When the Natural Toxin Concentration in Crotalaria Increase in Japan?

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Key words: [*Crotalaria juncea* L.; flowering; podding; Pyrrolizidine alkaloids; Trichodesmine]

Abstract

A green manure crop, sunn hemp (*Crotalaria juncea* L.), legumes, has a nematode-suppressive effect. It is also used as feed in India. However, it is not used as feed in Japan because it contains natural toxins: Pyrrolizidine alkaloids (PAs). PAs has the negative effects for not only livestock but also human health. The PAs, which are abundant in sunn hemp seeds and which are contained only slightly in leaves and stems. Therefore, if sunn hemp harvested before flowering, then no poison problem is posed by PAs. However, if it is harvested at the podding stage, a poisonous concentration of PAs occurs. For increasing yield, it is necessary to harvest it as late as possible. For obtaining both safe feed without PAs and increase in yield, we clarify when the PAs concentration will be increased from flowering to podding.

We cultivated the varieties of crotalaria which are distributed currently in Japan and USA for green manure during July–October 2019. We observed the period from flowering to podding. We harvested bud, flower, immature pods (under 15 mm) and pods. Then one PAs component that is abundant in seeds, the trichodesmine content, was analyzed.

In the case of late July seeding, the first flowers blossomed in about 50 days after seeding. No difference was found between varieties. The flowers wilted about two days after flowering. The ovaries grew to immature buds about five days after flowering. Trichodesmine in seeded seeds was greater than 1000 mg/kg. That in buds, flowers, and immature pods was less than 1.0 mg/kg. Also, that in pods (over 15 mm length) was greater than 10 mg/kg.

If harvested within one week from the first flower blooming, then sunn hemp can be used as forage with low PAs concentration.

Introduction

Sunn hemp (*Crotalaria juncea* L.), Legumes, with a nematode-suppressive effect (Kushida et al. 2003) is used worldwide as a green manure crop. Moreover, sunn hemp is used traditionally as feed in India (Narayanan and Dabadghao, 1972). Nevertheless, it is not used as feed in Japan because it contains natural toxins: pyrrolizidine alkaloids (PAs). PAs are abundant in sunn hemp seeds and are contained only in small quantities in leaves and stems (Mosjidis et al. 2012). If it is harvested before flowering, no poison problem is posed by PAs. Therefore, in recent years, research on feed use has become active in the USA (e.g. Lepcha et al. 2018, Jaramillo et al. 2020, Garzon et al. 2021). For increasing yield, it is necessary that it be harvested as late as possible. However, no report describes PAs concentrations between flowering to podding. In this study, we clarify when PAs concentration in sunn hemp will be increased from flowering to podding for forage use without PAs.

Study Site and Methods

Sunn hemp was cultivated at Kyushu Okinawa Agricultural Research Center, NARO, Kumamoto, Japan (32°53'N, 130°44'E, 78 m a.s.l.) during July–October 2019. The respective average monthly temperatures in January (lowest) and August (highest) were 5.1°C and 26.8°C in 2019. The annual mean precipitation was 1882 mm. As a basal fertilizer, a compound fertilizer (N: P_2O_5: K_2O, 16%:16%:16%) was applied at rates equivalent to 60 kg N/ha immediately before sowing seeds. The plot size was 3 × 4 m^2 with three replications. The three varieties of sunn hemp, Nekobukira, Nemakorori, and Kobutorisou, that are currently distributed in

Japan for green manure and the one variety from USA were sown on July 25, 2019. The seeding rate was 60 kg/ha. It was suppressed with a roller after sowing.

We recorded the day of first flowering and observed the period from flowering to podding for each plot. We harvested buds, flowers, immature pods (under 15 mm length), and pods (over 15 mm length) of the two varieties: Nemakorori and a variety from the USA on Oct 15 (Figure 1). Dried (60°C, 72 hr) and powdered 4 g samples were extracted with 150 ml extraction solvent (acetone: water (2:1)). The extracts were adjusted with acetone and diluted with ethanol. Then one PAs component that is abundant in seeds, the trichodesmine content in seeded seeds and harvested bud, flower, immature pods, and pod, was analyzed using LC-MS/MS (Triple Quad 6500+, AB Sciex Pte. Ltd., MA, USA).

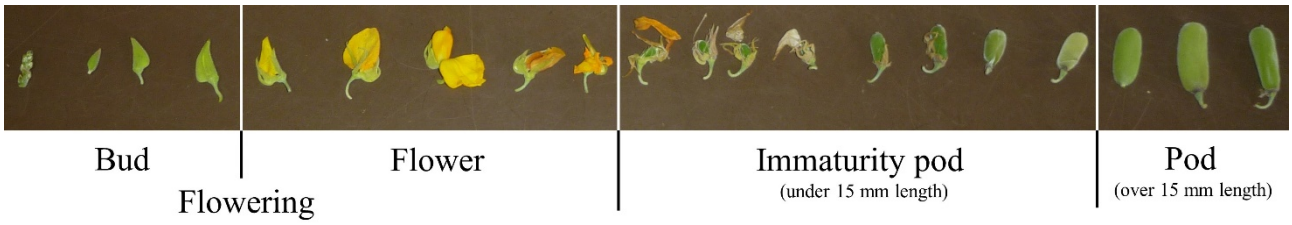

Figure 1. Bud, flower, immaturity pod (under 15 mm length), and pod (over 15 mm length) of crotalaria.

Results

The average days of first flowering of all varieties were found to be 47 days from seeding with no differences among varieties. Petals withered around 3–4 days after flowering with immature pod appearance. Pods grew to 10 mm length at 5 days from flowering and 15 mm length at 6 days from flowering.

Trichodesmine content of Nemakorori and a variety from USA were, respectively, 1800 and 1300 mg/kg in seeded seeds, 0.2 and 0.1 mg/kg in buds, 0.7 and 0.8 mg/kg in flowers, 0.8 and 0.7 mg/kg in immature pods (less than 15 mm length), and 24 and 13 mg/kg in pods (over 15 mm length).

Discussion

Sunn hemp grow under short-day conditions, flowering at 47 days, irrespective of variety. At around 4 days after flowering, the flowers withered; pods appeared and matured. At 5 days after flowering, pods are immature and of less than 15 mm length without an increase of trichodesmine. Our results show that trichodesmine does not increase until the seed-setting stage. Sunn hemp seeds include over 1000 mg/kg trichodesmine. Therefore, trichodesmine probably increases rapidly with seed maturity. In this study, the greater than 15 mm length pod division included various sizes and maturity levels. Pods of greater than 15 mm length include immature ones that might include less PAs. Further subdivision analysis will engender extension of the periods with low concentrations of trichodesmine.

Implications

The trichodesmine concentration of flower and immature pods was less than 1 mg/kg, similar to those of leaves and stems which can be used for feed (Mosjidis et al. 2012). Therefore, sunn hemp can be used as forage without toxicity within 5 days after first flowering.

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