka, Japan. The soil on the farm was Gray Lowland soil with a pH (H_2O) of 5.6, EC (Electric Conductivity) of 0.02 dS m^{-1}, TN (Total Nitrogen) ratio of 0.06%, TC (Total Carbon) ratio of 0.50%, Truog-P of 57 mg kg^{-1}, exchangeable K of 0.26 cmol kg^{-1}, exchangeable Mg of 0.24 cmol kg^{-1}, exchangeable Ca of 0.77 cmol kg^{-1}, and CEC (Cation Exchange Capacity) of 15.6 cmol kg^{-1}. Seeds of each species were sown on early May in 1992, and the plants were sampled at 40, 80, 120, and 160 days after sowing (DAS).

Seedlings of *C. juncea*, *C. spectabilis*, and *C. pallida* emerged at 3, 7, and 10 DAS, respectively. Each species had numerous yellow flowers at anthesis begun on mid-July to early August. The pods were produced at the end of September in all the species. Changes in plant height and diameter of the main stem are shown in Table 1. When *Crotalaria* species is grown for green manure, stem diameter may be an important factor for the incorporation into soil. The diameter of the main stem at 5 cm height in *C. pallida* was significantly thicker than that for two other species, and it was actually difficult for *C. pallida* to be harvested at the ground level by a sickle. On the other hand, this plant species continuously produced new leaves, and the foliage was dark green in color even at 160 DAS, when *C. juncea* and *C. spectabilis* showed drastic defoliation. *C. juncea* had a high dry weight and a great nitrogen content at early growing stage probably due to faster root branching and nodulation (Fig. 1). On the other hand, *C. pallida* grew slowly at the early stage due to slower root nodule formation, but had a high nitrogen content at the late stage.

We also examined the changes in growth and nitrogen content in *C. pallida* and *C. incana*[25]. This experiment was also carried out on the Experiment Farm at Osaka Prefecture University. Seeds of each species were sown in a seeding box on early May in 2001, and the seedlings were transplanted to each plot of the field at 18 DAS. Tops of the plants were sampled at 56, 87, and 120 DAS.

There was a difference in the growth rate between the two species. The length and diameter of the main stems of *C. pallida* were greater than those of *C. incana* throughout the growing period. On the contrary, the number of nodes on the main stem was larger in *C. incana* than in *C. pallida* from the early growing stage, which showed that the leaf emergence rate was higher in the former. At each sampling date, the dry weight and total nitrogen content of *C. pallida* were significantly higher than those of *C. incana*. A marked increase in dry weight and total nitrogen content was obtained from 56 to 87 days after transplanting (DAT) in *C. pallida*, and from 87 to 120 DAT in *C. incana*. The top dry weight ranged from 0.3 to 1.8 kg m^{-2} at 87 and 120 DAT, the possible time of incorporation into soil, respectively, and the nitrogen yield ranged from 8.6 to 40.0 g m^{-2} at that time.

The differences in the dry matter and nitrogen accumulation between *C. pallida* and *C. incana* could be attributed to the difference in the potential biomass pro-

Table 1. Changes in plant height and diameter of the main stem in the three *Crotalaria* species grown in the experimental field

	Days after sowing	*C. juncea*	*C. spectabilis*	*C. pallida*	LSD (P = 0.05)
Plant height (cm)	40	47.5	16.2	16.1	2.1
	80	166.3	79.1	81.3	8.8
	120	213.3	148.7	148.3	18.7
	160	235.8	150.9	171.4	16.2
Stem diameter[a)] (mm)	40	3.2	2.3	2.2	0.4
	80	9.8	7.9	6.9	1.4
	120	10.2	9.3	11.9	1.8
	160	10.9	10.4	14.1	2.3

a): Measured at 5 cm height. (From Daimon et al., 1995)

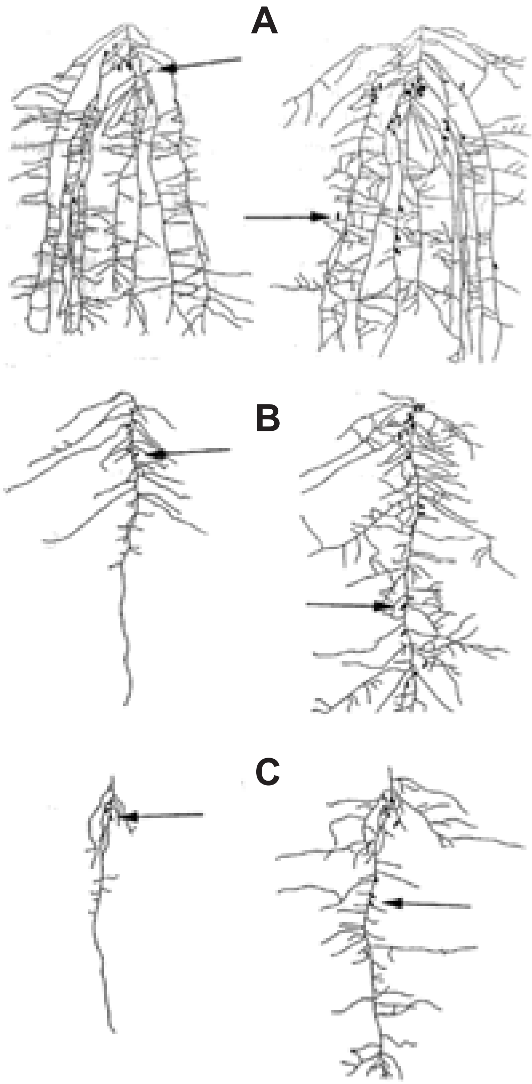

Fig. 1. The profiles of root systems of *C. juncea* (A), *C. spectabilis* (B), and *C. pallida* (C) grown under root box conditions at 20 (left) and 35 (right) days after sowing
Arrows indicate root nodules in each species.
(From Daimon et al., 1995)

ductivity and nitrogen fixing ability, but also to the difference in the defoliation rate of lower leaves due to different duration of vegetative growth. In *C. incana*, lower leaves defoliated continuously with emerging new leaves at the upper nodes after mid-August. In *C. pallida*, on the other hand, foliage was dark green even in late-September. Thus, *C. pallida* used as green manure may provide a larger amount of nitrogen to the succeeding crops than *C. incana*. However, it should be noted that the reduced dry matter production in *C. incana* is not necessarily undesirable as a green manure crop, because the defoliated biomass might contribute to the initial growth of the succeeding crop. Based on the pattern of defoliation, a suitable relay cropping pattern should be also examined.

Chemical properties of the green manure

The amount of nitrogen released from green manures incorporated into soil varies with the chemical properties of the plants. There are many reports showing that not only the C/N ratio of the incorporated plants but also other parameters, such as contents of lignin[23,27] and polyphenols[15,20], ratio of lignin to total N, ratio of polyphenols to total N, and ratio of (lignin + polyphenols) to total N[4,26], are determinants of green manure quality regarding the nitrogen release after incorporation. Plants with high ratios of these parameters are usually considered to decompose more slowly than those with low ratios.

We also investigated the changes in ratios of C/N and L (lignin)/N of *C. incana* and *C. pallida* grown in the field experiment described above. The C/N ratio of the plant tops in *C. pallida* was slightly lower than that in *C. incana* at 56 DAT, but was slightly higher than that of *C. incana* at 120 DAT (Table 2). The C/N ratio of leaves gradually decreased from 56 to 120 DAT, but that of stems markedly increased throughout the growth period

Table 2. Changes in C/N ratios of leaves and stems in the two *Crotalaria* species grown in the experimental field

Plant species	Leaf			Stem			Top		
	56DAT	87DAT	120DAT	56 DAT	87 DAT	120 DAT	56 DAT	87 DAT	120 DAT
C. pallida	10.1	8.8	8.7	23.0	38.4	49.2	13.0	16.5	22.1
C. incana	12.3	10.0	8.9	28.8	37.1	40.0	15.4	16.4	20.4
t-test	*	*	NS	*	NS	*	*	NS	*

*: Significant difference at 5% level. NS: Not significant. (From Uratani et al., 2004)
DAT: Days after transplanting.

in both species. The lignin contents of leaves and stems in *C. pallida* were significantly higher than those in *C. incana* at 87 and 120 DAT (Table 3). The L/N ratio of the plant tops increased from 87 to 120 DAT in both species, and was significantly higher in *C. pallida* than in *C. incana*. On the other hand, there was no difference in the L/N ratio of leaves between 87 and 120 DAT. Although there was a difference between the two species in the C/N ratio of the tops at 120 DAT, this may not be critical for nitrogen mineralization capacity after incorporation into soil, because green manures with a C/N ratio of lower than 25 are known to decompose rapidly[1,23]. The difference between the two species in either lignin content or L/N ratio of tops was similar to that in C/N ratio. These values might also not be critical for nitrogen release according to the findings by Vityakon et al.[26], who showed that nitrogen mineralization from residues of *Arachis* and *Sesbania* was not reduced even when the lignin content reached 9.9–11.1%, or L/N ratio 5.7–7.4. Some other factors such as polyphenols, cellulose and hemicellulose, which influence the rate of decomposition, should be analyzed with variation of the developmental stages.

Positive and negative effects of green manures on the succeeding crops

We have evaluated the effect of some leguminous crops such as *Crotalaria*, *Sesbania* and forage legumes on the succeeding crops such as wheat, corn and spinach[5,7,17,18,25,29]. In the case of *C. juncea*, *C. spectabilis*, *C. pallida*, and *C. incana*, the incorporation of each species provided a large amount of nitrogen necessary for the succeeding wheat crop. Regarding the contribution of fixed-nitrogen to the wheat crop, we calculated the percentage of nitrogen contribution from green manure (Table 4). The percentage recovery of nitrogen in the wheat crop ranged from 8 to 22%, and these values might be not necessarily low.

On the other hand, some reports indicated that growth inhibition of the succeeding crops by green manure and/or surface plant residues due to releasing allelopathic compounds during plant decomposition might occur in different cropping systems[9,10,21]. We also found inhibitory effects on root growth of the succeeding crop grown after incorporation of *Crotalaria* plants[7,16,18,25,29]. In order to clarify these inhibitory effects,

Table 3. Changes in lignin content and L/N ratio of leaves and stems in the two *Crotalaria* species grown in the experimental field

	Plant species	Leaf		Stem		Top	
		87 DAT	120 DAT	87 DAT	120 DAT	87 DAT	120 DAT
Lignin content (%)	*C. pallida*	7.9	7.5	11.8	14.7	10.3	12.9
	C. incana	5.3	5.5	10.6	13.2	8.1	11.1
	t-test	*	*	*	*	*	*
L/N ratio	*C. pallida*	1.5	1.4	10.2	15.4	3.8	6.1
	C. incana	1.3	1.1	9.5	11.7	3.2	5.0
	t-test	*	*	NS	*	*	*

*: Significant difference at 5% level. NS: Not significant. (From Uratani et al., 2004)
DAT: Days after transplanting.

Table 4. Contribution of nitrogen accumulated by *Crotalaria* species to nitrogen uptake of the succeeding wheat plant

Plant species	Experiment condition	Total N of *Crotalaria* plant	Total N of wheat plant	Percentage of N contribution from green manure (%)	Reference
C. juncea	Field	20.3 gm^{-2}	3.6 gm^{-2}	9.4	Yano et al.[29]
C. juncea	Wagner pot (1/5,000a)	550 mg $plant^{-1}$	54 mg $plant^{-1}$	9.8	Ohdan & Daimon[17]
C. pallida	Wagner pot (1/5,000a)	490 mg $plant^{-1}$	106 mg $plant^{-1}$	21.6	Ohdan & Daimon[17]
C. pallida	Small pot [a)]	78.6 mg $plant^{-1}$	6.2 mg $plant^{-1}$	7.9	Uratani et al.[25]
C. incana	Small pot [a)]	92.3 mg $plant^{-1}$	9.3 mg $plant^{-1}$	10.1	Uratani et al.[25]

a): Vinyl pot (10 cm diameter and 13 cm depth).

we examined the effect of the incorporated materials on the root growth of wheat plants by using the growth pouch culture technique.

Aqueous extracts were prepared from fresh leaves and stems of six *Crotalaria* species, *C. brevidens*, *C. juncea*, *C. lanceolata*, *C. pallida*, *C. sessiliflora*, and *C. spectabilis*, and they were put into seed pack growth pouches. Three-day-old seedlings of wheat were transferred to the pouches, and the growth was investigated after 21 days of culture[16]. Application of the extract remarkably depressed the top dry weight and the length of the longest root of wheat. Based on the control, the length was suppressed by 24% with 10 g of stem to 55% with 20 g of leaf.

We also evaluated the inhibitory effect of the incorporated materials of *C. spectabilis*, which had been sown at different seeding rates in the field[7]. Seeds of *C. spectabilis* were sown at the seeding rates of 1 (LD), 5 (MD), and 10 (HD) g m^{-2}. The plants were harvested and then used as materials for aqueous extracts in a growth pouch experiment. Application of the extract prepared from HD plants depressed the dry weights of top and root, and total root length with each application of leaf and stem (Table 5). The significant differences in dry weight and total

Table 5. Effect of aqueous extracts of leaves and stems of *C. spectabilis* grown under different seeding rates on the dry weight and total root length of wheat plants cultured under growth pouch conditions at 20 days after transfer

Plant part	Treatment	Dry weight (mg)			Total root length (cm)
		Top	Root	Whole plant	
Leaf	Low	24	23	47	130
	Medium	23	23	46	98
	High	20	21	41	85
Stem	Low	25	28	53	136
	Medium	25	28	53	132
	High	21	23	44	115

C. spectabilis was grown at low (1 gm^{-2}), medium (5 gm^{-2}) and high (10 gm^{-2}) seeding rates and then extracted.
(From Daimon & Kotoura, 2000)

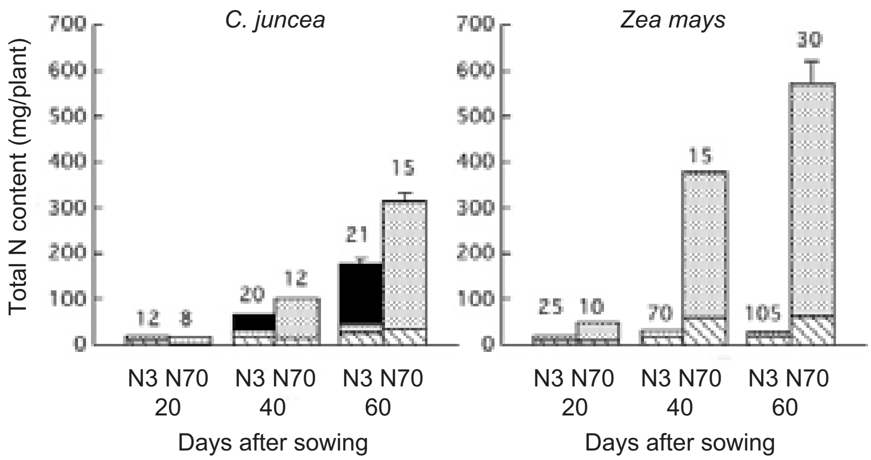

Fig. 2. Changes in nitrogen content derived from fixed-N (dffix), fertilizer-N (dffer), and soil-N (dfsoil) in *C. juncea* and *Zea mays* grown under different N application rates
N3: Applied N at 3 gm^{-2}. N70: Applied N at 70 gm^{-2}.
Values represent the C/N ratios of whole plant.
■ : N dffix, ⊡ : N dffer, ⧅ : N dfsoil. (From Ohdan & Daimon, 1999)

root length between the two plant parts indicated that leaf extract more severely inhibited the growth than stem extract.

Many crops could be affected by allelopathic compounds such as phenolic acids, alkaloids, fatty acids, and flavonoids produced by associated plants and the residues through their decomposition[11,24]. *C. spectabilis*, which has been reported to produce the toxic substance to livestock and nematode, pyrolozidine alkaloid "monocrotaline"[13,14], may also release phytotoxic substances to influence the crop growth. Those toxic substances and their qualitative and quantitative variations with the growth phases should be identified.

Nitrogen recovering ability as a cleaning crop

Since symbiotic nitrogen fixation in legumes associated with rhizobia is inhibited by nitrate nitrogen applied to the soil, the potential of absorbing nitrogen by *Crotalaria* plants showing vigorous root growth due to higher photosynthetic activity could alleviate nitrate-accumulated soils.

Nitrogen-absorbing activity of *C. juncea* grown in nitrogen-accumulated soil was compared with *Zea mays* in an experiment conducted with containers filled with volcanic ash soil[19]. Under the excess nitrogen condition (70 gN m^{-2}), total nitrogen content in the tops of *C. juncea* at 60 DAS was 55% of that in *Z. mays* (Fig. 2). In *C. juncea* grown under the excess nitrogen conditions, however, the proportion of fixed nitrogen to total nitrogen was less than 1% and nodulation was negligible, and the C/N ratio was significantly lower than that in *Z. mays*, suggesting that this plant species could be useful as a cleaning crop to accumulated-nitrogen, and then it would be used as faster decomposing organic matter.

Conclusion

Nitrogen cycling is one of the most important factors for a sustainable crop production system. Among various green manure legumes, *Crotalaria* has been recently evaluated as an important legume for the low input cropping system in Japan. To exploit the potentials of higher dry matter production, nitrogen fixation and nutrient recovery of this exotic green manure legume, genus *Crotalaria*, in sustainable agricultural systems, it is necessary to more effectively introduce them to each cropping system. Understanding the sequestration and recycling of C, N, P, and other nutrients derived from green manures is required to enable this plant genus to be used as an appropriate member even in the field management practices of an intensive agriculture system.

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