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

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ABSTRACT

Liquid nitrogen (LN₂) 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 LN₂ 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 LN₂ treatment. In a field experiment, 10 nests were treated by LN₂, while 9 were left as controls. The number of active nests significantly decreased to just one nest at the 14th day post LN₂ application and none was considered to be functional at the 21st day. These results suggest that LN₂ 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 (LN₂) 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 LN₂ y después excavados para el estudio extensivo y la sobrevivencia de las colonias fue monitoreada después de la aplicación. Después del tratamiento con LN₂, 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 LN₂, y 9 no tratados (control), los nidos activos disminuyeron de manera significativa a un nido a los 14 días después de la aplicación de LN₂ y todos los nidos se consideraron no funcional a los 21 días. Estos resultados sugieren que la congelación por medio del LN₂ 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: cryogénicas, 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; Loefgren 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 (Dermatophagoidea 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 LN2 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 CO2 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 LN2 Injection

A cryogenic tanks filled with LN2 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 cm³ (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 LN2 was injected into the nest by the internal pressure of the tank. The application ended as the mound was filled with LN2 and the leakage was observed on mound surface. The amount of LN2 applied was dependent on factors including the mound size, underground nest shape, soil properties, and water content of the nest. The actual usage of LN2 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 LN2 Treatment on Individual Red Imported Fire Ant Nests

To assess the performance of our methodology, we filled red imported fire ant nests with LN2 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 cm³. Ten nests were injected...
with LN₂, and the its usage was recorded, while the other nine were merely penetrated with the injection tubes but no LN₂ 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 LN₂ treatment. The survival rates were recorded and compared by Fisher’s exact test (α = 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 LN₂ Injection

Fig. 2a shows pressurized LN₂ being ejected from the openings of the injection tube that is connected to a LN₂ tank, and subsequently forming a ground mist of condensed water vapors. An example of hoar frost on an LN₂ 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
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 LN₂ Treatment on Individual Red Imported Fire Ant Nests

The efficacy of LN₂ treatment on individual mounds was evaluated by outdoor experiments involving 19 selected fire ant nests as described above. The total LN₂ usage was about 500 L per nest. On average, 5 to 8 min was required to treat a nest. After LN₂ 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).

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, LN₂ 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 LN₂ 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 LN₂ and vaporized N₂ gas generate a fast propagating cooling front that could cause a devastating chill-coma (Harris et al. 1965; Vinuela 1982). Meanwhile, the increased concentration of nitrogen gas throughout the nest might also cause hypoxia because of the displacement of O₂. These effects suggest that under certain condi-

![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 LN₂ injection could eliminate the sexual castes.](https://bioone.org/journals/Florida-Entomologist/96(3)/26809-figure3a.png)

![Fig. 4. Evaluation of LN₂ 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 LN₂ treated nests and the controls. Data were analyzed by Fisher’s exact test (α = 0.05).](https://bioone.org/journals/Florida-Entomologist/96(3)/26809-figure4.png)
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 LN₂ 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 handling LN₂, because it cools and hard-freezes everything to an extremely low temperature. Plastic containers generally become fragile once contacted with LN₂. Metal vessels and pipes with proper thermal insulation are suitable for handling this cryogen. LN₂ 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 LN₂ freezing against red imported fire ants. These results indicate that filling fire ant mounds with LN₂, 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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