Use of Liquid Nitrogen to Treat Solenopsis invicta (Hymenoptera: Formicidae) Nests

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USE OF LIQUID NITROGEN TO TREAT *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) NESTS

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ABSTRACT

Liquid nitrogen (LN_2) injection was used to against the red imported fire ant (*Solenopsis invicta* Buren) by taking the advantage of rapid killing, no side effects from residuals, and non-dependence on weather conditions of this method. Red imported fire ants workers were placed in glass bottles and treated by sub-zero temperatures for either two or zero minutes after thermo-equilibrium to determine the lethal low temperature. Fire ant nests were then treated by LN_2 and the survival of the ants was monitored. Thus -15 °C was shown to cause 100% of mortality of workers at 24 hours post treatment. Large numbers of ant corpses, which included larvae, pupae and queens, were discovered in the nest after LN_2 treatment. In a field experiment, 10 nests were treated by LN_2, while 9 were left as controls. The number of active nests significantly decreased to just one nest at the 14th day post LN_2 application and none was considered to be functional at the 21st day. These results suggest that LN_2 freezing is capable of eliminating individual fire ant nests effectively, and that this treatment is useful for areas of high human activity and for agricultural and other areas that have a low tolerance to conventional pesticides.

Key Words: cryogenic, liquid nitrogen, individual mound treatment

RESUMEN

Se utilizó la inyección de nitrógeno líquido (LN_2) en nidos de la hormiga roja de fuego importada (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) para eliminar las colonias. Este tratamiento tiene ventajas, que incluyen la muerte rápida, no hay efectos laterales de los residuos químicos tóxicos, la independencia del tratamiento de las condiciones climáticas y selectividad ecológica. Con el fin de determinar la temperatura necesaria para matar la plaga, se colocaron las hormigas rojas de fuego importadas en botellas a temperaturas bajo cero, ya sea para 2 o cero minutos después de la termo-equilibrio y se encontró que no podían sobrevivir a temperaturas inferiores a -15 °C. Los nidos de hormigas rojas fueron tratados por inyecciones de LN_2 y después excavados para el estudio extensivo y la sobrevivencia de las colonias fue monitoreada después de la aplicación. Después del tratamiento con LN_2, numerosos cadáveres de hormigas, incluyendo larvas, pupas y reinas, fueron descubiertos en el nido. Sin embargo, el tratamiento no creó ningún residuo químico. En un experimento con 19 nidos seleccionados, 10 tratados por LN_2, y 9 no tratados (control), los nidos activos disminuyeron de manera significativa a un nido a los 14 días después de la aplicación de LN_2 y todos los nidos se consideraron no funcional a los 21 dias. Estos resultados sugieren que la congelación por medio del LN_2 es capaz de eliminar eficazmente los nidos individuales de las hormigas de fuego, y debe ser útil para áreas de alta actividad humana o para las zonas agrícolas que tienen una baja tolerancia a los plaguicidas convencionales.

Palabras Clave: criogénicas, nitrógeno líquido, tratamiento montículo persona, red imported fire ant

Ever since the 1930s when the red imported fire ant was first discovered to have invaded the United States from the Southern America (Callcott & Collins 1996) and incubated for decades, this nasty pest expanded its territory to Australia and New Zealand in the year 2001 (Moloney & Vanderwoude 2002; Ward 2009). The pest's rapid global invasions not only created medical and health issues, but also caused serious economic and agricultural damage (Adams 1986; Lofgren 1986). In late 2003 the pest alarm rang hastily in Taiwan as farmers—helpless against this newly arrived pest—called for the assistance of agricultural experts. A year later in 2004, a red imported fire ant infestation was confirmed on a highway barrier in Taipei city, which bustles with human activities. The application of conventional pesticide in this highly populated area soon raised concerns over the risks of environmental pollution on health and food safety. Therefore, the development of a method without residual effects should fulfill the needs of fire ant management in some pesticide sensitive areas.

Physical methods of insect control function by altering environmental or physiological factors that exceed the survival limitations of insects to cause a repellent or lethal effect. For example, immersion of red ginger flowers (*Alpinia purpurata* Vieill. K.) in hot water at 49 °C for 12 to 15 min eliminated more than 95% of infested banana aphids, ants and mealybugs (Hara et al. 1996). Tschinkel & Howard (1980) also demonstrated that treating fire ant mounds with three gallons of hot waters at 90 °C achieved nearly 60% killing efficiency. Liquid nitrogen has previously been used as a cryogen to reduce the temperature of grain bins to disinfect stored products (Li et al. 2009), and as a freezing agent to eliminate house dust mites (*Dermatophagoides pteronyssinus* Trouessart) on mattresses (Colloff 1986).

In a preliminary study, it was found that the red imported fire ant could survive at temperatures ranging between -17 °C and 55 °C (Chen et al. 2007). The supercooling points of the fire ants ranged from -6 to -16 °C, depending on the body sizes, nest locations, and weather conditions (James et al. 2002; Quarles et al. 2005, Hahn et al. 2008). Microclimatic differences of nest sites also affect ant mortality and recovery rate from cold shock (Boyles et al. 2009). Short term exposure of the ants to -4 °C did not exhibit much lethality unless continuously applied for 5 days; the latter caused 100% mortality (James et al. 2002). Thus the exposure time and the lethal low temperature are critical in developing the technique of LN_2 freezing for fire ant control.

Material and Methods

Lethal Cryogenic Temperatures for Red Imported Fire Ants

Red imported fire ant workers were collected in Ching-Pu, Taoyuan County, Taiwan with tissue papers (Lin et al. 2011). Sample bottles for experiments were prepared as below. At first, 10 workers were paralyzed by CO_2 and then moved to a 5 mL glass bottle. Secondly, the bottle was sealed with a lid. Finally, a thermocouple was inserted through the lid to monitor the inner temperature. Two sample bottles were dipped into the cryogen at -10 °C. When the bottle reached the thermal equilibrium with the cryogen, one bottle was quickly withdrawn and held at room temperature (about 25 °C), while the second one was left for 2 additional min before removal. After the -10 °C treatment was completed, the temperature of the cryogen was set to -12.5 °C and -15 °C for similar subsequent experiments. Controls without cryogenic treatment were prepared by leaving bottles at room temperature.

When treatments were ended, wet cotton balls were put into the recovered bottles as the water supply, and lids were replaced with meshed caps for ventilation right after the cryogenic treatments. The survival rates of ants in bottles were measured for 24 h post treatment. The series of experiments at various temperature were repeated 3 times (n = 3), and the results were analyzed by t test (α = 0.05).

Freezing Red Imported Fire Ant Nests by LN_2 Injection

A cryogenic tanks filled with LN_2 was connected, through a flexible metal conduit, to a stainless injection pipe which was about 125 cm long, 3.4 cm in diam and with 20-30 injection holes (0.5 cm diam) at the tapered end. A mound 30 × 40 × 20 cm^3 (major, minor axes, and mound height respectively) was selected, a soil sampling tube (3.4 cm diam) was hammered into the center of the mound to create a vertical shaft Then, the injection tube was immediately inserted into the mound and LN_2 was injected into the nest by the internal pressure of the tank. The application ended as the mound was filled with LN_2 and the leakage was observed on mound surface. The amount of LN_2 applied was dependent on factors including the mound size, underground nest shape, soil properties, and water content of the nest. The actual usage of LN_2 was calculated according to the contents gauge on the tank. During the application, the cooling rate of the nest was monitored by a thermocouple inserted into the nest center. After 24 h, when the treated mound had thawed, the nest was excavated for the inspection of the survival of workers, larvae, eggs, alates, and queens.

Evaluation of LN_2 Treatment on Individual Red Imported Fire Ant Nests

To assess the performance of our methodology, we filled red imported fire ant nests with LN_2, and then monitored nest activity for 3 wk. The experiment was carried out on flat rice land with loamy soil in Dayuan, Taoyuan County, Taiwan, where 19 fire nests all larger than 20 × 10 cm (major and minor lengths, respectively) were selected. The average above-ground mound size was estimated to be about 5,600 cm^3. Ten nests were injected with LN_2 and the its usage was recorded, while the other nine were merely penetrated with the injection tubes but no LN_2 was injected. The nest were defined as active when rebuilding was observed within one m of the original nest, or when large numbers of swarming ants (> 10 ants) were observed upon disturbance. On the other hand, a treated nest without signs of rebuilding or without a large numbers of swarming ants upon disturbance was considered inactive. All the nests were inspected for 3 wk post LN_2 treatment. The survival rates were recorded and compared by Fisher's exact test ($\alpha = 0.05$).

Results

Lethal Cryogenic Temperatures for Red Imported Fire Ants

The cryogenic treatments that resulted in the rapid death of the red imported fire ants were observed (Fig. 1). For temperature at -10 °C only a slight lethal effect was observed. Meanwhile, for ants kept at -12.5 °C for 2 min, the average survival rate significantly decreased to 10-20%. At the even lower temperature of -15 °C, all ants were killed.

Freezing Red Imported Fire Ant Nests by LN_2 Injection

Fig. 2a shows pressurized LN_2 being ejected from the openings of the injection tube that is connected to a LN_2 tank, and subsequently forming a ground mist of condensed water vapors. An example of hoar frost on an LN_2 frozen mound is shown in Fig. 2b. Following injection, not only the nests themselves, but also the area surrounding each nest was frozen solid; the internal nest temperature was found to drop to -147 °C (see the inset of Fig. 2b). When the injection was completed, only hoar frost was observed at the nest site (Fig. 2b). Generally, at 24 h after treatment, large

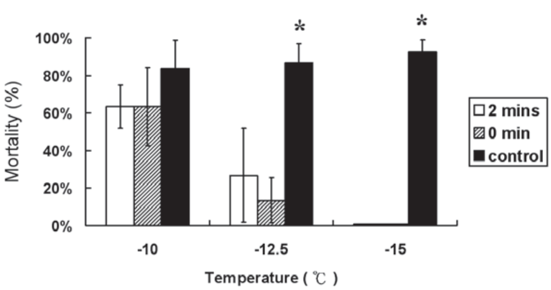

Fig. 1. The survival rate of the red imported fire ants one day after exposure to sub-zero temperatures for zero and 2 minutes after thermo-equilibrium (n = 3). The critical sub-zero temperature needed to freeze and kill the red imported fire ants. The results show that -15 °C treatment conferred the best killing efficiency on the fire ants. The asterisks indicate a statistically significant difference between the treated and control group in each temperature (t-test; $P > 0.05$).

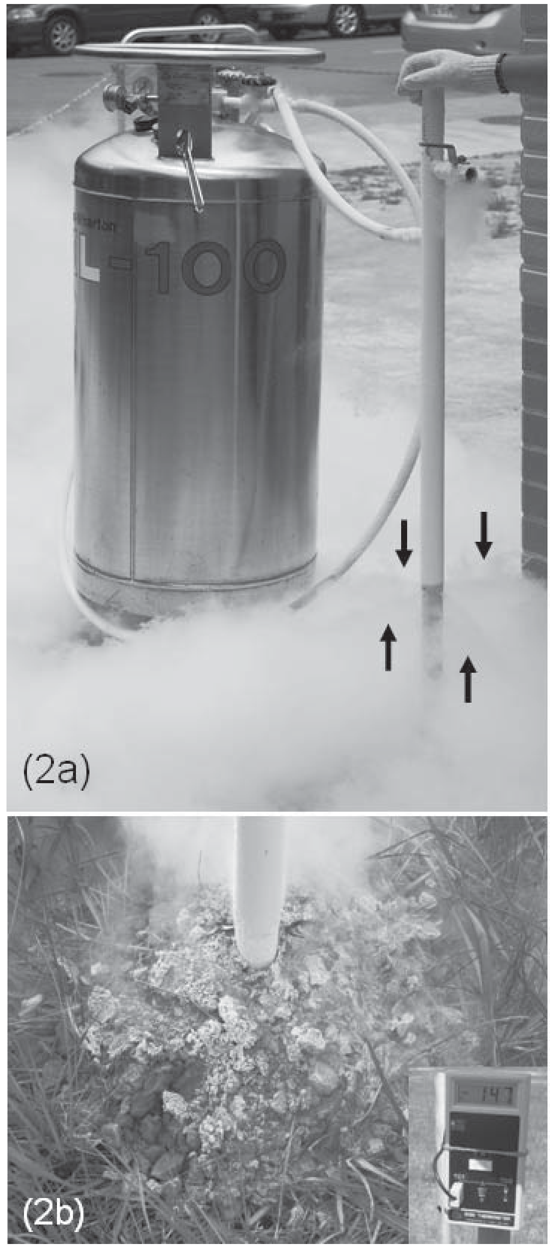

Fig. 2. (a) An injection tube was connected to a LN_2 pressure tank, LN_2 was ejected from the openings indicated by black arrows. (b) Frost was formed on the treated nest surface which indicated the drastic dropping of temperature. A thermometer showing that the temperature in the LN_2 treated nest reached -147 °C (inlet picture). Condensed water vapor along with nitrogen gas was observed evaporating from the mound top center. There was no other residue except for frost was found at the treated nests right after the treatment.

numbers of dead workers, alates, pupae, larvae, and queens were found in various corpse piles that accumulated around the nest (Fig. 3a).

Liquid nitrogen also killed the fire ants inside the tunnels and chambers as shown in an excavated nest (Fig. 3b), including dead bodies of alates and queens (Fig. 3c).

Evaluation of LN_2 Treatment on Individual Red Imported Fire Ant Nests

The efficacy of LN_2 treatment on individual mounds was evaluated by outdoor experiments involving 19 selected fire ant nests as described above. The total LN_2 usage was about 500 L per nest. On average, 5 to 8 min was required to treat a nest. After LN_2 application, the number of active nests started to decrease from 10 to 9 nests on the third day. It further decreased to 5 nests and finally to 1 on the 7th and 14th day, respectively. No activity was found in any of the treated nests after 3 wk, and no newly built nest was found nearby any of the treated mounds (Fig. 4).

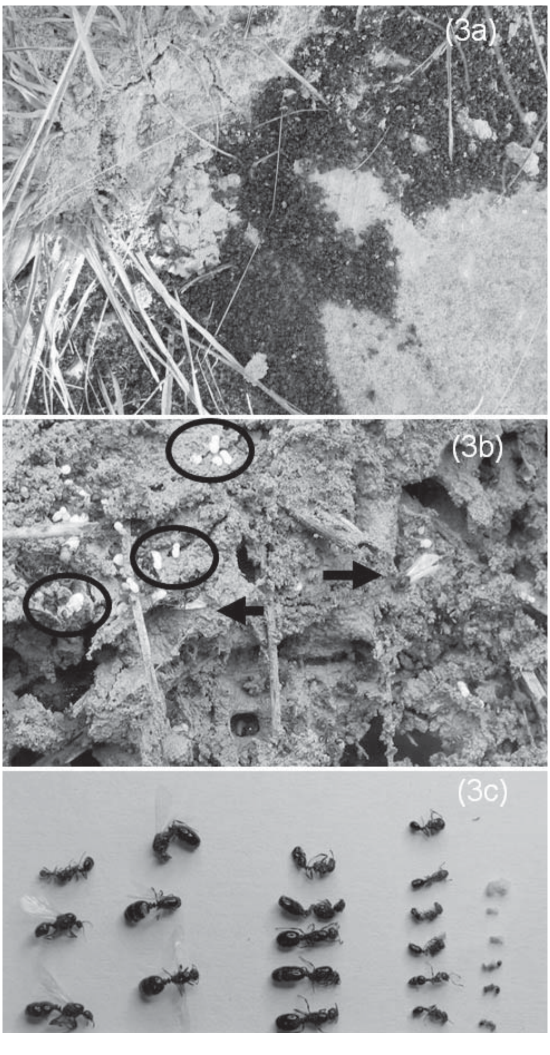

Fig. 3. (a) The ant graveyard formed by the remaining workers one day after. (b) Dead males (black arrows), larvae (black circles) and workers were also discovered in the treated nest. (C) Corpses of male, female alates and queens (from left to right) were collected from a treat nest suggesting the LN_2 injection could eliminate the sexual castes.

Discussion

The meandering distribution of tunnels and chambers facilitate the flow of liquids through fire ant nests (Green et al. 1999), and also in mounds of *Formica cinerea* Emery (Denning et al. 1977). Therefore, LN_2 injection could easily flow through and become evenly distributed throughout the entire nest-channel network. Liquid nitrogen not only removed heat from ants' bodies, but also froze soil particles and water in the inter-channel matrix, thus giving the nest a more rigid structure. Therefore, enough LN_2 could flow down to the deepest shafts in the nest through the hardened underground channels to kill the queens and alates at the lower depths of the nest. Liquid nitrogen injection also conferred indirect killing by lowering the nest temperature, thus establishing a lethal environment for the red imported fire ant. Although the fire ants in the wild are adapted to variations in temperature (Pinson 1980; Porter & Tschinkel 1987) and are capable of escaping from the cold (Morrill 1977; Morrill et al. 1978), injected LN_2 and vaporized N_2 gas generate a fast propagating cooling front that could cause a devastating chill-coma (Harris et al. 1965; Viñuela 1982). Meanwhile, the increased concentration of nitrogen gas throughout the nest might also cause hypoxia because of the displacement of O_2. These effects suggest that under certain condi-

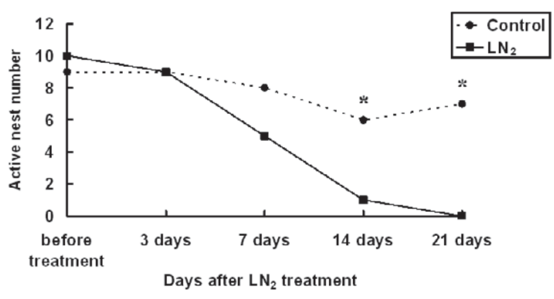

Fig. 4. Evaluation of LN_2 treatment on individual fire ant nests. The number of active red imported fire ant nest decreases with time after treatment. The asterisks indicate the statistical significant difference between the LN_2 treated nests and the controls. Data were analyzed by Fisher's exact test ($\alpha = 0.05$).

tions LN_2 freezing could be equally or even more effective than conventional insecticides that kill fire ants by direct contact (Lockley 2009). Although the mound built in sandy loam has less channel structures and spaces than in other soil types (Green et al. 1999), which may possibly affect the liquid transport in mound, the indirect killing effect of LN_2 could overcome this situation because the ants could still be killed by the cold. On the other hand, we found that the frozen nests were rigid and that they retained their intact structure during the excavation, which further provided a real model for detailed investigation of the spatial arrangement of the entire nest. Real time freezing also preserved all castes of the ants and the nest symbionts so that further studies on the inner ecosystem of nests could be conducted.

Nitrogen gas is chemically stable, confers no harmful effect on the environment and is not registered as a marine pollutant (The University of Iowa. 2011). Sites that were treated by LN_2 had no residual toxic chemicals, and the frozen ground recovered back to normal within h after the LN_2 had evaporated. The flora on or nearby the LN_2 treated nests withered, however few days post treatment, snails, beetles and spiders were discovered on the sites. The new emergence of moss and grass along with ant species other than the red imported fire ant were also observed about 2 wk post treatment. The appearance of non target insects and the re-growth of the flora suggested that the collateral damage of LN_2 injection was fleeting. To avoid harming valuable plants nearby the treated mound, the manner of LN_2 application or its combination with other control methods should be considered.

The corpse piles outside the treated nest could have been assembled by the foragers who were away from the nest during LN_2 treatment or by survivors within the nest. However, the reproductive portion of the colony could not escape or move under the rapidly killing of freezing event. Therefore even though ant activities may have continued in some treated nests, or surviving portions of colonies may have relocated, they soon perished as shown in our evaluation.

Liquid nitrogen is suitable for application in the areas that are less tolerant to insecticides such as urban locations, aquaculture farms, fruit and vegetable nurseries, and water supply catchments. Alternatively, integrating the LN_2 treatment with IGR-baits (insect growth regulators) might also make an effective “two-step” method for “greener” fire ant control.

Precautions should be taken when handing LN_2, because it cools and hard-freezes everything to an extremely low temperature. Plastic containers generally become fragile once contacted with LN_2. Metal vessels and pipes with proper thermal insulation are suitable for handling this cryogen. LN_2 may also cause cold burns to careless operators, therefore, thick cotton gloves and glasses were required for protection during operation. When liquid nitrogen evaporates, a huge amount of nitrogen gas is generated and builds up pressure in the container. Devices to regulate the pressure, like relief valves, should be installed onto the containers to avoid explosion. The labor costs to treat a nest was calculated about 0.7 USD (8.5 USD per h to treat 10-12 mounds), while the cost of liquid nitrogen per nest was 16.9 USD per nest. The total cost per nest was 17.6 USD, however, it may vary among different countries.

The aim of this research was to preliminarily assess the use of LN_2 freezing against red imported fire ants. These results indicate that filling fire ant mounds with LN_2 killed most of ants inside and inactivated the ant nest. This method has the potential to be an individual mound treatment method, or to become integrated into more environmentally friendly IPM programs.

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