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Hot water tolerance of soil animals: utility of hot water immersion in preventing invasions of alien soil animals

Shinji Sugiura*

Department of Forest Entomology, Forestry and Forest Products Research Institute; Tsukuba, Ibaraki 305–8687, Japan

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Abstract

Introduced soil animals have frequently affected native fauna on oceanic islands that have never been connected to a continental land mass. Alien soil animals can be unintentionally introduced via transfer in potted plants or by commercial trade among islands and continental landmasses. Hot water treatment to destroy pests has recently been used during the quarantine of ornamental plants. To examine the possibility of using hot water treatment for introduced soil animals in potted plants, an experiment was performed to determine whether hot water treatment (immersion in water at 40, 43, 45, 47, and 50°C for 5 min) kills soil animals. I examined four taxa (different phyla) of soil invertebrates that have been introduced to the oceanic Ogasawara (Bonin) Islands, approximately 1,000 km south of the Japanese mainland. The species used were: the invasive alien terrestrial flatworm *Platydemus manokwari* (Platyhelminthes); an unidentified alien species of earthworm (Annelida); the alien snail *Acusta despecta sieboldiana* (Mollusca); and the alien ant *Technomyrmex albipes* (Arthropoda). The water temperature required to kill flatworms (≥43°C) and earthworms (≥43°C) was lower than that to kill snails (≥50°C) and ants (≥47°C). Use of hot water for protection from alien soil animal invasions may mitigate their environmental impacts, particularly on oceanic islands where valuable biota could be threatened.

Key words: Hot water drenching; invasive alien species; Ogasawara Islands; *Platydemus manokwari*; quarantine

INTRODUCTION

Globalization has brought social, cultural, and economic benefits to many people, but it has also caused worldwide biological invasions of alien species (e.g., Reaser et al., 2007). Human activities such as travel, commercial trade, and agroforestry have increased the numbers of introduced organisms at regional and local scales (e.g., Mack et al., 2000). Many introduced species have negatively affected native ecosystems and species (e.g., Elton, 1958; Reaser et al., 2007). This is especially true on oceanic islands that have never been connected to a continental land mass (Elton, 1958; Reaser et al., 2007). For example, the accidental introduction of the brown tree snake, *Boiga irregularis* (Merrem), on Guam Island caused the extinction or dramatic reduction of many native vertebrates, including birds, bats, and reptiles (Fritts and Rodda, 1998); and intentional and accidental introductions of parasitoids to the Hawaiian Islands has caused heavy parasitism of many native moth species (Henneman and Memmott, 2001).

The effects of introduced soil animals have recently been recognized as a serious problem. For example, introduced earthworms have affected native ecosystems by changing nutrient cycling (Bohlen et al., 2004; González et al., 2006); introduced snails such as the giant African snail, *Achatina fulica* Bowdich, have become agricultural pests and have replaced native land snails on islands (Cowie, 2001b); snail-eating flatworms (e.g., *Platydemus manokwari* De Beauchamp) and predatory snails (e.g., *Euglandina rosea* (Férussac)), which were once frequently used for the biological control of introduced giant African snails (Muniappan, 1987, 1990; Eldredge and Smith, 1995; Civeyrel and Simberloff, 1996; Cowie, 2001a, b), have affected native land snail fauna on Pacific islands (Civeyrel and Simberloff, 1996; Cowie,

* E-mail: ssugiura@ffpri.affrc.go.jp
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2001a, b; Cowie and Robinson, 2003; Ohbayashi et al., 2007); and ants, which are easily introduced via human activities, have affected native vegetation and fauna on many oceanic islands (Holway et al., 2002; O'Dowd et al., 2003). Because soil animals are easily spread in imported potted plants, the transfer of potted plants for any purpose could cause invasions by alien soil animals. Intentional introductions of organisms as biological control agents are now considered more thoroughly. Accidental introduction should be more actively prevented.

Hot water treatment has been used as one quarantine method to destroy plant diseases and pests on cut and potted plants (Hara et al., 1993, 1994, 1996, 1997, 1999; Tsang et al., 1995, 2001; Follett and Neven, 2006); exposure to hot water at 49°C for 12 min kills most pests, including aphids, mealybugs, thrips, soft scale insects, and ants, with minimal effects on vase life and the quality of tropical ornamentals (Hara et al., 1996). Tsang et al. (1995) found that hot water treatment (49°C for 12 min) was not phytotoxic and actually extended the vase life of ornamental flowers and foliage. Therefore, hot water treatment may be useful for killing soil animals introduced with potted plants. To examine the utility of hot water treatment in preventing invasions by alien soil animals in potted plants, hot water treatment (immersion at 40, 43, 45, 47, and 50°C for 5 min) was applied to soil animals from four different taxa, including the invasive alien flatworm *P. manokwari* found on the oceanic Ogasawara (Bonin) Islands.

On the inhabited Ogasawara Island of Chichijima, the invasive alien flatworm *P. manokwari*, among the "100 World's Worst Invader Alien Species" (Lowe et al., 2000), is thought to have been introduced accidentally (probably via soils on plants) and became established in the early 1990s (Kawakatsu et al., 1999). Several studies have confirmed that invasive flatworms have eradicated the endemic land snail fauna on Chichijima (Ohbayashi et al., 2005, 2007; Sugiura et al., 2006b). Although the role of introduced earthworms in the native ecosystem in Ogasawara is unclear, dead earthworms are eaten by the invasive flatworm *P. manokwari*, suggesting that earthworms may support *P. manokwari* populations (Ohbayashi et al., 2005). The native land snail fauna of Ogasawara has been negatively influenced by the introduction of *P. manokwari* and alien land snails such as the giant African snail *A. fulica* (Tomiyama and Kurozumi, 1992). Furthermore, like introduced earthworms, introduced land snail species such as *Acusta despecta sieboldiana* (Pfeiffer) are eaten by *P. manokwari* (Sugiura et al., 2006b). The ant fauna of the Ogasawara Islands includes many alien species that invaded following human immigration (Terayama and Hasegawa, 1991). Some introduced ant species have been reported to influence plant–insect interactions on the Ogasawara Islands (Sugiura et al., 2006a). Flatworms, earthworms, land snails, and ants can readily be transported on or in various materials such as potted plants. Because plants may be transported to islands other than Chichijima for the purpose of vegetation restoration, quarantine methods to prevent their spread are essential.

MATERIALS AND METHODS

Site. The oceanic Ogasawara (Bonin) Islands are located in the northwestern Pacific Ocean, approximately 1,000 km south of the Japanese mainland (Ogasawara Village, Tokyo Metropolitan, Japan; Shimizu, 2003). The mean annual temperature was 23.2°C and the mean annual precipitation was 1,292 mm from 1987 to 1998 on Chichijima (Toyoda, 2003). The climate of the Ogasawara Islands is subtropical. Like other oceanic islands (Carlquist, 1974), the Ogasawara Islands support many endemic species (Shimizu, 2003), including vascular plants (137 species; Toyoda, 2003), insects (338 species; Ohbayashi et al., 2003), and land snails (82 species; Tomiyama and Kurozumi, 1992). However, many organisms are endangered because of human activities (e.g., Tomiyama and Kurozumi, 1992; Chiba, 2003; Toyoda, 2003; Karube, 2004). Furthermore, many alien organisms have invaded the islands, infiltrated the endemic ecosystems, and affected endemic biota (e.g., Yamashita et al., 2000; Ohbayashi et al., 2003; Karube, 2004; Sugiura et al., 2008).

All species samplings and experiments were conducted on the largest island, Chichijima (27°06′N, 142°11′E), in late June 2007.

Species investigated. Four species from different phyla were used: terrestrial flatworms (Platyhelminthes), earthworms (Annelida), land snails (Mollusca), and ants (Arthropoda). Both juveniles and adults of the invasive alien flatworm *Platydemus manokwari* were collected from the Kiyose Experimental Station of the Forestry and Forest Products Research Institute (FFPRI) on Chichijima (27°06′N, 142°11′E). *Platydemus manokwari* can be easily identified on the basis of its morphology and color (Kawakatsu et al., 1999; Sugiura et al., 2006b). Juveniles of an unidentified earthworm species were sampled from Kiyose. Because of the juvenile stage, the species could not be identified; however, all earthworms found in Ogasawara are introduced (Nakamura, 1994). Juveniles and adults of the alien land snail *Acusta despecta sieboldiana* were collected at Okumura on Chichijima (27°05′N, 142°12′E). Workers of the alien ant species *Technomyrmex albipes* (F. Smith) were collected at Asahiyama on Chichijima (27°05′N, 142°12′E). The body length of flatworms, earthworms, and ants, and the shell diameter of land snails were measured to the nearest 1.0 mm using a ruler.

Methods. The following experiments were conducted at the Kiyose Experimental Station or Okumura from 22 June to 12 July 2007. To examine the effective water temperature that kills the target species, individual animals were immersed in water of different temperatures (28, 40, 43, 45, 47, and 50°C) for 5 min. As a control for hot water treatment, water of 28°C was used. The ambient temperature ranged from 27 to 28°C. Soil animals were immersed in 300–500 ml of water in plastic cases (80×110×55 mm). Several treatments were conducted to determine the best water temperature to achieve 100% mortality in each animal group, although not all temperature treatments were applied to all species. I used 15–50 individuals per treatment per animal group. Water temperature was measured using a digital thermometer (Delta SK-1250MC; Sato Keiryoki Mfg. Co., Ltd., Tokyo). To maintain the temperatures (40.0±1.0, 43.0±1.0, 45.0±1.0, 47.0±1.0, and 50.0±1.0°C) during the experimental treatments, a small amount of hot water (ca. 60–70°C) was added, which was boiled in a kettle (3.0 *l*; CV-DT30; Zojirushi, Osaka). In plastic cases (35 mm diameter, 70 mm height), the death of each individual animal after hot water treatment was examined immediately after immersion and again after 1 h. Live and dead individual soil animals were differentiated by a change in body color and/or damage to the body after 1 h.

Data analysis. The body sizes of flatworms (body length, 8–62 mm), earthworms (body length, 20–91 mm), and land snails (shell diameter, 6–21 mm) varied considerably among individuals used in the experiment, but the body size of ants did not differ (body length, 2–3 mm). Therefore, the effects of animal body size, as well as hot water treatment, on survival in flatworms, earthworms, and land snails were analyzed using a generalized linear model (GLM) with binomial error distribution (JMP ver. 6.0; SAS Institute, 2005). Treatments immersed in different water temperatures and body sizes of each individual animal were used as explanatory variables. The response variable was whether an animal was killed (1/0). In ants, only water temperature was used as the explanatory variable, with death (1/0) as the response variable.

RESULTS

Different immersion temperatures significantly affected the mortality of flatworms, earthworms, and land snails, but body size and its interaction with treatment did not (GLM: flatworms: body size, df=1, $\chi^2=0$, $p=1.0$; water temperature, df=3, $\chi^2=80.7$, $p<0.0001$; body size×water temperature, df=3, $\chi^2=0$, $p=1.0$; earthworms: body size, df=1, $\chi^2=0$, $p=1.0$; water temperature, df=2, $\chi^2=93.2$, $p<0.0001$; body size×water temperature, df=2, $\chi^2<0.001$, $p\approx1.0$; land snails: body size, df=1, $\chi^2=0$, $p=1.0$; water temperature, df=4, $\chi^2=72.5$, $p<0.0001$; body size×water temperature, df=4, $\chi^2=0$, $p=1.0$). Exposure to hot water at ≥43, 43, and 50°C for 5 min resulted in 100% mortality of flatworms, earthworms, and land snails, respectively (Table 1). Immersion temperature significantly influenced the mortality of ants (GLM: water temperature, df=5, $\chi^2=207.7$, $p<0.0001$). Exposure to hot water at ≥47°C for 5 min resulted in 100% mortality of ants (Table 1).

DISCUSSION

Exposure to hot water at ≥43, 43, 50, and ≥47°C for 5 min resulted in 100% mortality of flatworms, earthworms, land snails, and ants, respectively (Table 1). In all animal groups, body size did not affect mortality under different treatments. Therefore, the water temperature frequently used in hot water treatment (ca. 50°C) could kill

Table 1. Hot water tolerance of alien soil animals on the Ogasawara Islands

Soil animal (species)	Mortality (%) at each water temperature[a] (n)					
	28°C	40°C	43°C	45°C	47°C	50°C
Flatworms (*Platydemus manokwari*)	0.0 (20)	25.0 (20)	100.0 (20)	100.0 (21)	— —	— —
Earthworms (unidentified)	0.0 (28)	3.7 (27)	100.0 (35)	— —	— —	— —
Land snails (*Acusta despecta sieboldiana*)	0.0 (15)	— —	0.0 (15)	0.0 (15)	0.0 (15)	100.0 (15)
Ants (*Technomyrmex albipes*)	0.0 (47)	— —	10.0 (30)	66.0 (50)	100.0 (44)	100.0 (41)

[a] Animals were immersed for 5 min.
—, no treatments at this temperature.

soil animals. Furthermore, the water temperatures that I used may not affect the survival of potted plants because exposure to hot water at 49°C for 12 min is not phytotoxic and has actually been shown to extend the vase life of ornamental flowers and foliage (Tsang et al., 1995). Tsang et al. (2001) designed the hot water drenching system and tested the effect of an increase in temperature using different potting media. The hot water drenching system proposed by Tsang et al. (2001) may be useful for killing soil animals that are frequently introduced with potted plants.

The isolated animals were directly immersed in hot water, but the ability of hot water to kill soil animals in potted soil masses was not examined; soil may protect the animals from direct contact with the hot water. Furthermore, some plant species may not tolerate water temperatures of approximately 50°C because the tolerance is species dependent (F. Kraus, pers. comm.). Therefore, further tests of the practicality of using hot water as a treatment method are needed at a local site (e.g., the Ogasawara Islands). However, hot water treatment is a promising method for preventing biological invasions by alien soil animals because it kills several animal groups such as insects (Hara et al., 1993, 1994, 1996, 1997, 1999; Tsang et al., 1995, 2001; Follett and Neven, 2006), as well as frog eggs (F. Kraus, pers. comm.). Because hot water does not negatively affect the environment, unlike pesticides, hot water treatment could mitigate biological invasions without causing pollution, particularly on oceanic islands that support valuable biota.

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