Resistance by the leaf shape of *Isodon umbrosus* var. *hakusanensis* (Lamiaceae) against the leaf processing by *Apoderus praecellens*

Yumiko Higuchi

Introduction

The shapes of plant leaves are diverse among angiosperms, but their roles in nature remain unclear. Reports on the effects of leaf shape on biotic factors such as herbivory are especially scarce, compared to those on abiotic factors. A few previous reports suggested that leaf shape functions as resistance against herbivorous insects that visually recognize leaf shape. However, because herbivorous insects generally do not rely on the visual perception of leaf silhouette for host selection, resistance against herbivorous insects via leaf shape is considered unusual. The present thesis explored the effect of the lobed leaf shape of *Isodon umbrosus* var. *hakusanensis* (hereafter, *I. umbrosus,* Lamiaceae) on host selection by the leaf-processing herbivorous insect, *Apoderus praecellens*. Plants in the genus *Isodon* host the oligophagous leaf-rolling weevil, *A. praecellens*, whose ovipositing females process an entire leaf into a leaf roll to serve as larval food and shelter. Among *Isodon* species in Japan, *I. umbrosus* is exceptional in that it has deeply lobed leaves. Because the leaf processing by female *A. praecellens* follows a sequence of complex behaviors, the unusual leaf shape of *I. umbrosus* among the host plant species may disrupt this process.

Materials and Methods

Throughout this thesis, I used *I. trichocarpus*, a close relative of *I. umbrosus* with non-lobed leaves for comparison. The two species often grow adjacently with each other in the field. In Chapter 2 following general introduction, I investigated whether the lobed leaf shape of *I. umbrosus* deters the leaf roll formation by *A. praecellens*. By combining field surveys and laboratory experiments, I evaluated the effects of both the shape and nutritional quality of *I. umbrosus* and *I. trichocarpus* leaves on leaf roll formation. In Chapter 3, I studied the leaf-processing behaviors of *A. praecellens* on the leaves of the two *Isodon* species to identify the timing at which female weevils abandon lobed leaves during leaf roll formation. Finally, in Chapter 4, I quantified the extent of

leaf loss caused by leaf rolls throughout a season in *I. umbrosus* and *I. trichocarpus* to explore how it can impact the growth of each plant species.

Results

In Chapter 2, female weevils created leaf rolls more on *I. trichocarpus* over *I. umbrosus* under both natural and laboratory conditions, despite their larvae developing equally well on the leaves of these plant species. Modifying the non-lobed *I. trichocarpus* leaves to mimic the shape of *I. umbrosus* leaves also discouraged leaf processing. In Chapter 3, I found that female weevils often abandoned lobed *I. umbrosus* leaves during inspectional walking, which takes place before leaf cutting. Modifying the lobed *I. umbrosus* leaves to non-lobed shape greatly increased the success rate of inspectional walking. During successful inspection, female weevils walk from the leaf base to the leaf apex along the leaf margin, turn at the leaf apex and return to the leaf base along the main vein. This behavior is repeated several times on both left and right sides of the leaf. However, on lobed leaves, they often turned around at the apex of the lateral lobe and walked toward the interior of the lateral lobe, failing to find the main vein. Consequently, their walking routes were much irregular. In Chapter 4, leaf loss due to leaf rolls was greater in *I. trichocarpus* than in *I. umbrosus*, but the amount was smaller than that caused by chewing insects. *I. trichocarpus* always showed greater vegetative growth than *I. umbrosus*, and leaf rolls did not have significant effects on the subsequent vegetative growth of *I. trichocarpus*.

Discussion

The results of Chapter 2 and 3 strongly suggest that the lobed leaf shape of *I. umbrosus* physically deters leaf processing by female *A. praecellens*. *A. praecellens* avoids making a leaf roll on *I. umbrosus* leaf because of its deeply lobed shape, rather than its nutritional property. The lobed leaf shape mainly hinders leaf roll formation at the inspectional walking stage preceding their leaf cutting. Leaf inspection on lobed leaves fails likely because the lobed shape disturbs weevil's regular walking pattern, which is considered important for determining where to cut leaves. The results of Chapter 4 suggest that the overall amount of leaf loss caused by leaf rolls is small, and

the greater amount of *I. trichocarpus* leaves lost to leaf rolls may be compensated for by its faster vegetative growth.

Altogether, the present thesis proposed a novel resistant mechanism against herbivorous insects via leaf shape. Leaf shape may be an overlooked but potentially important element that influences the working efficiency and quality of the construction in leaf-processing insects. Given the prevalence of insects that process leaves, other leaf shapes may also act in preventing herbivory by such insects. Although it is presently unknown whether the lobed shape of *I. umbrosus* leaves evolved specifically as defense, biological interactions may be an important factor that adds explanation to the diversity of leaf shape.