

# The Length and Density of Prickles on *Zanthoxylum ailanthoides* (Rutaceae): A Comparison of Japanese Islands with Different Sika Deer Browsing Pressures

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Received December 6<sup>th</sup>, 2013; revised January 11<sup>th</sup>, 2014; accepted January 24<sup>th</sup>, 2014

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## ABSTRACT

To determine the effects of sika deer (*Cervus nippon*) browsing on the physical defences of the Japanese prickly-ash "*Zanthoxylum ailanthoides* Sieb. et Zucc." (Rutaceae), we compared the length and density of prickles on Japanese islands which were under different browsing pressures. We measured the length and density of prickles on the midribs, leaf rachis, and stems. We found that the prickles of *Z. ailanthoides* on Kashima island were not significantly longer or at higher densities than those in the neighbouring areas; the longest pickles at the highest densities were found on Akune island. The density of sika deer on Akune (ca. 520 - 600/km<sup>2</sup>) was higher than that on Kashima (ca. 38.5/km<sup>2</sup>), and consequently, Akune was under greater browsing pressure. Our results suggest that the increased length and density of prickles on Akune is a response by *Z. ailanthoides* to the high density of sika deer found on the island.

## KEYWORDS

Deer; Defence; Density; Herbivore; Island; Length; Sika

## 1. Introduction

Plants have developed numerous physical and chemical defences against herbivores. Such defences are considered beneficial because they ultimately reduce herbivory, and increase plant fitness [1]. The physical defences of plants are required to withstand abiotic factors, such as wind damage, and biotic factors, such as herbivory. The Japanese sika deer (*Cervus nippon*) eats a wide variety of plants and is a gregarious species. Consequently the effects of sika deer on plant communities are increasing in the forests and alpine ecosystems of Japan [2], and local increases in sika deer numbers compound these effects [3-5]. Indeed, sika deer have a large impact on agricultural and forested vegetation in Japan [2], and on the islands of southern Japan, are important in seed dispersal, particularly the small seeds of graminoids [6,7].

The heavy browsing of sika deer has therefore altered the structure of some Japanese forests [2].

Prickles are thought to reduce the quantity of twigs or shoots eaten per unit of time by vertebrate herbivores by reducing bite size or biting rate [8-10], and are a defence primarily against mammalian herbivores [11]. Interestingly, some studies indicate that plants have developed long prickles as a defence against heavy browsing by sika deer on small Japanese islands. For example, Kin-kazan island (32°57'N, 132°27'E; area: 959 ha), which is situated approximately 1 km off the Pacific coast of Honshu, has a high density of sika deer (ca. 63.2/km<sup>2</sup>) [12]. As a result, *Cirsium amplexifolium* (Nakai) Kitam. var. *muraii* (Kitam.) Kitam. (Asteraceae) has a limited distribution on the island, and has longer prickles around its leaf edges and stems than those of *C. amplexifolium* var. *amplexifolium*, that occurs over a wide area of northern Honshu [13]. In addition, Kashima isl-

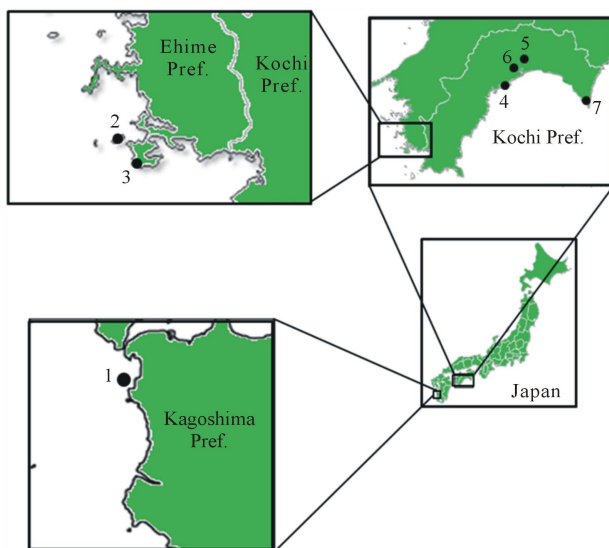
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and (32°57'N, 132°27'E; area: 45.1 ha) (Figure 1), which is located off southwest Shikoku, has a high density of sika deer (ca. 38.5/km<sup>2</sup>) [14], and the prickles of *Rubus sieboldii* Blume (Rosaceae) are significantly longer and at higher densities than those in ungrazed neighbouring localities, based on comparative morphological analysis [15].

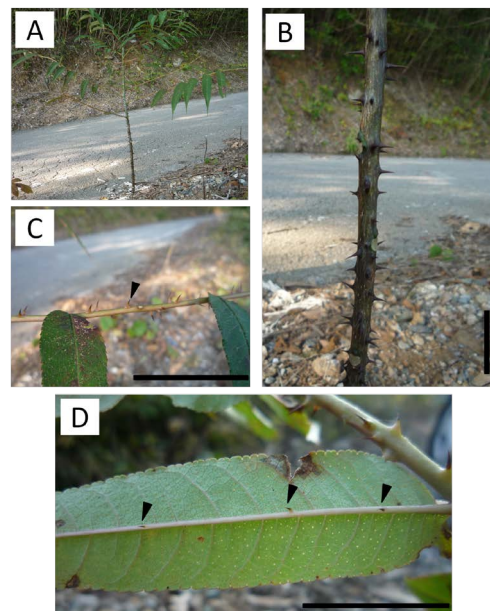
These results suggest that many other plant species may have developed long prickles under the high browsing pressure of sika deer, but which species in particular remains unknown.

On Kashima, several plant species exist that possess prickles [16]. Of these, *Zanthoxylum ailanthoides* Sieb. et Zucc. (Rutaceae) is a medium to large deciduous pioneer species that has pinnate leaves and conical prickles (Figure 2) and is found in Japan, Taiwan, Korea, China, and other parts of southeast Asia [13]. Further, it is one of the avoided plant species because of its prickles, which is a form of anti-herbivore defence [14]. This species may not only possess physical defences against herbivores, but also chemical defences, since it contains lignans, coumarins, quinolines, flavonoids, triterpenoids, and other substances [17]. However, this species is frequently damaged by sika deer on Mt. Kasugayama (ca. 950/km<sup>2</sup>) [4,18]. Therefore, the chemical defences of this species may be most effective against insect rather than deer attack, suggesting that it is possible to evaluate the effects of sika deer browsing by measuring the characteristics of the plants' physical defences (*i.e.* prickles) as has been carried out for other plant species.

Kamijo *et al.* (2001) [19] conducted a comparative study of seedling morphology of *Z. ailanthoides* on the



**Figure 1.** Sampling sites in this study. 1: Akune island; 2: Kashima island; 3: Komo Cape; 4: Yokonami; 5: Kurotaki; 6: Hitsuzan; and 7: Muroto.



**Figure 2.** *Zanthoxylum ailanthoides* (photographs were taken at Kurotaki, Kochi Prefecture). (A) *Zanthoxylum ailanthoides*; (B) Stem; (C) Leaf rachis; (D) Midrib. Bar = 1 cm. Arrowheads indicate prickles.

Izu islands, where there are no sika deer, and the Izu Peninsula, where sika deer are present. The percentage dry weight of prickles was lower on the *Z. ailanthoides* found on the Izu islands than those of the *Z. ailanthoides* found on the Izu Peninsula [19]. In addition, a non-prickle form, *Z. ailanthoides* var. *ailanthoides* f. *espinosum* Yonek, was found on Nii-jima island, which is one of the Izu islands [19]. It might therefore be possible for *Z. ailanthoides* to grow with no prickles, or few prickles, where sika deer are absent. Kamijo *et al.* (2001) [19] measured the relative weight of prickle to plant size using germinated seedlings derived from only one parent per population, under nursery conditions. However, no studies have been conducted that have used large numbers of samples derived from different parents, and grown under natural conditions with heavy browsing by sika deer.

In this study, we investigated whether *Z. ailanthoides* plants on Kashima have also developed long prickles to resist browsing by sika deer, as have *R. sieboldii* plants. The flora of Akune island (130°10'E 32°01'N) is similar to that of Kashima [14,20], and includes *Z. ailanthoides*. Akune is a small island (29.3 ha) off the southwest of Kyushu (Figure 1), and has a high density of sika deer (ca. 520 - 600/km<sup>2</sup>) [21,22]. It is possible that the prickles of *Z. ailanthoides* on Akune are also long as a defence against sika deer. The density of sika deer differs between Kashima and Akune, therefore the length and density of prickles of *Z. ailanthoides* may differ between

the islands. The aim of this study was to compare the prickle characteristics of *Z. ailanthoides* on Akune and Kashima with those of neighbouring areas.

## 2. Materials and Methods

*Z. ailanthoides* samples were collected from Kashima and Akune (that were heavily browsed by sika deer), and 5 areas close to Kashima (Komo Cape, Yokonami, Kurotaki, Hitsuzan, and Muroto) in 2012 and 2013 (Figure 1, Table 1). The height of individual plants included was less than 1.5 m, which is the maximum height reached by sika deer. To measure prickle length and density on the midribs and leaf rachis of *Z. ailanthoides*, five fully expanded leaves from the middle of each plant were collected. The mean length and density of prickles were calculated based on measuring the length and density of 5 prickles per leaf. The mean length of prickles on a stem was calculated as the mean length of 5 prickles from 10 locations near the middle of the stem. Prickle density on stems was measured by counting the number of prickles per square section of the stem surface area at 10 locations

on each plant. We measured leaf area, tree diameter at the base, and plant size, and analysed the prickle traits in comparison with the other traits. All statistical analyses were performed using SPSS Statistics 19 (IBM SPSS, Chicago, Illinois).

## 3. Results and Discussion

We analysed the length and density of prickles on the midribs, leaf rachis, and stem surfaces of *Z. ailanthoides* on Kashima and neighbouring areas, and on Akune (Table 2). The length and density of *Z. ailanthoides* prickles on Kashima were similar to those in the neighbouring areas (Figure 3, Table 2), suggesting that the grazing pressure of sika deer did not affect prickle characteristics on this island. The Akune population had the longest prickles, and the highest densities of prickles, of all the populations analysed (Figure 3, Table 2). These results suggest that *Z. ailanthoides* prickles on Akune had increased in length and density to counteract heavy browsing by sika deer. We did not find any relationships between prickle traits and the other traits measured (data

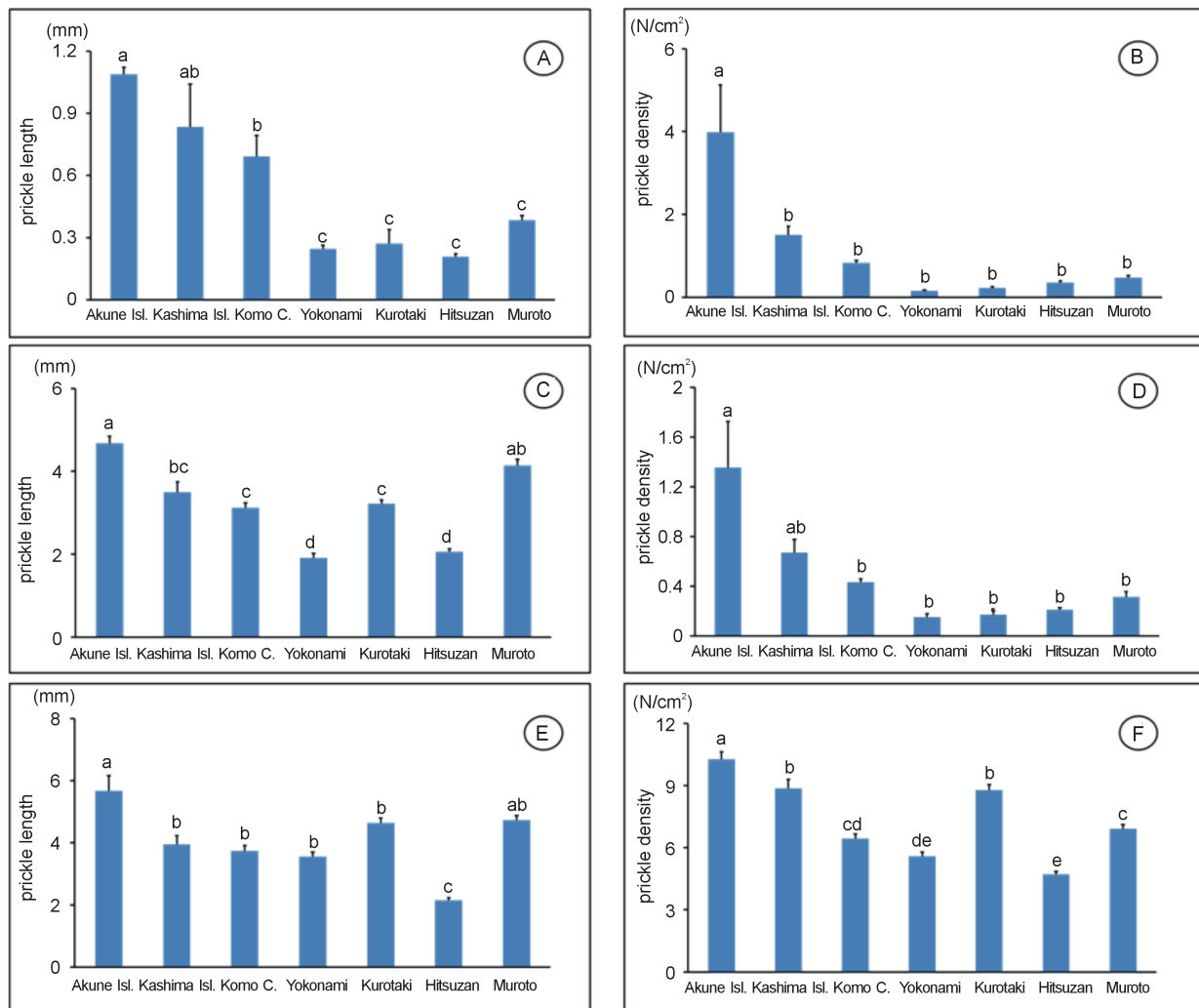
**Table 1. Sampling sites in this study.**

Location name	Location no.	Number of Individuals	Location	Latitude and longitude
Akune Island	1	30	Kagoshima Prefecture, Akune City	N 32°01' E 130°10'
Kashima Island	2	15	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho Kashima	N 32°56' E 132°27'
Komo Cape	3	30	Ehime Prefecture, Minamiuwa-Gun, Ainan-Cho Komo	N 32°54' E 132°28'
Yokonami	4	30	Kochi Prefecture, Susaki City, Uranouchi Yokonami	N 33°25' E 133°21'
Kurotaki	5	30	Kochi Prefecture, Nankoku City, Kurotaki	N 33°39' E 133°36'
Hitsuzan	6	30	Kochi Prefecture, Kochi City, Hitsuzan-Cho Hitsuzan	N 33°33' E 133°31'
Muroto	7	30	Kochi Prefecture, Muroto City, Murotomisaki-Cho Muroto	N 33°15' E 134°10'

**Table 2. Morphological measurements (average ± standard deviation) of *Zanthoxylum ailanthoides*.**

Trait	Akune Island	Kashima Island	Komo Cape	Yokonami	Kurotaki	Hitsuzan	Muroto
Midrib prickles							
Length (mm)	1.09 ± 0.03 a	0.83 ± 0.21 ab	0.69 ± 0.10 b	0.25 ± 0.02 c	0.27 ± 0.07 c	0.21 ± 0.01 c	0.38 ± 0.02 c
Density (N/cm <sup>2</sup> )	3.99 ± 1.14 a	1.51 ± 0.20 b	0.83 ± 0.06 b	0.16 ± 0.02 b	0.22 ± 0.03 b	0.35 ± 0.04 b	0.48 ± 0.04 b
Rachis prickles							
Length (mm)	4.68 ± 0.16 a	3.50 ± 0.25 bc	3.12 ± 0.12 c	1.91 ± 0.11 d	3.22 ± 0.09 c	2.06 ± 0.07 d	4.14 ± 0.15 ab
Density (N/cm <sup>2</sup> )	1.36 ± 0.37 a	0.67 ± 0.11 ab	0.43 ± 0.03 b	0.15 ± 0.03 b	0.17 ± 0.03 b	0.21 ± 0.02 b	0.32 ± 0.04 b
Stem prickles							
Length (mm)	5.67 ± 0.49 a	3.96 ± 0.27 b	3.75 ± 0.16 b	3.56 ± 0.14 b	4.65 ± 0.51 b	2.15 ± 0.09 c	4.73 ± 0.15 ab
Density (N/cm <sup>2</sup> )	10.26 ± 0.36 a	8.85 ± 0.44 b	6.45 ± 0.20 cd	5.59 ± 0.20 de	8.78 ± 0.27 b	4.71 ± 0.14 e	6.91 ± 0.22 c

Values that have the same letter within a column indicate no significant difference at the 5% level (Tukey HSD test).



**Figure 3.** Morphological measurements of the prickles of *Zanthoxylum ailanthoides*. (A) Length on the midrib; (B) Density on the midribs; (C) Length on the leaf rachis; (D) Density on the leaf rachis; (E) Length on the stem; (F) Density on the stem.

not shown).

On Kashima, the prickles of *R. sieboldii* are significantly longer and at higher densities than those in neighbouring areas, probably as a response to relatively heavy browsing by sika deer [15], but we found that the prickles of *Z. ailanthoides* were of a similar length and at similar densities to those in neighbouring areas (Figure 3, Table 2). Further, why were the prickles of *Z. ailanthoides* on Kashima not longer and at higher densities than those in the adjacent regions. One possibility is that the prickle length and density of this species on Kashima were already sufficiently great to inhibit browsing by sika deer, and there was no necessity for the plant to develop longer prickles at higher densities. The prickle length of *Z. ailanthoides* was longer than that of *R. sieboldii* in the areas with low densities of sika deer [15], suggesting that prickles play an important role in a

plant's defence against sika deer. Therefore, the prickle of them has physical defence to avoid browsing by sika deer in various evergreen forests including Kashima island.

In contrast to Kashima, the prickles of *Z. ailanthoides* on Akune were the longest and at the highest densities of all the regions included in the study, suggesting that such structural modifications could have occurred due to the high density of sika deer on the island. It is unknown why the *Z. ailanthoides* population of Akune had significantly the longest prickles; there was no significant difference in prickle length between the populations of Kashima and its neighbouring areas. The density of sika deer on Akune (ca. 520 - 600/km<sup>2</sup>) is approximately 15 times greater as that on Kashima (ca. 38.5/km<sup>2</sup>) [14,21, 22], suggesting that browsing pressure by sika deer on Akune is higher than on Kashima. Therefore, our results

suggest that in response to the high browsing pressure of sika deer, the *Z. ailanthoides* population on Akune has developed longer prickles.

*R. sieboldii* plants with relatively long prickles have been reported on Kashima [15], but we could not find *R. sieboldii* on Akune, even though this species is found in areas close to the island [23]. We suggest that this species might be extinct on Akune due to overgrazing by sika deer, since there is greater browsing pressure on Akune compared to Kashima. Recent studies have shown that forest damage by sika deer in Japan has increased, and the deer is now regarded as the most damaging mammalian pest species [2]. Indeed, some areas of forest are so heavily browsed that forest regeneration is prevented [24]; it is possible that plant species with long prickles can be found in these forests.

From the results of the present study and those of Kamijo *et al.* (2001) [19], it is probable that populations of *Z. ailanthoides* develop more prickles under heavy sika deer browsing pressure, and fewer prickles under low sika deer browsing pressure. Interestingly, a non-prickle variety (*Z. ailanthoides* var. *inerme* Rehder et E. H. Wilson) that has thin leaves grows on the Ogasawara islands, which are oceanic islands without sika deer [25]. The non-prickled and prickled varieties of *Z. ailanthoides* could have evolved on oceanic islands and small coastal islands respectively due to differential selection pressures caused by sika deer grazing.

In this study, no significant relationships were found between prickle traits and the other plant traits. However, future studies should investigate the relationship between prickles and relative growth rate per year, as there may be a trade-off between the dry weight biomass of individual plants and their corresponding prickle weights in this species (see [19]).

## Acknowledgements

We wish to thank Mr. Moriguchi Y. for providing useful comments concerning Kashima island, and Ohga K., Miyata H., Tsuchiya Y., Yoshimi Y., Muroi M., Isomoto S., Matsuyama K., Yokoyama N., Sunami T., Kumekawa Y., Kakimoto N., Matsui R., Inoue S., Uemoto C., Ozaki Y., Orito M., Kuribayashi S., Suzaki S., Fujiwara K., Minato Y., Miyake K. and Yamamoto F. for providing us with much needed assistance. This study was partly supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (to TF and JY).

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