［鹿大農学術報告　第 68 号, p1–7, 2018］

Do granivorous insects invade fallen acorns of *Pasania edulis* (Makino) Makino through exit holes of curculio weevil larvae?

Sone Koichi [1)], Oishi Keita [2)], Hata Kunihiko [1)]

[1)] *Laboratory of Forest Protection, Kagoshima University*

[2)] *The United Graduate School of Agricultural Sciences, Kagoshima University.*

Accepted October 1, 2017

Introduction

Pasania edulis (Fagacea) is a dominant tree species in many evergreen broad-leaved forests in southern Japan and produces many acorns every autumn. However, the establishment of its seedlings is hindered by a range of mortality factors [7, 10]. The curculio weevil, likely *Curculio hilgendorfi*, is an important mortality factor that negatively impacts germination and seedling establishment depending on the degree and the position of *cotyledonary damage* [9], as reported for other acorn-producing tree species [e.g., 1–6, 8, 11].

In late autumn, the larvae of curculio weevils, having grown in the *P. edulis* acorn, leave the fallen acorn through an exit hole they make and overwinter in the ground. These fallen acorns are then exposed to other mortality factors, such as the acorn borer (*Coccotrypes graniceps*), Tipulidae sp., the Nitidulid beetle (*Stelidota multiguttata*), and the Bell moth (*Cryptaspasma trigonata*) [7, 10, 12]. The acorn borer attacks the acorns through a crack at the top of the acorn from which a root or shoot protrudes or makes its own entrance hole, while the Bell moth uses the exit holes of the weevil larvae or a crack made during germination or by other factors [12]. The exit holes left by the curculio weevil larvae may be by these granivorous insects during their invasion or may facilitate predation by decomposers such as mites, springtails, and fungi. This may further lower the germination ratio and seedling establishment.

In 2015, we conducted a field experiment in an evergreen broad-leaved forest in southern Kyushu and examined whether other granivorous insects and decomposers enter the acorns through the exit holes of curculio weevil larvae. In April, we buried acorns without any holes (S-acorns), acorns with a hole bored into them with the same diameter as the exit holes of curculio weevil larvae (H-acorns), and acorns with exit holes from curculio weevil larvae (W-acorns). In November (the end of the first growing season), we compared the germination ratio, the condition of cotyledons, and the degree of invasion by the acorn borer and the Bell moth among the three categories of acorns. We then discussed whether or not the exit holes of the curculio weevil larvae on *P. edulis* acorns were subsequently used by other granivorous insects and decomposers.

Methods

The field study was conducted in an evergreen broad-leaved forest of the Takakuma Experimental Forest of Kagoshima University (TEFKU) (31°31′ N, 130°46′ E, and 550 m in altitude) in Kagoshima Prefecture, southern Japan. The annual mean temperature and precipitation of this area during the last decade were 14.3 °C and 3,460 mm, respectively. The crown layer of the forest was closed, and *P. edulis* was the most dominant species of tree, occupying about 50% of the canopy. Other dominant tree species were *Castanopsis cuspidata*, *Machilus thunbergii*, *Neolitsea sericea*, *Distylium racemosum*, *Quercus acuta*, and *Q. salicina*. The understory vegetation was composed of *Cleyera japonica*, *Symplocos lucida*, *Camellia japonica*, and *Illicium anisatum*. On the floor, vegetation was poor, and 3–5 cm thick leaf litter had accumulated uniformly through the forest.

In October and November 2014, we collected fallen *P. edulis* acorns, wrapped them in wet paper towels and stored them in a refrigerator (5 °C, dark conditions). In April 2015, we classified these acorns into W-acorns and S-acorns. We made H-acorns by drilling a hole 2 mm in diameter and 5 mm long in the area about 1/3 from the bottom of half of the S-acorns.

On April 9, 2015, we put the 100 S-, W-, and H-acorns in 30×30×10 cm cages, and buried them about 5 cm deep in the soil in TEKFU's evergreen, broad-leaved forest. These cages were made of 4 mm wire mesh to prevent the buried acorns from being pilfered by granivorous *Apodemus* mice and predation by large or medium-sized omnivorous mammals such as the wild boar (*Sus scorofa leucomystax*), the badger (*Meles meles anakuma*), and the raccoon dog (*Nyctereutes procyonoides viverrinus*). We buried one cage each of S- and W-acorns and two cages of H-acorns at intervals of 3–10 m; a total of 100 S-, 100 W-, and 200 H-acorns were buried. Sone *et al*. [10] and Nakamura *et al*. [7] reported that the acorn borer attacked the germinated *P. edulis* acorns in the soil, and attacked acorns were observed here and there in the forest, suggesting that the acorn borer uniformly inhabited the forest and the probability of the buried acorns being attacked by the acorn borer did not differ with the cages.

On November 2, 2015, we collected the buried acorns and examined them for appearance, the emergence of shoots and roots, cracks at the top of the acorn, and cracks at parts other than the top (nutshell cracks). In this study, acorns with an emerged root or shoot and root and those with a crack at the top were regarded as germinated acorns. We dissected each acorn and examined the degree of invasion by the acorn borer or the Bell moth and the condition of the cotyledons. The condition of the cotyledons was recorded as being sound, rotten/deteriorated, or absent (acorns were empty because the cotyledons were completely consumed), and any other alterations were also noted. The cotyledons of some H-acorns had deteriorated around the artificial hole or were rotten in limited sections along the artificial hole. We recorded the condition of these cotyledons as sound.

Data analysis

We compared the percentages of ungerminated acorns with sound cotyledons in the three categories of acorns using Fisher's exact probability test. We conducted Bonferroni's correction to examine the significance of the difference between the values of each pair of acorn categories.

Results

In October, we collected a total of 99 S-, 98 W-, and 200 H-acorns and examined their conditions (Table 1). Of the S-acorns, 34.3% had an emerged root or root and shoot and 10.1% had a crack at the top. Of the W-acorns, 6.1% had an emerged root or root and shoot and 3.1% had a crack at the top. Of the H-acorns, 31.5% had an emerged root or root and shoot and 29.0% had a crack at the top. In addition, the nutshell of 6.5% of the H-acorns was cracked vertically (nutshell crack).

Table 1 The percentage of acorns in various conditions

Condition	S-acorns	W-acorns	H-acorns
Cracked at the top*	10.1	3.1	29.0
Emerged root*	13.1	1.0	8.0
Emerged root and shoot*	21.2	5.1	23.5
Cracked other than at the top			6.5
Unchanged in apperarence**	55.6	90.8	33.0

* regarded as germinated
** regarded as ungerminated

Attacks by the acorn borer were confirmed in 34 (25 germinated and nine ungerminated) S-acorns and 18 (13 germinated and 5 ungerminated) H-acorns. The acorn borer had bored into all 25 germinated S-acorns and 11 of the 13 H-acorns through a top crack. The acorn borer made its own entrance hole in two H-acorns. The acorn borer made an entrance hole on five of the nine ungerminated S-acorns and two of the five ungerminated H-acorns. The acorn borer did not use the artificial holes to enter the acorns (Table 2).

Table 2 the number of S- and H-acorns attacked by the acorn borer through different sites

Entrance site	S-acorns		H-acorns	
	Germinated	Ungerminated	Germinated	Ungerminated
Crack at the top	25	0	11	0
Entrance hole made by the borer	0	5	2	2
Artificial hole	0	0	0	0
Others/unknown	0	4	0	3

The Bell moth attacked only the H-acorns (five germinated and five ungerminated acorns). The larvae entered through the crack at the top of four of the germinated H-acorns and the artificial hole of the other germinated H-acorn. The larvae entered one of the ungerminated H-acorns through the artificial hole and through an undetected path in the other four ungerminated H-acorns (Table 3).

Table 3 The number of H−acorns attacked by the Bell moth through different sites

Entrance site	Germinated	Ungerminated
Crack at the top	4	0
Artificial hole	1	1
Others/unknown	0	4

Cotyledons in the acorns attacked by the acorn borer and the Bell moth were completely rotten or absent. The cotyledons of 21.7% of the ungerminated S-acorns were completely rotten and 78.3% were sound. Cotyledons were completely rotten and/or absent in 97.8% of the ungerminated W-acorns and only 2.2% were sound. The cotyledon deterioration present in some of the H-acorns had progressed around the artificial hole. The cotyledons were completely rotten and/or absent in 58.9% of the ungerminated acorns and were sound in 41.1%. The percentage of ungerminated acorns with sound cotyledons varied significantly according to the category of the acorns, and the difference in the percentage of ungerminated acorns with sound cotyledons was significant for any combination of acorn categories (in all cases, Fisher's exact probability $P < 0.01$).

Discussion

Curculio weevil attacks have detrimental effects on the seedling establishment of acorn-producing tree species [3, 5, 6, 9]. In this study, 44.4%, 9.2%, and 60.5% of the S-, W- and H-acorns were regarded as germinated and 21.2%, 5.1%, and 23.5%, had emerged shoots and roots, respectively. These results indicate that, as reported by Sone *et al*. [9], curculio weevil attacks greatly suppress the seedling establishment of *P. edulis* while the artificial holes did not.

After the acorns drop to the ground, insects attack the acorns before and after germination [1, 2, 4–8, 10–12]. Four species of insects have been reported to attack the acorns of *P. edulis*: the acorn borer, the Tipulidae sp., the Nitidulid beetle, and the Bell moth [12, 13]. The acorn borer uses the crack at the top of germinated acorns or makes an entrance hole and the Bell moth larvae attacks through a crack made during germination on the nutshell or a hole made by other insects [12].

In this study, the acorn borer attacked S- and H-acorns and the Bell moth larvae attacked only the H-acorns. The cotyledons in the acorns attacked by these two insects were completely consumed or absent. These results suggest that the attacks of these insects have devastating effects on seedling establishment, which is consistent

with the report by Sone *et al*. [10] and Nakamura *et al*. [7] that suggests the attack by the acorn borer is a mortality factor.

The acorn borer attacked both germinated and ungerminated acorns. This insect used the crack at the top or made an entrance hole for its entry, but it did not use the exit holes of the weevil larvae or artificial holes, as has been reported by Ueda *et al*. [12]. The Bell moth larvae entered some H-acorns through the artificial holes. This suggests that although we did not verify the entry of the Bell moth larvae through the exit holes of the weevil larvae, this possibility may remain. However, the ratio of acorns attacked by the Bell moth was very low in this study. Therefore, in the forest, the exit holes of the curculio weevil larvae may be used for invasion only by a few granivorous insects on the ground.

The percentage of ungerminated acorns with sound cotyledons was significantly higher in S-acorns than in either W- or H-acorns. The deterioration of the cotyledons of H-acorns progressed around the artificial hole. These results show that the fungal and arthropod decomposers invaded the acorns through the exit holes of weevil larvae. In conclusion, the exit holes of the weevil larvae are not necessarily used by granivorous insects to invade the acorn on the ground, but may facilitate the deterioration of cotyledons due to the invasion of fungal and arthropod decomposers, resulting in the inhibition of germination and seedling establishment.

Acknowledgement

This study was financially supported by a Grant-in-Aid for Scientific Research (No.25660119).

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Sone Koichi[1)†], Oishi Keita[2)] and Hata Kunihiko[1)]

[1)] *Laboratory of Forest Protection, Kagoshima University*

[2)] *The United Graduate School of Agricultural Sciences, Kagoshima University*

Summary

Pasania edulis (Fagacea) is a dominant tree species in evergreen broad-leaved forests in southern Japan. Predation by insects is harmful to seedling establishment. The larvae of the acorn weevil, likely C*urculio hilgendorfi*, feed on the acorns of *P. edulis* and exit the fallen acorns to overwinter in the ground. The fallen acorns are then invaded by other granivorous insects. To examine whether the exit holes of the weevil larvae are used for invasion by other insects, we buried the acorns in the ground in April 2015 and compared the germination ratio, the condition of the cotyledons, and the invasion of the acorn borer, *Coccotrypes graniceps*, and the Bell moth, *Cryptaspasma trigonata*, in acorns in November 2015. The germination ratio was significantly higher in acorns without holes (S-acorns) and acorns with artificial holes mimicking the exit hole of weevil larvae (H-acorns) than for acorns with an exit hole (W-acorns). The acorn borer did invade S- and H-acorns, but not through the exit holes or the artificial holes. The Bell moth attacked some H-acorns through the artificial holes. The percentage of ungerminated acorns with intact cotyledons was significantly higher in S-acorns than in H- or W-acorns. These results show that the exit holes of the curculio weevil larvae may contribute only slightly to invasion by other granivorous insects, but may significantly facilitate the invasion of fungal and arthropod decomposers into the fallen acorns.

Key words: Curculio weevil-attacked acorns, Exit holes, *Pasania edulis* acorns, The acorn borer, The Bell moth

[†]: Correspondence to : Koichi Sone (Laboratory of Forest Protection Laboratory, Department of Environmental Sciences and Technology, Faculty of Agriculture, Kagoshima University)
Phone : 099-285-8580, E-mail : sonesun@agri.kagoshima-u.ac.jp