報告番号	※　　　第　　　号

主　論　文　の　要　旨

論文題目	Flora of fungi associated with an ambrosia beetle (*Euwallacea interjectus* (Blandford)) attacking fig tree, tree-pathogenic risk of the fungi, and predatory behavior of a natural enemy of the beetle （イチジクに穿孔するアイノキクイムシの共生菌相とその病原リスク、および天敵昆虫の捕食行動）
氏　　名	姜　自如

論　文　内　容　の　要　旨

The recognized causal agent of wilt disease on fig tree (*Ficus carica* L.) is *Ceratocystis ficicola* Kajitani & Masuya in Japan. The first occurrence of fig wilt disease (FWD) dates back to 1982 in Aichi prefecture. Since then, FWD has spread progressively to many fig orchards. In 2017, areas infested by FWD are estimated to have reached 33 prefectures of Japan. This devastating epidemic of FWD has spread in stands of fig variety – 'Masui Dauphine', 'Horaishi' and 'Toyomitsuhime'.

From now on, the dispersal process of *C. ficicola* has not been fully clarified. In the beginning, application of fungicide was tried to polluted soil in order to control *C. ficicola* as a soil-borne plant disease, which is not always effective to reduce the damage. Then, an ambrosia beetle, *Euwallacea interjectus* (Blandford), was found in the fig trees infested with FWD, and was extensively controlled as suspected vector of *C. ficicola*. This insect comes from forest where it is harmless, secondary wood-boring beetles. *E. interjectus* played a primary role in FWD, contributing to the symptom development. However, the real way by which *E. interjectus* declined fig trees was unknown.

Ambrosia beetles have specialized structures termed mycangia (singular form: mycangium) where fungal spores or hyphae are contained and allowed to proliferate. Hence, the spores or hyphae are protected against desiccation and contamination until dispersal. They are released from mycangia and inoculated on the gallery walls. Once established, the fungi form a symbiotic relationship with the beetle by serving as the primary food resource for larvae and adults. Mycangia occur in many parts of the beetle's body such as the elytra, pronotum, mesonotum, prothoracic-pleura, prosternal-subcoxa and mandible. *C. ficicola* was most frequently isolated from the elytra of *E. interjectus* as compared to other body parts. However, this previous study did not investigate the role of mycangia in transmitting the pathogenic fungi.

My Ph.D. research work mainly focused on exploring: (1.) the location and structure of mycangia, (2.) fungal symbionts of adult female *E. interjectus*, (3.) the role of mycangial fungi of *E. interjectus* in FWD, and (4.) the efficiency with which natural enemy (earwig) prey on ambrosia beetles and its prey preference.

1. X-ray microtomography has been applied successfully to obtain reliable microstructural information of many insect species. Nonetheless, the technique has not been widely applied to ambrosia beetles. The ambrosia beetle *E. interjectus* was first recorded as a vector of plant-pathogenic fungus *C. ficicola*. Previous studies of *E. interjectus* have not described the mycangia (fungus-storing organ) in detail. In this study, I non-destructively examined the internal structure of an adult female of *E. interjectus* through micro-CT scans. Paired mycangia were observed on typical CT cross-sections of the head. Each mycangium, ovoid in shape, was located in oral (mouth) tissues just posterior to emarginated notch of eyes, adjacent to pharynx. Three dimensions (length × width × depth) of the mycangia were measured on stereography. I confirmed the absence of mycangia in the other body parts, such as elytra, prothorax, and coxa of legs.

2. I have found the female adult has mycangia in its oral rather than elytron. However, the mycangial fungi of *E. interjectus* are still unclear. Thus, further investigation of fungal associates of *E. interjectus*, especially head, would be helpful to understand its ecology and resultant tree damage. To identify the primary symbiotic fungi of female adults of *E. interjectus*, I isolated the fungi from the adults of both wild and rearing populations. Dispersal female adults of *E. interjectus*, which were collected from logs of infested fig tree (51 individuals) caused by *C. ficicola* and from artificial diets (54 individuals), were used for fungal isolation. Isolated fungi were identified based on morphological characteristics and DNA sequence data. For all tested female adults, 13 filamentous fungi were detected in the body of wild population. Specific fungus, *Fusarium* sp., was dominant in head, probably because of its oral mycangia. By contrast, 9 filamentous fungi and 1 yeast were found in rearing population, showing that *Fusarium* sp., *Neocosmospora metavorans* and *Meyerozyma guilliermondii* (yeast) were more frequently isolated from head than thorax and abdomen. Regardless of the wild and rearing populations, *Fusarium* sp. is closely associated with female adults of *E. interjectus*. The present experiment also shows *C. ficicola* is not transmitted via mycangia of *E. interjectus*.

3. According to my previous result (2.), *E. interjectus* is probably a secondary pest for FWD because it cannot really carry *C. ficicola* within its mycagia. However, of the 22 species of filamentous fungi isolated directly from wild and/or rearing *E. interjectus*, *Fusarium* sp. and *N. metavorans* (the mycangial fungi) have the ability and chance to affect the symptom development for FWD when fig tree was bored by *E. interjectus*. To verify a role of the two fungal species, 4 kinds of inoculation treatment (T1: *Fusarium* sp., T2: *N. metavorans*, T3: *C.*

ficicola, T4: *Fusarium* sp. + *C. ficicola*) were used for fig saplings. As a control, sterilized toothpicks were also inserted into the saplings. As an initial external symptom, the saplings in T3 and T4 started wilting around 12 days after inoculation, and all of the saplings finally died. The wilting speed of fig saplings in T4 was 7 days faster than that in T3. No wilt symptom was observed on T1 and T2, including the control.

To evaluate water-transporting function in the main stems, xylem sap-conduction test by injection of acid fuchsin solution was performed 28 days after inoculation. The sap flow decreased around the inoculation sites in all of the treatments including the control, but water was supplied in the upper part of the saplings in T1, T2 and the control. In contrast, the saplings in T3 and T4 died owing to dehydration of the upper part of the saplings. Xylem discoloration turning brown (necrosed tissue) was also observed near the inoculation site in all of the treatments including the control. In T1, T2 and control, the maximum discoloration rate (discolored area/cross-section area) of each sapling was from 1.2% to 40.7%, and longitudinal range of the discoloration extended 10 cm above and 10 cm below the inoculation site. The highest rate in T3 and T4 equally have reached 99% around the inoculation site, but the range in T4 (40 cm) was much longer than that in T3 (30 cm), in terms of the upward distance from the inoculation site. These facts suggest that *Fusarium* sp. may be a potential causal agent of xylem dysfunction in fig tree, although it did not seem to directly kill the tree.

4. Earwigs (Dermaptera) such as *Forficula auricularia* L. are important euryphagous predators for a wide variety of prey, and can markedly deplete the populations of orchard pests. Most previous studies on its feeding behavior have used eggs, larvae, and soft-bodied adults of the prey species, and hardly have focused on prey preference. Some fragments of beetle exoskeleton and an earwig adult, *Anisolabella marginalis* (Dohrn), were found in the same cage, where adults of ambrosia beetle, *E. interjectus*, were emerging from the logs of a fig tree infected with *C. ficicola*. Thus, *A. marginalis* was suspected of being a predator of *E. interjectus*. To shed light on this issue, in the laboratory, I set up a test arena, and observed and recorded behavioral interactions between *A. marginalis* and *E. interjectus*, which were collected from the logs of fig tree and reared on an artificial diet, along with other 6 different ambrosia beetle species, which were collected from a trap (baited with ethanol) and a fallen maple tree. A series of laboratory experiments demonstrated that *A. marginalis* is actually a predator of *E. interjectus* and other species of ambrosia beetle, indicating its high potential for use in effective pest control in the field. The predators strongly select their prey depending on prey size, rather than sex and beetle species. Furthermore, earwig has alternative predatory strategies for dealing with such prey of 7 different species, although it uses its forceps to cut the body of most tested beetles.