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〈Short Communication〉

Induction of Conditioned Taste Aversion to Korean Pine Nuts (*Pinus koraiensis*) Treated with Lithium Chloride in Red Squirrels (*Sciurus vulgaris*)

Kim, Eui Kyeong[1], Won Myeong Kim[2], Yung Chul Park[1], Byung Ho Yoo[3] and Jong Kuk Kim[1]*

[1]*Division of Forest Resources, College of Forest & Environmental Sciences, Kangwon National University, Chuncheon 200-701, Korea*

[2]*Ecosystem Assessment Division, Nature and Ecology Research Department, National Institute of Environmental Research, Incheon 404-170, Korea*

[3]*Ecological Restoration Division, Nature and Ecology Research Department, National Institute of Environmental Research, Incheon 404-170, Korea*

ABSTRACT: We investigated the responses of red squirrels to pine nuts (*Pinus koraiensis*) treated with Lithium Chloride (LiCl) and the potential of the chemical for inducing conditioned taste aversion (CTA) in red squirrels. In red squirrels, nut feeding declined dramatically during the first 4 days after feeding with LiCl-treated nuts. The ratio of LiCl-treated nuts eaten to total nuts eaten declined from the 1st day in LC-1 and the 2nd day in LC-2, along with a general reduction in quantity eaten. Thus, feeding with LiCl-treated nuts induced CTA from the 2nd day after feeding, and CTA remained constant until the 4th day, but disappeared on the 5th day. The squirrels ate an average of 757.0 ± 106.1 mg ($n = 2$, range 682.0～832.0) of LiCl before dying on the 16th day of the study. The lethal dose of LiCl was 2.32 mg LiCl/g body weight, and the average amount of LiCl needed to induce CTA was 23.0 ± 4.24 mg (20 mg in LC-1 and 26 mg in LC-2).

Key words: Conditioned taste aversion, Lithium Chloride, *Pinus koraiensis,* Red squirrels, *Sciurus vulgaris*

INTRODUCTION

Korean white pine trees, *Pinus koraiensis*, grow in a broad area from the middle to the north of Korea and are one of most popular silvicultural species in Korea. Korean white pines were planted over extensive areas of Korea in the 1970's and 1980's (Han and Yi 1996, Yi et al. 1999, Song and Yun 2006) and the nut harvest from the pine trees has increased recently (KFS 2007). Since pine nuts are one of the main food items for red squirrels as well as being a popular human food, the red squirrel population has also been increasing rapidly due to increased nut production by pine trees, resulting in a reduced nut harvest for humans. Various mechanical methods, such as shooting and trapping, have been employed to attempt to control red squirrel populations, but have not resulted in a decline in red squirrel populations.

In this study we tested the efficacy of CTA (conditioned taste aversion) for prevention of nut damage by red squirrels. CTA is a learned behavior that is associated with avoidance of harmful foods (Garcia et al. 1974, Welzl et al. 2001). CTA occurs when an animal associates the taste of a food with sickness, vomiting, or nausea and avoids consuming that food in subsequent encounters (Garcia et al. 1974). Thus it has probably evolved as defense to prevent consumption of the same harmful item in the future (Massei et al. 2002). Since CTA can modify animal feeding behavior, it could in theory be used to protect pine nuts from red squirrels. When a CTA agent is applied to pine nuts, red squirrels feeding on the treated nuts should subsequently avoid consuming those foods, resulting in reduced nut losses. We investigated the response of red squirrels to nuts treated with LiCl (Lithium Chloride) and describe the potential of LiCl for inducing CTA in red squirrels

MATERIALS AND METHOD

We captured 2 adult red squirrels (*Sciurus vulgaris*) in the natural forests of Kangwon National University (San 61-1, Bukbang-1ri, Hongcheon-gun, Kangwon-do, South Korea) and kept each individual (named LC-1 and LC-2) in a separate 1 m^3 cage in a laboratory for 30 days to allow them to acclimate to the laboratory environment. At the time of the experiments, the body masses of the squirrels were 312.9 g (LC-1) and 338.3 g (LC-2), respectively. CTA tests were conducted from 19 Nov to 15 Dec, 2003. During

* Corresponding author; Phone: +82-33-250-8363, e-mail: jongkuk@kangwon.ac.kr

preliminary observations, the red squirrels ate 5 to 100 pine nuts per day. We provided each squirrel with 50 nuts per day as food during the CTA tests.

For treatment with the chemical solution, we bored a hole 1 mm in diameter in each pine nut, and then injected 5 ul (2.0 mg) of LiCl solution (0.4 mg/uL) into the hole with a micropipette (Jaeger and Mucha 1990 and also refer to Prendergast et al. 1996). We subsequently fed the nuts injected with LiCl to LC-1 and LC-2.

We conducted CTA tests on each squirrel for 15 days. For the first 9 days, 50% of nuts (25 of 50 nuts) provided to LC-1 in a day were treated with LiCl. Subsequently, from the 10^{th} to the 15^{th} day, all nuts provided to LC-1 were treated with LiCl. For LC-2, during the first four days of the study 50% of nuts (25 of 50 nuts) were treated with LiCl, and from the 5^{th} to the 15^{th} day all of the nuts provided to LC-2 were treated with LiCl.

Nuts were provided to each squirrel in a plastic food bowl at 10:00 am every day. Before new nuts were provided, the nuts remaining in the food bowls and other parts of the cages were counted and removed from the cages. Every morning, we observed the squirrels' behavior patterns, including feeding, for 2 hours after providing the nuts. We also recorded indications that the red squirrels had vomited nuts.

RESULTS AND DISCUSSION

The two red squirrels showed similar reactions to LiCl-treated nuts. LC-1 ate slightly more nuts on the 2^{nd} day than the 1^{st}, then the number of nuts that LC-1 ate declined from the 2^{nd} to the 4^{th} day and then increased again from the 5^{th} day on (Fig. 1A).

During the first 9 days, LC-1 ate an average of 13.66 ± 8.99 (n = 9, range 2～25) LiCl-treated nuts/day and 23.11 ± 2.15 (n = 9, range 18～25) of the LiCl-untreated nuts (Fig. 1A). The difference was even more pronounced on the first 4 days, when LC-1 ate 5.50 ± 3.42 (n = 4, range 2～10) LiCl-treated nuts and 22.0 ± 2.83 (n = 4, range 18～24) untreated nuts (Fig. 1A).

The ratio of LiCl-treated nuts to total nuts eaten decreased for the first 4 days (averaging 19.58 ± 11.76% during the first 4 days) and then increased from the 5^{th} day on (averaging 44.92 ± 7.13% from the 5^{th} to the 9^{th} day; Fig. 1A and 3B). However, LC-1 vomited repeatedly starting on the 7^{th} day and finally was found dead on the 16^{th} day of the experiment.

LC-1 ingested an average of 11.00 ± 6.83 mg (n = 4, range 4～20) of LiCl during the first 4 days of the experiment and 40.4 ± 11.44 mg (n = 5, range 26～50) from the 5^{th} to the 9^{th} day (Fig. 1B). When all of the food was chemical-treated, from the 10^{th} to the 15^{th} day of the study, LC-1 ingested an average of 72.67 ± 19.54 mg/day (n = 6, range 40～92), with a total ingestion of 682 mg during the study, or 45.47 ± 29.14 mg/day (n = 15, range 4～92) (Fig. 1B).

The feeding patterns of LC-2 were similar to those of LC-1. LC-2 ate a decreasing number of nuts from the 2^{nd} to the 4^{th} day, including only 50% of the LiCl-treated nuts provided, and then ate an larger number of nuts after the 5^{th} day, when all of the nuts were treated with LiCl (Fig. 2A). On the 1^{st} day, LC-2 ate only 5.6 % nuts that had been treated with the chemical, but the proportion of treated nuts eaten increased to 37 % on the 2^{nd} day and then decreased to 17.9 % on the 3^{rd} day (Fig. 2A and 3A). On the 4^{th} day, LC-2 ate fewer nuts in total (Fig. 2A), but all of the nuts that LC-2 fed on had been treated with LiCl (Fig. 2A and 3A). During the first 4 days, LC-2 ate an average of 5.75 ± 5.12 (n = 4, range 1～13) LiCl-treated nuts and 15.50 ± 10.66 LiCl-untreated nuts per day (n = 4, range 0～23) (Fig. 2A). On average, LC-2's diet included 40.14 ± 41.97% (n = 4, range 5.56～100) of treated nuts during the first 4 days (Fig. 3A). Thus, LC-2 appeared to avoid

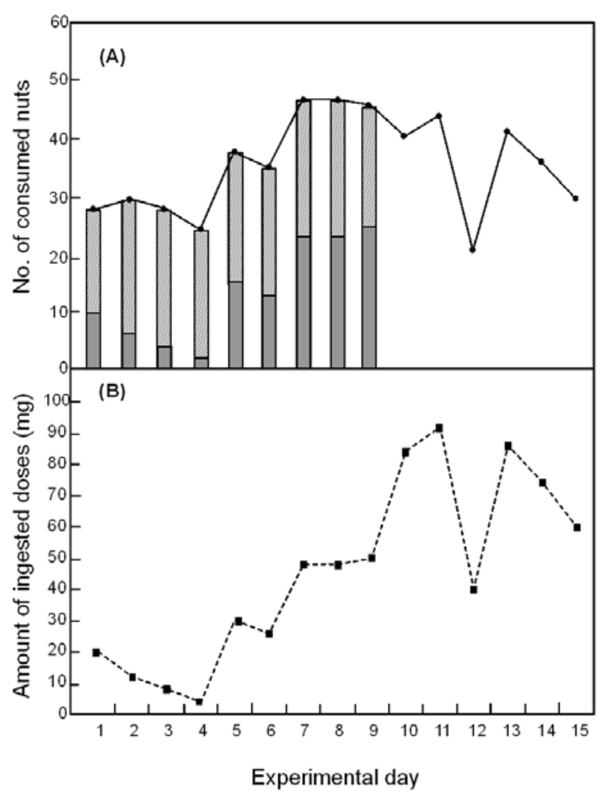

Fig. 1. The number of nuts that LC-1 consumed on each experimental day (●-●) and the numbers of LiCl-treated nuts (▥) and LiCl-untreated nuts (▇) that LC-1 ate during the first 9 days (A). The amount of LiCl ingested by LC-1 on each day (B). During the first 9 days of the experiment, only 50% of the nuts provided for LC-1 were treated with LiCl. From the 10^{th} to the 15^{th} day, all of the nuts were treated with the chemical.

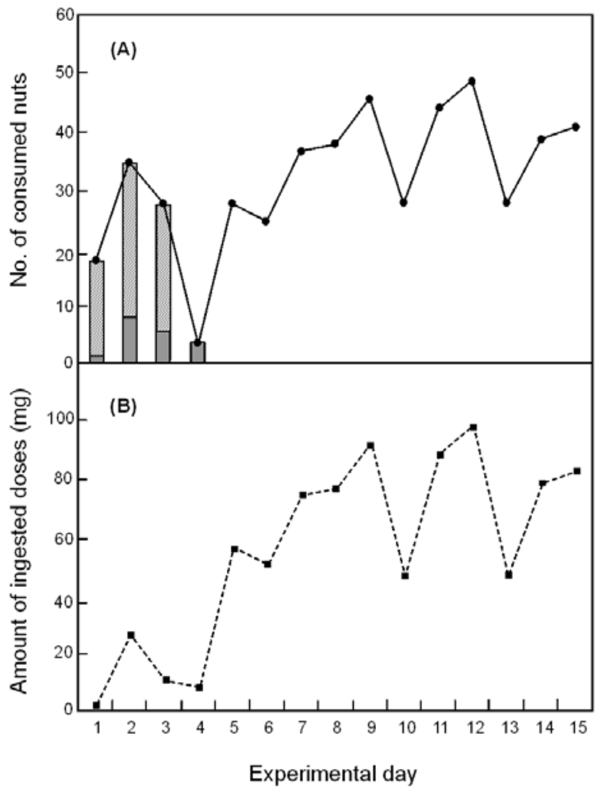

Fig. 2. The number of nuts that LC-2 consumed on each experimental day (●–●) and the numbers of LiCl-treated nuts (▥) and LiCl-untreated nuts (■) that LC-2 ate during the first 4 days (A). The amount of LiCl ingested by LC-2 on each day (B). During the first 4 days of the experiment, only 50% of the nuts provided for LC-2 were treated with LiCl. From the 10^{th} to the 15^{th} day, all of the nuts were treated with the chemical.

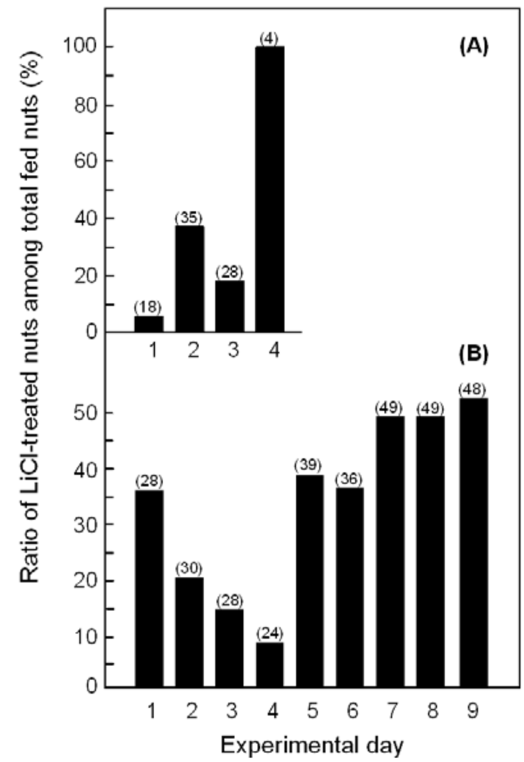

Fig. 3. Ratio of LiCl-treated nuts to total nuts eaten by LC-1 in the first 9 days (B; LC-1) and LC-2 in the first 4 days (A; LC-2). 25 LiCl-treated nuts and 25 LiCl-untreated nuts were provided during the first 9 days of treatment for LC-1 and during the first 4 days for LC-2. The numbers in parentheses indicate numbers of nuts eaten each day.

LiCl-treated nuts during the first 4 days when only 50% of nuts provided were treated with LiCl. Though nut feeding increased after the 5^{th} day, when only LiCl-treated nuts were provided, LC-2 vomited repeatedly from the 5^{th} day and finally died on the 16^{th} day.

The average amount of LiCl that LC-2 ingested was 11.5 ± 10.2 mg ($n = 4$, range 2～26 mg) during the first 4 days (Fig. 2B), and 71.5 ± 18.9 mg ($n = 11$, range 46～98 mg) from the 5^{th} to the 15^{th} day, resulting in a total accumulation of 832 mg and a mean daily accumulation of 55.47 ± 32.11 mg ($n = 15$, range 2～98) (Fig. 2B). From the starting day of the test until the termination of the test upon LC-2's death, LC-2 ate a total of 488 nuts and an average of 32.53 ± 11.7 nuts/day ($n = 15$, range 4～49) (Fig. 2A).

The average amount of LiCl accumulated in the bodies of LC-1 and LC-2 at the time of death was 757.0 ± 106.1 mg ($n = 2$, range 682.0～832.0). Since their average body weight was 325.6 ± 17.96 g ($n = 2$, range 312.9～338.3), we estimate that a lethal dose of LiCl is about 2.32mg LiCl /g body weight.

As both squirrels ate fewer treated nuts starting on the 2^{nd} day of the experiment, we conclude that CTA was induced from the 2^{nd} day after consumption of LiCl-treated nuts and that CTA persisted for 3 days (Fig. 1A and 2A) and disappeared on the 5^{th} day (Fig. 1A). We estimate that the average amount of LiCl needed to induce CTA was 23.0 ± 4.24 mg (20 mg in LC-1 and 26 mg in LC-2) (Fig. 1B and 2B), or 0.0639 mg/g body weight in LC-1 and 0.0769 mg/g body weight in LC-2. In a previous study, the maximum aversion effect was induced at 0.01 mg/g for LiC1 in Dawley rats (the lowest effective dose was 0.0025 mg/g for taste aversion and 0.01 mg/g for place aversions) (Mucha and Herz 1985). Thus, CTA induction appears to require larger doses of LiCl in red squirrels than in rats.

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