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A Newly Invasive Long-Horned Beetle from Mainland Kyushu Attacks Citrus Trees in the Amami Islands

SAKAMAKI Yositaka, TSUDA Katsuo and MIYAKE Masataka

Faculty of Agriculture, Kagoshima University

Abstract

In the Amami Islands many kinds of citrus have been introduced from continental China and Taiwan. As there are few indigenous insect pests, introduced citrus species have been well-kept and have diversified in Kikaijima, Tokunoshima and Okinoerabujima islands. As a result, we can now find a plentiful variety of citruses in home yards in these areas. About 2008, however, many of the trees appeared to weaken and then died over the course of one or two years. The problem spread rapidly. For example, in one village in Kikai Island the total number of citrus trees drastically decreased from 2,800 to 2,000 over a period of four years. These dead trees had many distinct 2cm-circular exit holes of long-horned beetle at ground level. The islands have long been home to a type of long-horned beetle, *Anoplophora oshimana*, that had never attacked citrus trees. From 2011, we started to simultaneously examine the species of long-horned beetles in the citrus orchards and to conduct new control strategy against the beetles. The new strategy involves a wide-area application of biopesticide band, *Beauveria bassiana*, Bio-Lisa®, conducted in the abovementioned village. It took three years to decrease long-horned beetle numbers by 90%. DNA analysis revealed that the long-horned beetle in the orchards was hybrid population between *A. oshimana* and *A. malasiaca*. *Anoplophora malasiaca* is a notorious pest of citrus trees in main island Japan. We estimate that the latter long-horned beetle established itself in the islands before 2008 and hybridized with the endemic long-horned beetle.

Keywords: *Anoplophora malasiaca*, *A. oshimana*, biopesticide band, DNA analysis, hybrid population

Introduction

The history of citrus cultivation on the Amami Islands, which include Amami-Oshima I., Kikaijima I., Okinoerabujima I., Tokunoshima I. and Yoron I., is ancient. The people living on these islands have cultivated many types of citrus trees from China and mainland Japan, but sports and natural hybrids of these citrus trees have been cultivated and handed down within individual homes for centuries. Consequently, each farmer has unique cultivars of citrus in his orchard or residential yard, which can be considered valuable genetic resources (YAMAMOTO *et al*. 2006). For example, in two local citrus cultivars on Kikaijima I. (*Citrus keraji* and *C. keraji* var. *kabuchii*), the polymethoxy flavone content of the juice is four-fold higher than

that of satsuma mandarin (*Citrus unshu*), and these local citruses are considered to be a good source of phytonutrients (YAMATOMO *et al*. 2008). Few studies have examined the phytonutrients in local citruses from the Amami Islands or examined the cultivars from the islands in detail. YAMAMOTO *et al*. (2006) reported that severe citrus damage caused by the citrus long-horned beetle on Kikaijima and Tokunoshima islands is worsening and becoming a serious threat to local citrus cultivation. In this report, we interviewed people living on the islands who have experienced citrus damage caused by the long-horned beetle to confirm YAMAMOTO's report. Second, we assessed the damage status in 2011 and began to control the long-horned beetle in an experimental model village in Kikaijima in 2012. Here, we provide an interim report of these actions to protect local citruses against long-horned beetle damage and discuss the future of these control methods to conserve local citrus populations.

The citrus long-horned beetle, *Anoplophora malasiaca*, is one of the well-known wood-boring species of the family Cerambycidae that is distributed across mainland Japan. This species usually attack many types of hardwood trees, as well as citrus trees, willow, poplar, walnut, ficus, sycamore, rose, pear, peach and winter hazel (HAYASHI 1984, MAKIHARA 2007). The closely related species *Anoplophora oshimana*, which is distributed across Amami-Oshima, Okinoerabujima, Tokunoshima, Okinawajima, Ishigakijima and Iriomotejima islands, is also known to have a broad spectrum of host plants (HAYASHI 1984, MAKIHARA 2007), but it is thought to prefer winter hazel over citrus; thus, *A. oshimana* is not commonly regarded as an insect pest for citrus cultivation on these islands.

We tried to interview a number of citrus farmers and local government officials from Kikaijima I. regarding citrus damage caused by the long-horned beetle in 2011–2012. They stated that a severe outbreak of the beetle started about 10 years ago and that the damage was obvious from 2006–2008. Another farmer that observed damage more than 10 years ago answered that the beetle rarely attacked citrus trees, but did observe damage to winter hazel trees used as a windbreak in orchards. In the area one official had been aware a phenomenon where white spots pattern on back of the long-horned beetle changed from yellowish to bluish-white was reported on Amami-Oshima I. (MAKIHARA 2007), which suggests that the native *A. oshimana,* which has yellowish white spots, was replaced by *A. malasiaca*, which has bluish white spots, originating from mainland Japan, Kyushu, Shikoku and Honshu islands.

Example of Decreased Citrus Trees in a Village on Kikaijima I.

Oasato-Nishime is a small village (approximately 25 ha) in the center of Kikaijima I. There are fewer than 100 houses in the village, and half of the houses are empty. In the village, a tree suffering from citrus greening disease (Citrus Huang-Long-Bin) was first observed in December 2003. Since this was the first record of the disease on the island and some additional trees suffering from the disease were found in the village, all citrus trees cultivated and present (about 2,800 trees in total) were counted, identified, numbered and mapped using

a GIS system by the local government of Kikai Town in 2005 as part of a citrus greening disease eradication program. Using this map, a mortality survey of all citrus trees in the village was traceable. Thus, we traced the change in number of citrus trees in the village using the map and numbering system. Additionally, fewer than ten trees were felled by citrus greening disease after 2005.

The change in number of citrus trees in the village during 2006–2008, which decreased slightly but was generally stable, was about 2,800. The number of trees then decreased to 2,600 by the end of 2006 and to 2,500 by 2010. Only 1,600 trees were thicker than about 2 cm in diameter, meaning we could use a microbial insecticide containing *Beauveria brongniartii* (Biolisa Kamikiri Slim®; Idemitsu Kosan Co. Ltd., Tokyo, Japan). When we established control of the long-horned beetle using this microbial insecticide, we excluded trees narrower than 2 cm in diameter to reduce the use of the expensive insecticide and the labor required to set the control measures. In addition, we did not count the number of narrow trees. If we assume that the trees we excluded accounted for approximately 20% of the citrus trees in the village, the total number of citrus trees would be decreased to about 2,000 (Fig. 1). In this case, the village lost 1/3rd of the trees over four years.

Choice of a Suitable Control Method

We avoided using chemical insecticides to control the long-horned beetle because the village has a water well that many people living in adjacent villages use, and we had to conserve the village's insect fauna, which was damaged by a chemical insecticide used to control an insect vector of citrus greening disease during 2004–2008.

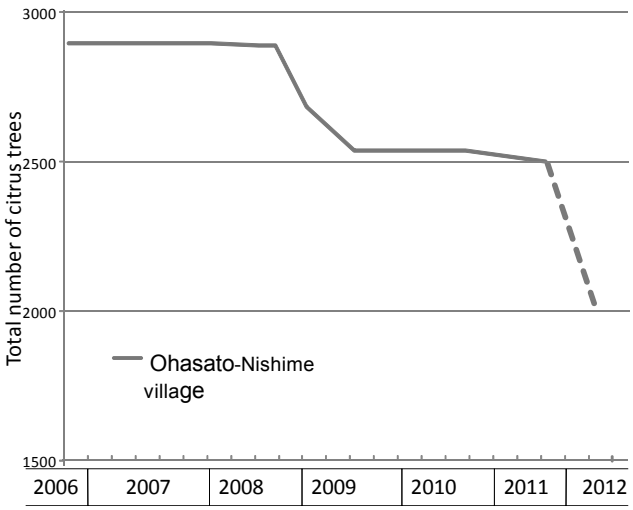

Fig. 1. Change of the number of citrus trees in Oasato-Nishime Village of Kikai Town, 2006–2008. The Oasato-Nishime Village lost 1/3rd of citrus trees from 2008 to 2011.

Alternatively, we chose a microbial insecticide (Biolisa Kamikiri Slim®), which consists of a non-woven fiber band impregnated with a fungus (*B. brongniartii*) to control the beetle on citrus trees. The reason we chose this particular microbial insecticide is that the fungal strain originated from Japan, ensuring that the fungus would die in the local environment. Thus, the use of microbial agents to control the long-horned beetle is one form of environmental load-reducing control.

However, the microbial band takes 10 days to kill an adult beetle, so the female beetle can still lay a portion of her eggs. Since the longevity of an adult female is typically 2–3 months, the fungus can reduce the beetle longevity time to 1/9th to 1/6th if the fungus infects just after emergence.

Since the female beetle typically has 200–300 eggs in her ovary and the preoviposition period of the beetle is 7–15 days, a female exposed to the insecticide just after emergence will lay few eggs. The fungal infection rate using the microbial band is approximately 1 month. Therefore, an effective technique for setting the microbial band is to fasten it to an infected tree at about 40 cm above the ground during the onset of the adult emergence season.

An efficacy report on the microbial band from Okinawajima I. (KYUSHU AGRICULTURE PROMOTION COUNCIL 1994) stated that 50–100% of adult beetles captured in experimental orchards (about 11–15 ha) containing the microbial band were infected by *B. brongniartii*, but the larvae boring into the citrus tree did not decrease during the later season. Consequently, the report concluded that control by the band was limited on Okinawajima I. because the subtropical island has more essential host tree species adjacent to the forest than mainland Japan. The report estimated that many non-infected females invaded from surrounding forests and deposited eggs within the experimental orchard (Fig. 2). The report did not perform a trial of the band on the other islands in the Okinawa Islands and their adjacent Amami Islands, and people living in the isolated island area have abandoned use of the band. Since Kikaijima I. belongs to the Amami Islands, whose climate is subtropical and similar to that on Okinawajima I., a similar phenomenon may occur if we set the band in some citrus orchards. To avoid similar failures, we set a microbial band on all citrus trees in the village (about 25 ha). Using this widespread approach, we hoped to determine the actual efficacy of the microbial band (at least in the central area of the village). It may be possible to identify invader females at the edge of the setting village to decrease the likelihood of invader females reaching the central area of the village.

An Effect of Wide-Area Control by an Entomopathogenic Fungal Pesticide

During our first visit, we observed that many residents were unaware of the correlation between citrus tree damage and the citrus long-horned beetle. We first distributed a leaflet to all residents of Kikaijima I. to discuss the correlation between citrus damage and the long-horned beetle, the life cycle of the long-horned beetle, the morphology of the beetle at all developmental stages (egg, larva, pupa and adult) and control methods. Simultaneously, we

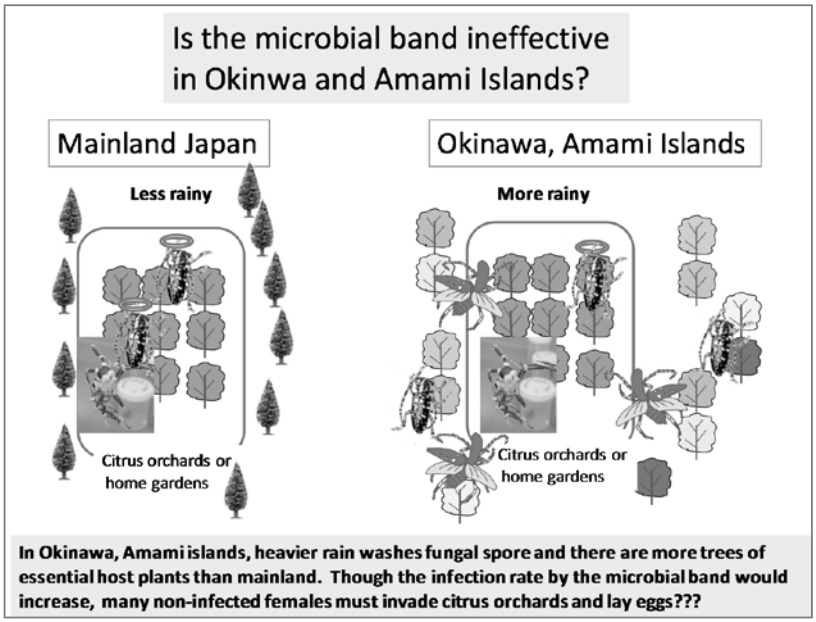

Fig. 2. Schematic comparison of environmental conditions for setting the microbial band between mainland Japan and subtropical isolated islands (Okinawa, Amami).

held a meeting to explain the long-horned beetle control method using the microbial band and we identified participants to set bands the next day in the Oasato-Nishime. These actions increased the residents' awareness of control of the citrus long-horned beetle.

When we set the microbial bands in May 2012 and in May and June 2013, more than ten residents participated. Since they knew the position of every citrus tree, setting the bands on more than 1,500 trees only took half a day. The infection rate of *B. brongniartii* in the wild beetle at 2 weeks after setting the bands was more than 70% in May and June 2013 (MIYAKE *et al*. unpublished data). This indicated that most of the female beetles that emerged in the village could lay few or no eggs and died. In autumn, when the damage caused by larvae became visible, we searched for freshly damaged trees and counted the number of damage signs by larvae, which exhausted frass like sawdust. The larval density, which was calculated based on the number of damage signs, was 1.3 larvae/citrus tree before setting the bands in 2011, but was 0.11 larvae/tree at 2 years after setting the bands (in 2013; MIYAKE *et al*. unpublished data). Thus, we were able to achieve high efficacy for the control of long-horned beetle larvae. This control efficacy was completely different from that on Okinawajima I. (KYUSHU AGRICULTURE PROMOTION COUNCIL 1994). This may be because we set the microbial bands in a larger area than on Okinawajima I. (i.e., broader effect).

These results suggest that microbial band control can be extremely useful in isolated island areas if people use this approach with considering a broader effect. Unfortunately, during our 2-year survey 30% of the fixed observation trees (n=108) died. To protect the citrus trees throughout Kikaijima I., we need to rapidly expand the treatment area.

DNA Analyses of the Citrus Long-Horned Beetle in Kikaijima I.

We conducted DNA analyses of Mitochondrial COI region (about 800 bp) and nuclear ITS2 region (about 500 bp) of the long-horned beetle from Kikaijima I. The COI sequences of those samples were similar to the sequence of *Anoprophora oshimana*, but the ITS2 sequence were intermediate status between *A. malasiaca* and *A. oshimana.* These results must explain as introgressive hybridization of the citrus long-horned beetle inhabit in Kikaijima I. by *A. malasiaca*'s gene.

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