Effect of Harmonic Radar Tagging on Lycorma delicatula (Hemiptera: Fulgoridae) Nymphal Mobility and Survivorship

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Effect of harmonic radar tagging on *Lycorma delicatula* (Hemiptera: Fulgoridae) nymphal mobility and survivorship

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Abstract

The spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), has recently emerged as an invasive pest in the United States, threatening the grape and tree fruit industries. The biology of *L. delicatula* is poorly known. This invasive pest has rapidly spread and established itself in new regions (e.g., South Korea), taking advantage of a wide range of host plants, but virtually no studies of the dispersal capacity and patterns of the species have been published. Harmonic radar tracking provides a new tool to study the dispersal of small animals such as insects in natural field settings. In this study, we discuss procedures that allow secure attachment of harmonic radar tags on the 4th instar nymphs of *L. delicatula*. The adhesive bond strength between the radar tag and the nymph was sufficiently great for operational use, yielding 431.10 ± 43.37 *g* forces (mean ± SE). Variations in *L. delicatula* body size of did not affect the adhesive bond strength. Neither horizontal mobility nor vertical climbing ability of *L. delicatula* was affected by radar tagging when compared with untagged controls. Also, there was no significant difference in the jumping ability of radar-tagged and untagged insects. Finally, radar tagging did not affect the survivorship of *L. delicatula* over a 3 d test period. The results of this study indicate that the harmonic radar system can be applied successfully to 4th instars of *L. delicatula* without changing their mobility and survivorship. This technology facilitates study of the dispersal capacity and behavior of this invasive species under natural field conditions, and this information can serve as a basis for the development of effective monitoring and management strategies.

Key Words: spotted lanternfly; invasive species; dispersal; tracking; pest management

Resumen

La mosca linterna manchada, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), ha surgido recientemente como una plaga invasora en los Estados Unidos, amenazando las industrias de uva y de árboles frutales. La biología de *L. delicatula* es poco conocida. Esta plaga invasiva se ha extendido rápidamente y se ha establecido en las nuevas regiones (por ejemplo, Corea del Sur), tomando ventaja de una amplia gama de plantas hospederas, pero prácticamente no hay estudios publicados sobre la capacidad y los patrones de dispersión de la especie. El seguimiento por radar armónico provee una nueva herramienta para estudiar la dispersión de pequeños animales como los insectos en condiciones naturales de campo. En este estudio, se discuten los procedimientos que permiten una fijación segura de etiquetas radar armónicas en las ninfas del 4 instar (estadio) de *L. delicatula*. La fuerza de la unión adhesiva entre la etiqueta de radar y la ninfa fue suficientemente fuerte para el uso operacional, dando una fuerza de 431.10 ± 43.37 g (media ± DE). Las variaciones en el tamaño del cuerpo de *L. delicatula* no afectaron la firmeza de la unión adhesiva. Tanto la movilidad horizontal como la capacidad de acenso vertical de *L. delicatula* no se vieron afectadas por el etiquetado de radar en comparación con los controles sin etiquetar. Además, no había ninguna diferencia significativa en la capacidad para saltar de insectos con etiquetas radares y los no etiquetados. Por último, las etiquetas radares no afectaron a la sobrevivencia de *L. delicatula* durante un período de prueba de 3 dias. Los resultados de este estudio indican que el sistema de radar armónico puede ser aplicado con éxito a las ninfas de 4° estadio de *L. delicatula* sin cambiar su movilidad y sobrervivencia. Esta tecnología facilita el estudio de la capacidad de dispersión y el comportamiento de esta especie invasora en condiciones naturales de campo, y esta información puede servir como base para el desarrollo de estrategias eficaces de control y manejo.

Palabras Clave: mosca linterna manchada; especies invasivas; dispersión; rastreo; manejo de plagas

The spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), which is native to China, is an invasive species in the United States. In the literature, this species is also known as spot clothing wax cicada or Chinese blistering cicada. In the USA, it was first detected in Pennsylvania in Sep 2014, where it fed and laid eggs on willow, maple, poplar, and sycamore trees, as well as on fruit trees including plum, cherry, and peach (USDA-APHIS 2014). As of Dec 2014, the distribution of *L. delicatula* was restricted to Berks County (2,243 km^2) in eastern Pennsylvania. Based on anecdotal evidence, the infestation is believed to be at least 2 to 3 yr old (Barringer et al. 2015).

Lycorma delicatula threatens the grape and tree fruit industries in North America (USDA-APHIS 2014; Barringer et al. 2015). It is an invasive pest that has rapidly spread in South Korea since the mid-2000s and become a serious problem of grapes, fruit trees, and shrubs (Shin et al. 2010; Lee et al. 2011; Kim et al. 2013; Park 2015). Notably, damage by *L. delicatula* was recorded on 67 plant species, including *Ailanthus altissima* (Miller) Swingle (Sapindales: Simaroubaceae), *Toona sinensis* (A. Juss.) M. Roem. (Sapindales: Meliaceae), *Picrasma quassioides* (D. Don) Benn. (Sapindales: Simaroubaceae), *Parthenocissus quinquefolia* (L.) Planchon (Vitales: Vitaceae), *Vitis coignetiae* Pulliat ex Planchon

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(Vitales: Vitaceae), and *Vitis vinifera* L. (Vitales: Vitaceae), 34 of which are found in the U.S. (25 in Pennsylvania) (Park et al. 2009; Barringer et al. 2015). Although *L. delicatula* has not yet become a major pest in cultivated grapes in Pennsylvania, the insect has been observed feeding on wild *Vitis* species in the region (Barringer et al. 2015). This pest's preferred host in North America is the introduced tree *A. altissima*, otherwise known as tree of heaven (Barringer et al. 2015). The presence of this tree is believed to contribute substantially to the settlement and proliferation of *L. delicatula* in South Korea (Kim et al. 2013).

Lycorma delicatula had been studied very little until it emerged as an outbreak pest in South Korea in 2006 (Han et al. 2008; Barringer et al. 2015; Park 2015). Only 22 publications were listed in Scopus (Elsevier) in a search conducted in Sep 2015 (the search term was '*Lycorma delicatula*'). This insect remains poorly studied; there is virtually no information regarding the dispersal of this species. Information regarding dispersal behavior and capacity is essential in the effort to identify the extent of the infestation and its capacity for spreading into adjacent ecosystems. Given that there are no trapping methods (e.g., pheromone or light traps) established for this species, direct observation and tracking are the most effective methods of assessing the dispersal patterns of *L. delicatula*.

Traditional tracking tools for small animals such as insects are mostly limited to mark-release-recapture and mark–capture methods (Hagler & Jackson 2001). However, as is the case with many insect species, low recapture rates can severely affect the quality of the data (Boiteau et al. 2011). Recently, harmonic radar tracking techniques have substantially improved and, thus, we are provided with new technology to study the dispersal behavior and ecology of insects under natural field conditions (e.g., Mascanzoni & Wallin 1986; Wallin & Ekbom 1988; Riley et al. 1996; Roland et al. 1996; Chapman et al. 2004, 2011; O'Neal et al. 2004). Harmonic radar technology tracks insects by illuminating radar tags with a high-power microwave signal and recapturing the 2nd harmonic of the transmitted signal radiating from the tag (Riley & Smith 2002; Boiteau et al. 2009; Chapman et al. 2011). A unique feature of the radar systems is that the transponder of the radar tag does not require a battery, so the tag weighs only about 3 mg, which is light enough that many insects can carry it (Lee et al. 2013). Recent studies have demonstrated that harmonic radar systems can be used to study hemipteran insects, including *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) (Lee et al. 2013, 2014), *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Pilkay et al. 2013), and *Riptortus pedestris* (F.) (Hemiptera: Alydidae) (Lee 2016). These studies confirm that radar tags can be attached securely to the adult stage of several insects without affecting their survival, walking mobility, or flight capacity.

To our knowledge, previous studies on harmonic radar systems have applied radar techniques only to adult insects; no attempts have been made to evaluate the potential of radar systems for immature insects. Given that many insect pests, including *L. delicatula,* are known to damage crops and disperse in all stages of life, it is important to understand the dispersal ecology of nymphal insects. Thus, the objectives of this study were to apply harmonic radar tag attachment procedures (Boiteau et al. 2009; Lee et al. 2013) to the 4th instars of *L. delicatula* and to identify any potential negative effects on survivorship and behavior.

Materials and Methods

INSECTS

Fourth instars of *L. delicatula* were collected from *A. altissima* in Gwangju-si, Gyeonggi-do, South Korea (37.4244917° N, 127.3122972° E). The insects were brought to the laboratory immediately and maintained on *A. altissima* (28 °C, 60% RH, 16:8 h L:D photoperiod). The nymphs were used in the experiments within 12 h post-collection.

RADAR TAG ATTACHMENT

Harmonic radar tags were made as described in previous studies (e.g., Boiteau et al. 2009; Lee et al. 2013). The dipole radar tags were made of copper wire (0.3 mm in diameter). Tags were 9 mm high with a 1-mm-diameter loop at the pole and a 1 mm foot that was bent 90° (Fig. 1). Because the current study did not require activation of radar tags, a droplet of cyanoacrylate glue (Loctite 401 Prism Instant Adhesive, Henkel Corporation, USA) was applied to the loop in order to replace a diode and compensate for its weight. The final weight of the radar tag was about 3.0 mg. In order to attach the tags, *L. delicatula* nymphs were individually covered with a piece of paper (50 × 50 mm) with a square hole (5 × 5 mm) in the center. A droplet of cyanoacrylate glue was applied to the middle of the pronotum through the hole, and the radar tag foot was gently mounted on the pronotum in an upright orientation (Fig. 1). The insects were restrained for 5 min to let the glue dry and then transferred individually into 350 mL plastic cups. The insects were maintained in the vented cups, in which *A. altissima* branches and wet tissues were provided as food and water resources, at 29 °C and 56% RH.

ASSESSMENT OF TAG RETENTION STRENGTH

The adhesive bond strength between the radar tag and the pronotum of *L. delicatula* was measured with a tension measurement system (±3.1 g accuracy) (Economy Force Sensor, PASCO, USA). A Plexiglas panel (5 × 5 cm) with a hole (6 mm diameter) in the center was affixed upright 30 cm from the measurement system. Each radar-tagged nymph was positioned facing up on the opposite side of the panel from the measurement system, and the radar tag was inserted through the hole. A nylon thread (35 cm long) (Coats & Clark Upholstery Thread, COATS Corporation, USA) was tied to the radar tag, and the opposite side of the thread was pulled by the measurement system until the radar tag was dislodged from the insect. The measurement system recorded the force required to break the adhesive bond between radar tag and in-

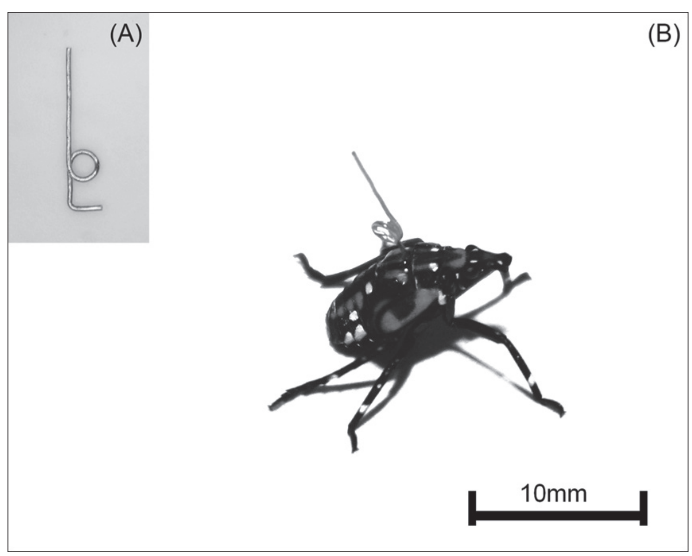

Fig. 1. (A) A 9 mm dipole harmonic radar tag. (B) An example of a 4th instar *Lycorma delicatula* nymph with a 9 mm dipole harmonic radar tag attached to the pronotum.

sect. The pronotum size and body length of each insect were recorded. Correlation coefficients were calculated for the relationship between pronotum size and body length versus adhesive bond strength (SigmaPlot Version 10, Systat Software Inc., USA). Thirty insects were tested for adhesive bond strength.

ASSESSMENT OF MOBILITY AND SURVIVORSHIP OF *L. DELICATULA*

Possible negative impacts of radar tagging on horizontal walking ability, vertical climbing ability, jumping capacity, and survivorship were assessed by comparing radar-tagged and untagged *L. delicatula* nymphs in the laboratory. To measure horizontal mobility, we observed the walking patterns of *L. delicatula* on the wood floor of the gymnasium (35 × 20 m) at Gachon University, Gyeonggi-do, South Korea (25 °C, 70% RH). In each trial, radar-tagged and untagged nymphs were followed separately by human observers. At the onset of each trial, the nymphs were gently placed on the floor and tracked by observers for 15 min. Observers were positioned >2 m away from the nymphs to avoid possible human disturbances to nymph behavior. The locations of the nymphs were recorded every 5 min, so that the total distance of the walking path could be measured at the end of the observation. Twenty-five individuals were tested for each treatment.

The vertical mobility of *L. delicatula* was measured in radar-tagged and untagged individuals in the laboratory (23 °C, 47% RH). At the onset of each trial, the nymphs were placed individually inside and at the base of clear polycarbonate cylinders (6 cm diameter, 36 cm tall). The distance that *L. delicatula* nymphs climbed was recorded for 5 min; the cylinder was inverted if the insect reached the top of the cylinder. Thirty individuals were tested for each treatment. Distance was analyzed using a *t*-test to compare the distances between radar-tagged and untagged individuals (SigmaPlot Version 10, Systat Software Inc., USA).

The jumping behavior of *L. delicatula* was assessed by measuring jumping distance and time duration from release to jumping under laboratory conditions (30 °C, 64% RH). In each trial, radar-tagged and untagged individuals were tested individually. Preliminary observations demonstrated that *L. delicatula* nymphs readily jumped when they were released from an elevated place. Thus, a piece of white paper (5 cm diameter) was attached at the top of a wooden stick (30 cm tall) as a release stand. At the onset of each trial, nymphs were placed individually on the release stand and observed for 3 min to record jumping behavior. When nymphs jumped from the stand, the jumping distance and time duration to jumping were recorded. Each individual was tested 3 times, and the median distance was used for data analysis. Thirty individuals were tested for each treatment. Data were analyzed with a *t*-test to measure the difference between radar-tagged and untagged individuals (SigmaPlot Version 10, Systat Software Inc., USA).

Survivorship of radar-tagged and untagged *L. delicatula* nymphs was evaluated in the laboratory (29 °C, 56% RH, 16:8 h L:D photoperiod). Each individual was placed inside a plastic cup and provided with an *A. altissima* branch in wet floral foam. The survivorship of each individual (alive versus dead) was determined every 4 h for 3 d. Thirty individuals were tested for each treatment.

Results

RADAR TAG RETENTION STRENGTH

The strength of the adhesive bond between the radar tag and the pronotum of *L. delicatula* averaged 431.10 ± 49.37 *g* forces (mean ± SE) (Fig. 2). This bond strength is sufficiently strong for operational use considering that, in 14 out of 30 individuals, the pronotal plate was dislodged from the insect body. Neither body length (r = 0.0338, P = 0.8591) nor pronotum size (r = 0.0477, P = 0.8024) correlated with bond strength (Fig. 2).

MOBILITY AND SURVIVORSHIP OF *L. DELICATULA*

Horizontal mobility of *L. delicatula* was not affected by radar tagging (t = 0.6027, df = 48, P = 0.5495) (Fig. 3A). The horizontal distance traveled by radar-tagged and untagged individuals was 1,430.12 ± 134.76 and 1,322.56 ± 117.00 cm (mean ± SE), respectively, in 15 min. Similarly, there was no significant difference between radar-tagged and untagged individuals in vertical climbing ability (t = 0.6165, df = 58, P = 0.5400) (Fig. 3B). Radar-tagged *L. delicatula* climbed 164.86 ± 38.55 cm, and untagged individuals climbed 134.79 ± 29.89 cm (mean ± SE) during a 5 min observation period. In the jumping test, the same number of radar-tagged and untagged individuals (24 out of 30) successfully jumped from the release stand within 3 min. Neither jumping dis-

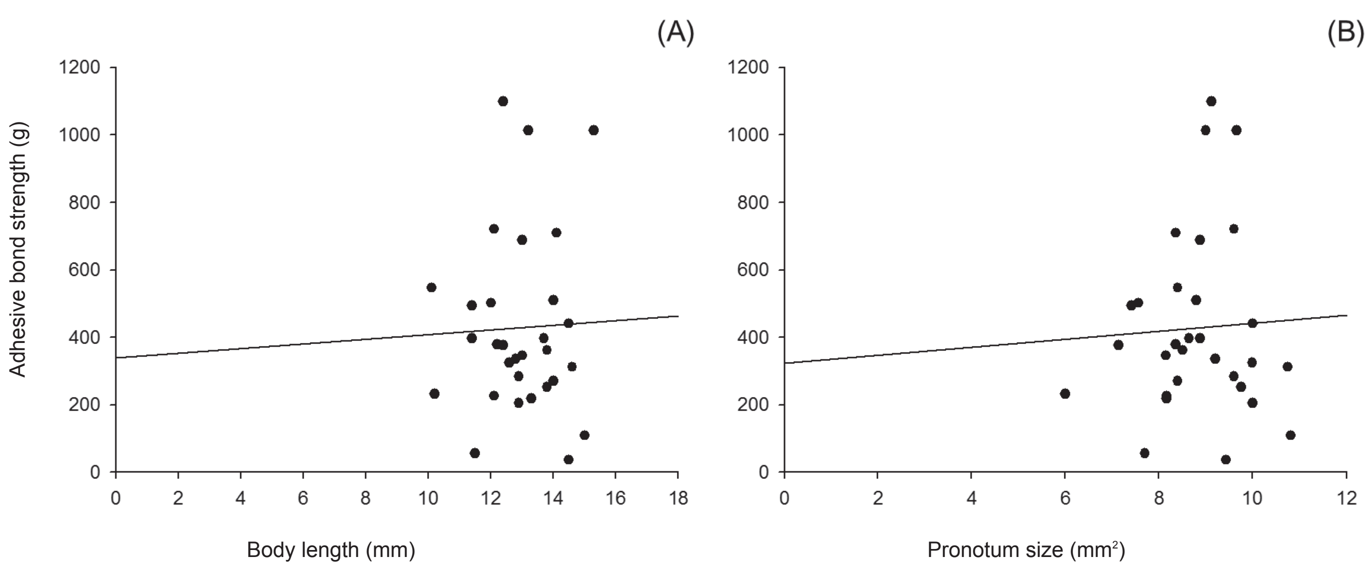

Fig. 2. The strength of the adhesive bond between the radar tag and the pronotum of *Lycorma delicatula* in relation to (A) body length and (B) pronotum.

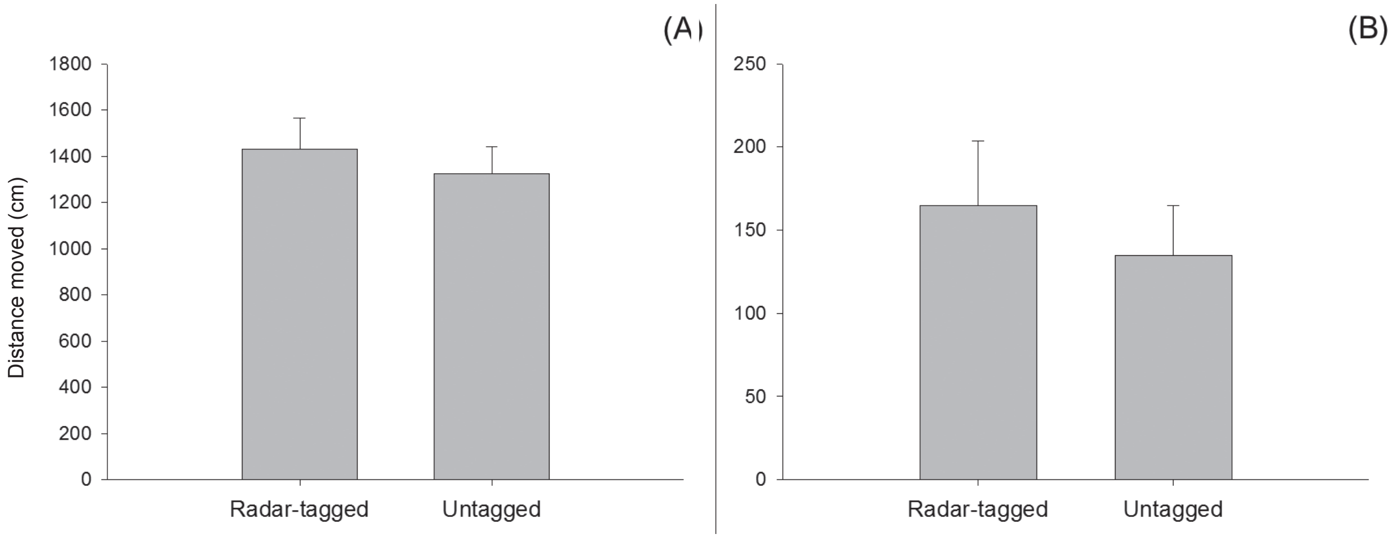

Fig. 3. (A) Horizontal distance moved by radar-tagged and untagged *Lycorma delicatula* in 15 min. (B) Vertical distance climbed by radar-tagged and untagged individuals in 5 min.

tance (t = 0.0145, df = 58, P = 0.9884) nor time duration from release to jumping (t = 0.2745, df = 58, P = 0.7847) was affected by radar tagging (Fig. 4). There was no difference in survivorship between radar-tagged and untagged *L. delicatula*; 24 out of 30 individuals survived after 3 d in both treatments.

Discussion

Our results suggest that harmonic radar systems can be applied to *L. delicatula* nymphs in order to track them without affecting their walking mobility and survivorship. In the laboratory, 4th instars exhibited strong walking abilities regardless of the presence of radar tags. Radar-tagged nymphs moved horizontally at about 95 cm/min on a wood floor, and this mobility was not significantly different from that of untagged individuals. Likewise, the climbing test showed that *L. delicatula* nymphs had strong vertical mobility, and there was no adverse effect of radar tagging on climbing capacity.

We also evaluated whether the presence of a radar tag would affect the jumping ability of *L. delicatula* nymphs. Field observations indicate that both nymphs and adults use jumping as an escape behavior; jumping allows them to cover a longer distance in a shorter time than does walking. Our results showed that there was no adverse effect of a radar tag on the jumping capacity of *L. delicatula*; both radar-tagged and untagged individuals jumped, on average, about 64 cm. Given that the extra radar tag weight (about 3 mg) is only 6.5% of the average body weight of *L. delicatula* nymphs, it is understandable that radar tagging had no measurable impact on mobility; this is consistent with findings from other insects including beetles (Boiteau et al. 2011; Gui et al. 2012) and stink bugs (Lee et al. 2013; Pilkay et al. 2013).

Harmonic radar systems require reliable attachment of the radar tag to the target insect. However, wax molecules or secretory compounds on the insect cuticle can result in a weak bond (Chapman 1998; Boiteau et al. 2009; Lee et al. 2013). For this reason, sanding the cuticular wax layer has been used to remove waxes and secretory

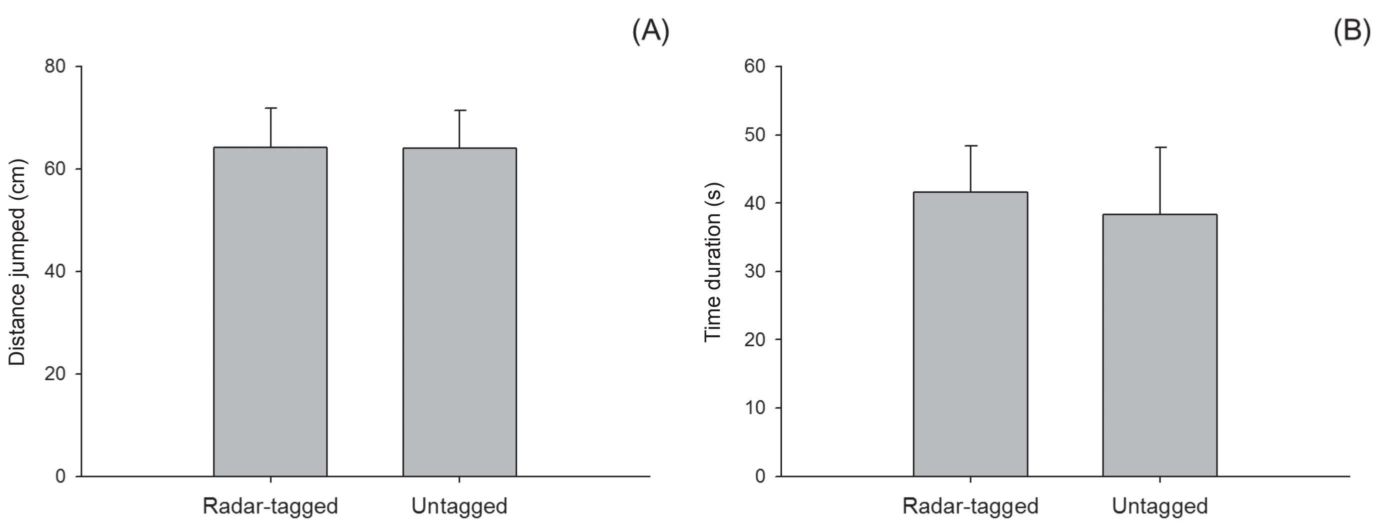

Fig. 4. (A) Jumping distance and (B) time duration from release to jumping of radar-tagged and untagged *Lycorma delicatula*.

compounds before applying glue in order to increase the quality of the adhesive bond (Boiteau et al. 2009; Lee et al. 2013). However, our results indicate that there is no need for sanding because the adhesive bond strength between the radar tag and untreated pronotum was sufficiently strong (>400 *g* forces) for operational use of the radar systems. Considering that the body weight of *L delicatula* nymphs is generally less than 46 mg (Pyo 2011), the attached tag is very unlikely to be dislodged from the insect by self-generated forces if the tag becomes entangled in the field.

Radar tracking of invasive pest such as *L. delicatula* will provide critical information useful for enhancing monitoring and management strategies. Dispersal plays a central role in insect invasion dynamics, especially during the early stages of establishment and spread. For *L. delicatula*, the radar systems that we studied can be particularly useful in the effort to better understand when and how the insects disperse and colonize wild and cultivated crops. For instance, tracking *L. delicatula* individuals in and around cultivated grapes, which are known as principal hosts late in the growing season, would provide detailed information on the timing and movement patterns of *L. delicatula* from wild hosts such as *A. altissima*. Radar tracking can be used to address adult dispersal dynamics including determination of potential egg-laying sites for overwintering. This phenological information will be useful in development of management programs to control invasive *L. delicatula*.

In practice, it is challenging to track the same individual over a very long distance. For example, 47% of tagged Colorado potato beetles were not recovered in a field study due to individuals leaving the experimental arena, resting behind or within objects (e.g., soil cavities), or because radar tags were lost or damaged (Gui et al. 2012). Although technical limitations still affect the recovery rate of tagged individuals, radar tracking provides direct and continuous measurement of insect dispersal with a much higher recovery rate than that possible with conventional mark–recapture approaches (Pilkay et al. 2013). This study confirms that harmonic radar tagging can be applied successfully to *L. delicatula* nymphs, and, therefore, it can serve as a useful tool to address dispersal capacity and behavior of this species and probably many others.

Acknowledgments

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