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病毒感染對入侵紅火蟻及臺灣本土螞蟻種間競爭之影響

Virus infection affects interspecific competition among the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae) and native ants in Taiwan

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摘要

入侵紅火蟻撲滅計畫自 2004 年實行以來，有效地限制了入侵紅火蟻之擴散及其族群建立於重點防治區域。然而單蟻后族群婚飛造成之新發生點以及防治區域之再次入侵事件等，均持續發生。目前新的防治策略著重在整合性蟲害管理，結合餌劑及生物防治性天敵之防治方法。本研究目的為探討入侵紅火蟻體內新發現之單股 RNA 病毒 (*Solenopsis invicta* virus 1) 其作用在新生族群之入侵紅火蟻及兩種本土性螞蟻，熱烈大頭家蟻 (*Pheidole fervens*) 以及中華單家蟻 (*Monomorium chinense*) 種間競爭之影響，分別從個體與族群間干擾競爭能力探討。族群間干擾競爭之結果顯示，兩種本土性螞蟻在擁有數量優勢時，無論入侵紅火蟻感染病毒與否，的確有能力殲滅入侵紅火蟻之新生族群。中華單家蟻花費較少入侵時間去殲滅病毒感染之入侵紅火蟻相較於健康之火蟻。當入侵紅火蟻與相同數量之熱烈大頭家蟻競爭時，健康之入侵紅火蟻可完全殲滅熱烈大頭家蟻族群，但是經病毒感染之入侵紅火蟻，則需花費較長時間才能入侵成功。從試驗觀察結果，推測病毒感染造成入侵紅火蟻覓食行為及入侵能力之改變。遭受病毒感染之入侵紅火蟻與健康之入侵紅火蟻相比，派出之覓食工蟻較少，尤其在與其競爭之本土螞蟻出現的情況下。個體間干擾競爭之結果顯示出中華單家蟻造成病毒感染入侵紅火蟻小型工蟻較高之死亡率；然而入侵紅火蟻之大型工蟻憑藉著本身化學防禦及體型優勢，相對於本土性螞蟻表現高度競爭力。另外，熱烈大頭家蟻之兵蟻的確具備防禦功用，其造成入侵紅火蟻之高死亡率。由以上結果推測，遭受病毒感染或許造成入侵紅火蟻競爭能力之下降。中華單家蟻之化學防禦可能是造成遭病毒感染之入侵紅火蟻高死亡率之影響因子，但是熱烈大頭家蟻大顎攻擊之物理性防禦，並不會造成健康或病毒感染之入侵紅火蟻死亡率之差異。本研究突顯了整合競爭性及病原性防治天敵於火蟻區域性防治之可行性。

關鍵詞：入侵紅火蟻、入侵紅火蟻病毒、種間競爭、熱烈大頭家蟻、中華單家蟻

Abstract

The fire ant (*Solenopsis invicta*) eradication program has been carried out in Taiwan since 2004. It certainly effectively restrains the populations of fire ant on treatment areas. Unfortunately, the reinfection or new infection site by natural nuptial flight of monogyne colony are still promulgated. Current new approaches to manage fire ants are integrating biological control agents with baits applications. This study aims to investigate alternative suitable biological control agents in Taiwan, and examines how SINV-1 (*Solenopsis invicta* virus-1) infection affects interspecific competition between incipient *S. invicta* against two native ants, *Pheidole fervens* and *Monomorium chinense*, by conducting two levels of trial, colony interference and individual confrontation, in laboratory conditions. The results from colony interference study showed that both native ants owing numerical advantages were capable to kill either infected or healthy queens of *S. invicta*. There was a significant less time for *M. chinense* to eliminate SINV-1 infected *S. invicta* compared to healthy ones. All *S. invicta* could repulse the invading of equal worker numbers of *P. fervens*. Compared with healthy *S. invicta*, SINV-1 infected *S. invicta* spent longer time to terminate *P. fervens* colonies. Virus infection was observed to have significant effects on foraging behavior and invading willingness of *S. invicta*. SINV-1 infected *S. invicta* recruited lesser number of foragers than healthy one, and in particular on competitive native ants were present. In confrontation trial *M. chinense* caused significant greater mortality on infected *S. invicta* minors than did on healthy ones. However, *S. invicta* majors (either infected or healthy) performed high competitive abilities against *M. chinense*, which might account for chemical defense and/or size advantages. In dealing with *P. fervens*, one cannot but admit that soldiers indeed function in defense, which caused *S. invicta* high mortality. These data suggest that virus may somehow weaken the competitive ability of infected *S. invicta* and make them prone to be terminated by *M.*

chinense but not by *P. fervens*. Chemical interference by *M. chinense* might be a more likely influencing factor on mortality of infected *S. invicta* than the physical combat by *P. fervens*. Results from this study highlight the likely success on area-wide control of *S. invicta* in Taiwan by an alternative strategy that involves integration of competitors and pathogen.

Key words: *Solenopsis invicta*, *Solenopsis invicta* virus, interspecific competition, *Monomorium chinense*, *Pheidole fervens*

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1. Introduction

Since the eradication program of *Solenopsis invicta* initiated, the density and spread rate of *S. invicta* have been controlled in Taiwan. According to previous study, the range of the polygyne colonies was small and assembled in the middle of Taoyuan County, but the range of the monogyne colonies was larger and randomly distributed, especially in outlier area of Taoyuan (Yang *et al*., 2008). The polygyne colony has been reported more susceptible for bait treatments because of frequent intercolony food exchange and high nest density (Matthew *et al*., 2006). Therefore 80 % of fire ant control rate was mainly resulted from the core polygyne area. According to this fact, monogyne colonies could be a loophole in the fire ant control program. Future control program should consider the other alternative control strategies to cooperate with chemical control of *S. invicta*.

The *S. invicta* are suppressed by the wide varieties of natural enemies especially parasitoids, pathogens and competitors in the native range (Porter *et al*., 1997; Keck *et al*., 2005). Therefore, it is possible to apply predators, or other competitors as biological control agents for preventing outward expansion and reducing the density of *S. invicta* (Mottern *et al*., 2004). Among more than ten natural enemy species, phorid flies and microsporidia are two major groups that have been developed for main biological control agents in area wide control of *S. invicta* in USA (Vander Meer *et al*., 2007; Oi *et al*., 2008).

It has been reported that main predators of *S. invicta* probably are other ant species (Wilson, 1971). Due to predation by other species, the mortality of newly mating queen of *S. invicta* was expected over 99 % (Whitcomb *et al*., 1973). The interspecific competition among different ant species also restrained

the spread abilities of *S. invicta* (Hung and Vinson, 1978). Hence, using native ant species to control fire ants might be an alternative concept of biological control of fire ants.

Recent progress on developing of biological control of fire ant is to use virus as a management agent since the first discovery of the picorna-like virus (SINV1) in 2004 (Valles *et al*., 2004). One distinct genotype of SINV-1 (SINV-1A) and another new positive strand virus SINV-2 have been isolated from *S. invicta* in 2005 and 2007, respectively (Valles and Strong, 2005; Valles *et al*., 2007a). Both viruses infect all stages of fire ants in the nest (eggs, larvae, pupae, and queens) and may induce significant mortality of brood, otherwise no apparent symptom or negative impact on colony performance could be found in infected colonies (Valles *et al*., 2007b). Indeed, it should not neglect the probable effects of SINV-1 / SINV-2 on *S. invicta* given the fact that viruses do impact on honey bee population when they are exposed under stressful conditions or with infection by unrelated pathogens (Tentcheva *et al*., 2004).

Although biological control by using either pathogens or competitors has been widely applied, few studies exactly characterized how effective it is if two types of agents are integrated into a *S. invicta* biological control program (Keck *et al*., 2005). Previous data showed that there were no pathogens infected populations of *S. invicta* in Taiwan except virus (SINV-1 and SINV-2, Yu, 2009). Combined with virus and several ant species with strong competitive ability were found co-existing with *S. invicta* (Tsai *et al*., 2008) may become potential biological control agents.

1.1 Biology and control of imported fire ants

The red imported fire ants, *S. invicta* were native to South America and have been accidently introduced into two areas of Taiwan, Taoyuan and Chiayi Counties since October 2003 (Chen *et al*., 2006). The fire ant colonies have two social forms, monogyne and polygyne. In the monogyne colonies, colony reproduction certainly through mating flights followed by colony founding. A monogyne newly mated queen reserve 50 % body fat to take flight. Mating flight aerially occur midday in warm, moist weather after substantial rainfall day (Tschinkel, 2006). Newly mated queens usually fly less than 400 m but sometimes fly up to 1.6 km or more (Tschinkel, 2006). A female alate mates once with one male alate (Ross and Fletcher, 1985; Ross, 1993) and store the sperm in the female's spermatheca and found new colony independently. Workers of monogyne colonies are aggressive and willingly recognize non-nestmate intruders, and they will attack and kill them.

In contrast, polygyne colonies are not by one queen independent founding but by multiple fertile queens instead. The female alates from polygyne colonies weigh (about 10-11 mg) less than monogyne alates (about 14-16 mg), as a result, decrease mating flights ability due to insufficient body reserves and tend to mate near natal nest. Because these female alates can not found colonies independently, they probably are adopted into a polygyne nest (Glancey and Lofgren, 1988; Porter, 1991). Additionally, in polygyne colonies, exchange of workers and trophallaxis frequency among nests are probably high. Since 80 % of the polygyne male alates are diploid and sterile, only have 30 % of the egg-laying queens in polygyne colonies are uninseminated due to mating with diploid male (Tschinkel, 2006). As a result, polygyne colonies always occur

accompany with monogyne colonies (Tschinkel, 1998).

The most effective method to control fire ants is using the insect growth regulating baits, fast-acting baits, and toxic baits Amdro® which contained the active ingredient hydramethylnon, once-refined soybean oil, and corncob grits. Such chemical control methods were achieved by individual mound treatment or by broadcast treatments on infection areas. Although certainly acute mammalian toxicity didn't be observed clearly, many environmental scientists and ecologists concerns the use of baits may affect on non-target native ants as well as the environment (Markin *et al*., 1974; Calixto *et al*., 2007). Additionally, if control method of fire ants were single used the chemical control, only have ≦ 85 % control efficacy can be achieved (Vander Meer *et al*., 2007; Oi *et al*., 2008). Consequently, fire ants continued to spread and reinfestations on bait-treatment areas that abundance of native ants has been decreased. Thus the development of species-specific control methods, such as biological control agents, should be emphasized in fire ant control, which may increase the control efficacy.

Compared to population density of *S. invicta* in native South America, the average density of *S. invicta* in the United States were more than 5 times higher. Meanwhile, the absent of natural enemies, included predators, parasites, pathogens, and competitors, in the United States may be the major reasons for this difference in population density (Porter *at al*., 1997). According to prior study, there are many natural enemies in South Ameica consists of least 18 species of parasitic phorid flies, 10 or more microorganisms, 3 species of nematodes, a parasitic wasp, a parasitic ants , and many of other symbionts (Porter *at al*., 1997). However, it is the front burner to search self-sustaining biological agents to suppress fire ant populations in lone time scale. The recent potential natural enemies could be release to field are two species of phorid flies, *Pseudacteon tricuspis* Borgmeier and *P. curvatus* Borgmeier (Porter *et al*., 2004; Vazquez *et al*., 2006) and one

species of microsporidium, *Kneallhazia* (= *Thelohania*) *solenopsae* (Oi and Williams, 2002; Sokolova and Fuxa, 2008).

Phorid flies in the genus *Pseudacteon* owned high host specificity which specialized parasitized the genus *Solenopsis* (Porter *et al*., 1995a; Porter, 2000). Phorid flies certainly act on stopping the foraging behavior, and only one phorid fly may affect hundreds of fire ants workers (Porter *et al*., 1995b). As a result, decapitating flies decreased the exploitative ability of fire ants and probably increased the probabilities for other ants to monopolize or recruit food resources (Feener, 1981; Morrison, 1999; LeBrum, 2005). The microsporidium, *Kneallhazia solenopsae*, was an intracellular obligate pathogen which though vertical transmission from the queen to its progeny (Valles *et al*., 2002). *Kneallhazia solenopsae* has been shown to reduce the weights of workers, reproductives, and queens (Cook *et al*., 2003).

As a matter of fact, combining biological and chemical control for the managements of fire ants is a large progress in the history of fire ants control. The IPM research for fire ants in Florida and Texas indicated that combine chemical insecticide, fipronil, with two biological control agents, microsporidium and decapitating phorid fly, could increase control efficacy from ~ 85 % (only use chemical treatment) to ~ 95 % at the integrated site (Vander Meer *et al*., 2007; Oi *et al*., 2008).

1.2 Biotic resistance

Any invasion proceeds through two primary stages: the first is the introduction, colonization, and establishment of a non-indigenous species in a new area; the second is the dispersal, spatial distribution, and spread (Allendorf and Lundquist, 2003). The probability of an exotic species successfully established in unoccupied regions is certainly associate with recipient environment and intrinsic characterize of the invasive species (Sagata and Lester, 2009). All successful invasive species may perform two

characteristics: superior competitive ability to occupy limited food resource or less susceptibility to unfavorable environment which restrict other species (MacDougall and Turkington, 2005). Abiotic environment conditions play an important role on triggering the invasion of exotic species. The invasive species must overcome the environment stress (temperature, disturbance, and so on) to arrive, survive, and establish itself (Gibb and Hochuli, 2003). Additionally, the number of exotic species individuals (or called propagule size) in an invasion front has a strong relationship with success invasion. Larger incipient populations have a higher chance to invade in a new area by their numerical advantages to easily occupied at food resource and successfully compete the nesting site. In contrast, small incipient populations may hard to reproduce, or may not resist unfavorable environment and activity of native species populations (Sagata and Lester, 2009).

The local activity of native ant community may influence the success of incipient invasive species by their relative numerical advantages, especially affect on distribution phase. Elton (1958) first proposed the biotic resistance hypothesis; the activities of local species may hinder the spread of invasive species, especially by predation and competition (Walters and Mackay, 2005). While the invasion happened in a new area, those invasive species meet some resistance by nature. The recipient community structure existed dominant or functional similar native species to repel those invasive species and significantly affect the survival and spread of invaders. Species richness poor communities have been believed to be more invasibility due to lack of biotic resistance (Elton, 1958). However, the level of disturbance, such as fire, grazing, logging, and human activity, may significantly change the community structure and resulted in decreasing the total richness and abundance (Gibb and Hochuli, 2003; King and Tschinkel,

2008). Once competitive/predatory species disappear with fast rate by repeated disturbance resulted intensity of competition reduced, the invasive species may have more chance to invade the recipient community and displace native species (MacDougall and Turkington, 2005).

Walters and Mackay (2005) used the native dominant ant, *Iridomyrmex* to test the biotic resistance hypothesis against Argentine ants in Australia. They indicated biotic resistance form the dominant native ant genus, *Iridomyrmex*, may affect the invading and limit the distribution of Argentine ants in South Australia. However, while the colony size of Argentine ants became larger, Argentine ants significantly affected the foraging success of *Iridomyrmex* and finally overcame and excluded the native ants by their high recruitment numbers. In addiction, Wilson (1971) mentioned that the greatest enemies of ants are other ants as well as the major predators of ant species are frequently other ant species (Wilson, 1971; Hölldobler and Wilson, 1990). There are some research reported some native ants were capable to destruct and prey small colonies of invasive species (O'neal, 1974; Rao and Vinson, 2004). For example, Rao and Vinson (2004) observed the native ant species *Monomorium minimum* (Buckley), *Pheidole dentate* Mayr, and *S. molesta* could attack and eliminate small *S. invicta* colonies in the laboratory experiments. At the same time, another native ant *Forelius* sp. can prevent *S. invicta* workers from leaving their nest to forage.

Besides, we should not neglect that the survival of newly founding queens of monogyne colonies play an important role in the life cycle of fire ant societies. The newly founding queens of *S. invicta* are more vulnerable to predation and suffered high mortality during the nuptial flight until the first workers have a chance to forage and defense. The mortality due to predation can

be expected to exceed 99% (Whitcomb *et al*., 1973; Nickerson *et al*., 1975). A major cause of mortality of newly founding *S. invicta* queens is certainly predation by other native ants and workers from nearby other colonies (Whitcomb *et al*., 1973; Hung, 1974; Nickerson *et al*., 1975; Kaspari and Vargo, 1994). The recorded predators of queens in the low *S. invicta* density areas in Florida included least three native ant species (e.g., *Conomyrma insane* (Buckley), *Lasius latreille* Emery, and *Pogonomyrmex badius* Latreille) (Whitcomb *et al*., 1973).

Even if newly mated *S. invica* queens successfully began a nest and their first generation of workers eclose and started to forage, these colonies should compete with other native competitive/predatory ants. Competition with native ant species for food was reported a key phenomenon in ant community and resulted in the competition hierarchy associated of subordinate ants and dominant ants (Hölldobler and Wilson, 1990).

1.3 Competition mechanism among ants

In order to understand the competition hierarchy among ants, researchers pay more attentions on interspecific competition mechanisms to explain why some exotic ants could quickly displace native ants (Holway, 1999; Morrison, 2000; LeBrum *et al*., 2007). In general, competitive mechanisms can be simply divided into two categorizations as either exploitation or interference. Exploitation refers to resource competition among different species, which one species skilled at discovering and using limiting resources resulted in decreasing resources acquisition of another species. Interference is kinds of direct competition for a species to prevent others from accessing resources by aggressive behaviors, chemical defense, and so on (Schoener, 1983; Begon *et al*., 1986).

At the same time, Schoener (1983) further classified the competitive mechanisms

into six categories of interactions: consumptive, preemptive, overgrowth, chemical, territorial, and encounter competition. Additionally, the mechanisms of competition leading to displacement generally could be identified the following case. (1) differential resource acquisition, (2) differential female fecundity, (3) differential searching ability, (4) resource preemption, (5) resource degradation, (6) agonistic interference competition, (7) reproductive interference, and (8) intraguild predation. Actually, one species has abilities to displace other species must due to four factors: (1) resource monopolization, (2) release from natural enemies, (3) metapopulation structure, (4) highly environment adaptations (Reitz and Trumble, 2002). According to such competitive interaction, relative abundance, colony size, body size, and foraging strategy may caused the competitive hierarchies are common outstanding asymmetric and lead to one species monopolize resources, at the same time, so that displace the other species (Hölldobler and Wilson, 1990; Reitz and Trumble, 2002).

Asymmetric competition is defined as competitions staged in no equilibrium population density or body size. Abundant or larger species have a disproportionate advantage over smaller ones regardless of individual or population level (Persson, 1985; Bauer *et al*., 2004). Asymmetric competition is suggested the natural outcome of local competition among species (Bauer *et al*., 2004). Additionally, asymmetric competition was not only caused by inherent species characteristics but also trait-mediated indirect interactions (TMIIs). The body size, colony structure, aggressive behavior, and resources acquisition ability can direct affect the competition relationship between each species. Nevertheless, the interactions between two species probably are affected by the existence of an intermediary species (Morrison, 1999).

A species will modify their developmental, morphological, physiological, life historical or behavioral traits in response to the presence of another species (Werner and

Peacor, 2003; Miner *et al*., 2005). Most parasites can affect host populations by modifying the host mortality, fecundity, growth, physiology, and behavior of their host and may eventually weaken the competitive ability, and alter the outcomes of competitions between their host and competitors (Poulin, 1994; Hudson and Greenman, 1998; Poulin and Thomas, 1999; Wood *et al*., 2007). For example, phorid flies exhibited high performance on TMIIs. In the presence of phorid flies, the host ant species would change behavior into defensive posture and immobile (Feener, 1987; Feener and Brown, 1992) which possible resulted in decreasing exploitative efficiency, reducing the asymmetries in dominance hierarchy and altering the outcome of competitions (Feener, 1981; Morrison, 1999; LeBrum, 2005).

1.4 *Solenopsis invicta* virus

Velles *et al*. (2004) made the first report of virus infection in *S. invicta*, which named *S. invicta* virus 1 (SINV-1). SINV-1 was belonging to picorna-like virus (encoded two large open reading frames); single positive stranded RNA whose genome was consisted of 8026 nucleotides (Velles *et al*., 2004). Afterward, the new species or genotype virus (*S. invicta* virus-1A, SINV-1A) and another positive-strand RNA virus (*S. invicta* virus 2, SINV-2) were recorded in 2005 and 2007, respectively (Velles and Strong, 2005; Velles *et al*., 2007a). It is problematic to definite SINV-1A is a distinct species or genotype of SINV-1 due to 89.9 % nucleotide identify (near the threshold identify to definite new species, 90 %) and 97 % amino acid identity between SINV-1 and SINV-1A (Velles and Strong, 2005). On the other hand, SINV-2 seems to be a different virus from SINV-1, which has four major open reading frames and five minor open reading frames in the sense orientation (Velles *et al*., 2007a).

The *Solenopsis invicta* virus (SINV-1, SINV-1A, and SINV-2) could infect all *S.*

invicta (polygyne and monogyne social forms) caste members including eggs, larvae, pupae (worker and sexual), workers, sexual alates, and queens (Velles *et al*., 2004; Valles and Strong, 2005; Hashimoto *et al*., 2007; Hashimoto and Valles, 2008). According to QPCR detection, virus infection expressed positive reaction on head, thorax, and abdomen of the workers. And *S. invicta* gut epithelia were more susceptible to virus infection, especially the midgut have high virus performance (99.4 ± 0.9 %) (Hashimoto and Valles, 2007). Transmission of virus to uninfected *S. invicta* workers reported could be through the trophallaxis. As a result, it was successfully transmitted virus into healthy *S. invicta* colonies by feeding homogenate of virus infected individuals (homogenized with food source) (Valles *et al*., 2004; Valles *et al*., 2007b; Hashimoto and Valles, 2008).

However, virus infection rate were variable in either intra-colony or inter-colony, which from 0 % to 100 %. Valles *et al*. (2004) surveyed field *S. invicta* colonies (168 nests) in Florida and obtained mean SINV-1 infection rate about 22.9 % from 0 % to 87.5 %. For another thing, the infection rate of SINV-1A was 2.2-fold higher (55 %) than SINV-1 in Florida (Valles and Strong, 2005). Similarly, the infection rate of SINV-2 was lower than SINV-1 and SINV-1A about 5.5 % from 1.6 % to 16.4 % (Hashimoto and Valles, 2008). In Taiwan, Yu *et al*. (2009) founded higher virus prevalence than in Florida. SINV-1/1A infection rate was up to 52.1 % and 16 % SINV-2 infection rate. On the other hand, few survey data recorded the virus infection rate in intra-colony, Hashimoto and Valles (2007) recorded about mean 86.7 % and 60 % SINV-1 infection rate in winter and summer, respectively.

In addition, we have not known the effect of *S. invicta* virus so far. The *S. invicta* virus did not produce any overt symptoms on their hosts in the field.

When SINV-1 and SINV-2 infected *S. invicta* colony were excavated from the field to the laboratory, these colonies seemed to exhibit brood die-off during rearing (Valles *et al*., 2004; Valles *et al*., 2007b). However, although no symptoms were observed, it may mediate the behavior and affect the competition relationships (Hudson and Greenman, 1998, Igbal and Mueller, 2007). From the IPM point of view, such fire ant virus could be occupied an imported position in IPM strategy.

Since, SINV-1 and native ants are available in fire ants distributed area. It can be hypothesized that there must be some interaction between native ants and *S. invicta* either SINV-1 infection or not. However, no researchers study focus on the possible effects of SINV-1 and the indirect interaction induced by SINV-1 to affect the competition between *S. invicta* and native ants. The present study analyzes biotic resistance of native ants and takes into account the virus infection effects.

Therefore the objectives of this study were designed to 1) evaluate the effects of SINV-1virus on interspecific competition of *S. invicta* at either individual inference or colony interference with native ants and, 2) determine whether native ants are able to invade the nest of *S. invicta* with/without SINV-1 virus infection. Data from this study may provide not only preliminary evidence that how virus infection affects its host's defense abilities and susceptibility toward attacks from other ants also provide an alternative strategy for controlling *S. invicta* in Taiwan.

2. Materials and methods

2.1 Survey of potential competitive native ant species

This survey was investigated in the campus of National Taipei University. According to previous study, results from large-scale surveillances revealed that after applications of the baits, population density of *S. invicta* has been significant reduced with almost 98% control rate (Hung *et al*., 2006). Moreover, only 2 *S. invicta* individuals were captured in recent investigation (Tsai, 2008). Such population reductions in the campus of National Taipei University can result in reinvasion of *S. invicta* and provides an opportunity to survey potential competitive native ant species by artificial planting *S. invicta* female alates.

The vials containing a *S. invicta* female alate were set up to survey the potential candidate native ant species, which were able to prey or attack newly mating queens of *S. invicta*. Treatment trails were constructed from two 50 ml central tubes. One tube was modified by cutting a hole (~ 0.2 mm diameter) at the bottom and coated Fluon®. And that tube was plugged into another tube without a cutting hole to prevent the ants from escaping that apparatus. The apparatus allow workers of most ant species to enter but prevent the alate escaping out. There were three treatments for experimental apparatus, First vial containing a *S. invicta* female alate, second vial containing potato chip, and the third empty vial.

Three transects which separated by 100 meters in the campus of National Taipei University were selected for this experiment during August, 2008 (A, B, and C, respectively). Seven centimeter depth depression was dug in the soil to mimic initial nest site of newly mating queen (3-12 cm deep, Markin *et al*., 1973) after the nuptial flight and established the experiment apparatus, then kept some rocks to cover it. At each transects, 15, 17, 19 experimental apparatus (included female alate, potato chip,

and control treatments) respectively were placed separated by fifty meters intervals. Data collected after 24 hours and recorded the number of ant species which were capture in the experimental apparatus and percentage of vials occupied by ants. Percentages were converted using an arcsine transformation to better approach normal distribution, and analyzed using Shapiro-Wilk test. Relative percentages in each transect and each treatment was compared with Kruskal-Wallis test (SAS Institute Inc.).

Fig. 1. The transect map of study area in the campus of National Taipei University.

2.2 The collection and rearing of ants

All *S. invicta* colonies were collected from infected sites in Taoyuan and Taipei Counties, Taiwan by excavating mounds, and then transported to laboratory. Ants were separated from soil by exposing under lamps heating and then transferred to plastic boxes (22 by 26 by 6 cm high) with a moist plaster nest. Two native ant species, *Pheidole fervens* and *Monomorium chinense*, were collected from rotten logs in ChangHua County, Taiwan and the campus of National Taiwan University campus, respectively. Both of *P. fervens* and *M. chinense* were transferred to plastic boxes as did for *S. invicta*. All plastic boxes were coated Fluon® to prevent the ants from escaping. All colonies were held at 30 ± 1 ℃ and 65 ± 10 % relative humidity and were exposed to a 12 L: 12 D photo-phase. Each ant colony was fed with fresh water, sucrose solution, and freeze-killed crickets. Total 14 *S. invicta* colonies, including 7 healthy colonies and 7 SINV-1 infected colonies, 10 *P. fervens* colonies and 7 *M. chinense* colonies were chosen for experiments.

Both *M. chinense* and *P. fervens* are native to tropical Asia including Taiwan. *P. fervens* is polygynous. Their colonies can include more thousand workers. *P. fervens* is dimorphic consisted of large-sized headed workers (majors or soldiers) and proportionately much narrower heads workers (minors) without intermediates. Their body colors are light reddish brown, head darker, and gaster blackish brown. Total body length is around 4.5 mm in majors, around 3 mm in minor workers. *P. fervens* is a numerically and behaviorally dominant species, which can recruit a large of workers to occupy food, and aggressively attack their competitors. *P. fervens* is more abundant locally in wet regions with a semi-closed canopy and some light penetration and inhibited dead branches. *P. fervens* is a primary scavenger to eat human foodstuffs and dead insect remains, but may also is omnivores to kill insects or collect seeds (Herris *et*

al., 2005).

In contrast, *M*. *chinense* is monomorphic which only have worker caste. Their average body length is 1.3 mm and body colors are blackish brown. *M*. *chinense* is also polygyne, while the numbers of worker may exceed more than thousand. *M*. *chinense* live around the warmer regions of the world and are often among the abundant ant species in semi-arid and seasonal arid regions. It is belong to ground-dwelling ant species and certainly found in woodland margins and grassland, nesting in the soil near the bases of plants. Some species of *Monomorium* are primary omnivores and relative slow moving and less aggressive ants. They can not only recruit a larger of workers but perform high interference ability to monopolize food resource. The genus of *Monomorium* is conspicuous ant species for using chemical repellency as interference mechanisms against their competitors. At the same time, species of genus of *Monomorium* are very closely-related to *Solenopsis*, and both of them contain major alkaloid in venom (Adams *et al*., 1981; Jones *et al*., 1982; Andersen *et al*., 1991; Jones *et al*., 2003).

2.3 The detection of *Solenopsis invicta* social form

According to the protocols reported by Valles and Porter (2003) to determine the social form of *S. invicta*, the social form was confirmed using a modified of *Gp*-9 polymerase chain reaction. The primer sets are 26Bs/16Bas (~517 bps) and 25bs/24bAs (~423 bps). Total 25-µl of PCR reaction included 3.33µl Gold Thermal 10 × PCR buffer, 1.6 µl dNTPs (2.5 mM), 0.5 µl 26 *Bs*, 0.5 µl 16 *BAs*, 0.5 µl 25 *bs*, 0.5 µl 24 *bAs*, 0.2 µl Gold Thermal hot-start Taq (5 U), 1 µl sample DNA, and 16.87 µl Deionized Water, was carried out on an ABI 9700 thermal cycler with the conditions of initial denaturation at 94 °C for 2 min followed by 35 cycles of 94 °C for 15 s, 55 °C for 15 s and 68 °C for 30 s, and a final extension at 68 °C for 5 min. If running 1.2 % agarose

gels exhibit two amplification specific fragments by photographing under UV light, we recognized these as the polygyne form (*Gp-9*^{Bb} heterozygous); likewise, only have one specific fragment were recognized as the monogyne form (*Gp-9*^{BB} homozygous).

2.4 The detection of *Solenopsis invicta* virus

2.4.1 RNA extraction

To screen if fire ant infected SINV-1, RNA for subsequent RT-PCR was extracted from 10 to 15 ants using TRIzol reagent (Invitrogen, Carlsbad, CA). Ant workers were homogenized in 150 µl of TRIzol reagent in 1.5 ml centrifuge tube, and then added additional 650 µl in the same tube to make 800 µl volumes in total, and vortex briefly. The homogenized samples were incubated for 5 min at room temperature. Add 160 µl Chloroform, shook tubes vigorously by hand for 15 s, and incubated for 3 min at room temperature. Centrifuge the samples with 13000 rpm for 15 min at 4 °C, resulting in separations of layers. The aqueous phase were transferred to the fresh tubes with 400 µl Isopropanol, inverted a few times, incubate for 10 min at room temperature, and centrifuge the samples with 13000 rpm for 10 min at 4 °C. The supernatant was removed into the beaker specific for organic waste. Then wash the RNA pellet once with 1 ml of 75 % ethanol, mixed the sample by vortex, centrifuged the samples with 13000 rpm for 5 min at 4 °C. The RNA pellet were dried for 30 min, and then dissolve the BNA pellet in 50 µl RNase-free water, store at -80 °C.

2.4.2 cDNA synthesis

All cDNA of *S. invicta* colonies were synthesized and amplified by Two-step RT-PCR. First step, add 20 µl RNA sample into reaction reagent which consisted of 6 µl Oligo (dt) (100 µM) and 2 µl dNTPs (10 mM) at 65℃ for 2 min. Second step, mixed the product from the first step with reaction reagent which consisted of 4 µl 10× RT First-Strand buffer, 2 µl DTT (0.1 M), 1 µl MMLV RT (200 units/µl),

and 5 µl Deionized Water (DEPC treated) at 37℃ for 30 min.

2.4.3 SINV-1/SINV-1A and SINV-2 detection

All cDNA were used to screen virus-infected *S. invicta* ants with SINV-1-specific oligonucleotide primers p341 and p343 to detect SINV-1/SINV-1A infection under the following PCR program: 1 cycle at 95 °C for 10 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 68 °C for 90 s, followed by a final elongation step of 68 °C for 5 min. The reaction reagent was included 5 µl Gold Thermal 10× PCR buffer, 2 µl dNTPs (2.5 mM), 2 µl P341, 2 µl P343, 0.2 µl Gold Thermal hot-start Taq (5 U), 2.5 µl cDNA samples, and 36.3 µl Deionized Water.

Additionally, primers p64 and p65 were used to detect SINV-2 infection, PCR program: 1 cycle at 95 °C for 10 min, 35 cycles of 94 °C for 15 s, 56 °C for 15 s, 68 °C for 30 s, followed by a final elongation step of 68 °C for 5 min. Similarly, these PCR products were also stored at 4 °C. The reaction reagent was included 2 µl Gold Thermal 10× PCR buffer, 0.8 µl dNTPs (2.5 mM), 1 µl P64, 1 µl P65, 0.2 µl Gold Thermal hot-start Taq (5U), 3 µl cDNA samples, and 12 µl Deionized Water.

2.4.4 Restriction Fragment Length Polymorphism analysis

In order to distinguish the genotype of *S. invicta* virus-1 (SINV-1/SINV-1A), we used restriction fragment length polymorphism (RFLP) to analyze the positive p341/p343 primer reaction PCR product by *Ava*I or *Bgl*II restrict enzyme to confirm whether *S. invicta* were infected with SINV-1 or SINV-1A. If *S. invicta* were infected by SINV-1, samples were displayed two fragments on a agarose gel by *Ava*I and *Bgl*II (*Ava*I: 550 bp and 1030 bp; *Bgl*II: 710 bp and 870 bp) (Valles and Strong, 2005).

2.5 Individual interference competition

Each pair competition was staged in small arenas to assess the effects of virus and size advantage to *S. invicta*. Interference competition test were quantified by placing 10 workers of each ant species into 9 cm diameter plastic Petri dish with inner side coated Fluon®. This test was observed the behavioral interactions and recorded mortality of the each ant species workers in 1 hour by counting the numbers of dead *S. invicta* and native ants. To calculate the competitive ability of *S. invicta*, I used the following equation:

$$\text{Competitive ability (\%)} = \frac{S}{ST}, \qquad (1)$$

where *S* is the survival numbers of *S. invicta* and *ST* is the survival numbers of *S. invicta* and competitive ants.

The following pair competition conditions were designed for discriminating whether *S. invicta* infected by virus as function of body size: (1) SINV-1 infected/healthy *S. invicta* majors vs *P. fervens* soldiers, (2) SINV-1 infected/healthy *S. invicta* majors vs *P. fervens* workers, (3) SINV-1 infected/healthy *S. invicta* minors vs *P. fervens* soldiers, (4) SINV-1 infected/healthy *S. invicta* minors vs *P. fervens* workers, and (5) SINV-1 infected/healthy *S. invicta* mixed size (five minors and five majors) vs *P. fervens* mixed size. Each treatment was replicated ten times using new plastic Petri dishes. Additionally, because *M. chinense* workers are monomorphic, one size of *M. chinense* were paired up with SINV-1 infected/healthy majors, minors, and mixed size of *S. invict*a. The *M. chinense* workers are much smaller than *S. invicta* workers (1.3 mm versus 2-7 mm in length). As a result, choose 75 *M. chinense* workers to do the test by equal biomass. The mortality data were transformed by an arcsine square-root transformation to better approach normal distribution, and analyzed using Shapiro-Wilk

test. Differences in mortality were compared by a Wilcoxon-Mann-Whitney U test (PROC NPAR1WAY, SAS Institute Inc.).

2.6 Colony interference competition

The experiment was set up to assess the effects of SINV-1 on inter-specific competition relationships between *S. invicta* and native ants at colony level. Both *P. fervens* and *M. chinense* colonies were tested against SINV-1 infected and healthy *S. invicta* colonies, respectively. In order to simulate the incipient *S. invicta* colonies, 20 minor workers, 10 broods, and one queen were used for *S. invicta*. However it was hard to capture the queen of monogyne *S. invicta* colony, therefore one polygyne queen was selected instead of monogyne queen. The experimental composition of *P. fervens* colonies were included 200, 100, and 20 worker/soldier combination (1/5 soldiers and 4/5 workers) with one queen. At the same time, the colonies of *M. chinense* consisted of 150, 750 workers and one queen. Each foraging arena consisted of two connections to colony container of *S. invicta* and agonistic ant species. Besides the pair of 150 M. *chinense* and 20 S. *invicta* was replicated three times, other pair combinations were replicated five times. In each trail, each experimental colony container was connected via 50-cm plastic tubes to a foraging arena. One cricket nymph was placed in the center of foraging arena, which was used to do exploitative competition of *S. invicta* and native ants. As a control to these experiments, single ant colonies without opposite competitive ant species were tested with each colony size category. Following data was recorded: (1) the time required by either ant species to entered into the food arena, (2) the recruitment and dead numbers of each ant species were recorded at each 15 minutes for first 2 hours, then every one hour until 12 hours, and follow by once every 12 hours, (3) the time required by either ant species to kill the each other's queen and invade opposite nest. The recruitment and mortality data were transformed by an arcsine

square-root transformation to better approach normal distribution, and analyzed using Shapiro-Wilk test. And the invading time was on means derived by average over days within a replicate. Differences in level of foraging activity, invading time, and worker mortality were compared by a Wilcoxon-Mann-Whitney U test (PROC NPAR1WAY, SAS Institute Inc.).

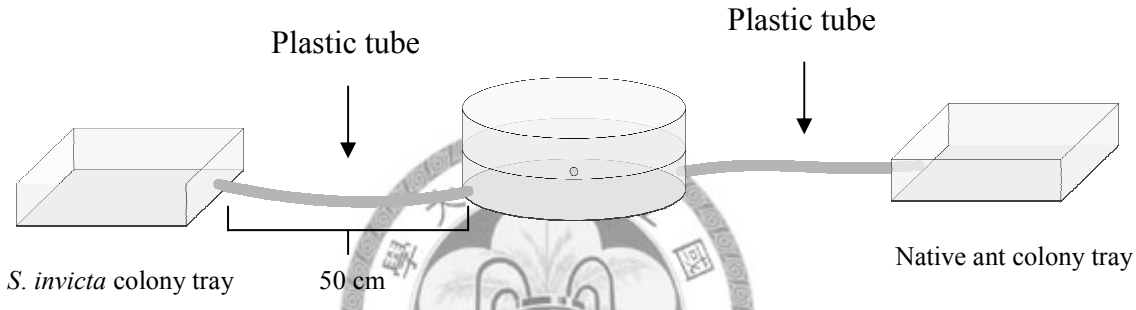

Fig. 2. Design of the colony-level interference competition experimental setup. All colony trays were 18 × 10 × 4 cm. Food arena were 15 cm diameter and 4 cm high.

3. Results

3.1 Potential competitive native ant species

Ants captured from experimental apparatus were used to survey the potential competitive native ants. For all transects, only nine ant species were collected, represented three subfamilies: Formicinae, Myrmicinae, and Dolichoderinae. There were one arboreal species, *Crematogaster sbmuda formosae*, two endemics (*Pheidole ernesti* and *Crematogaster sbmuda formosae*), and two tramp species (*Tapinoma melanocephalum* and *Paratrechina longicornis*) (Table 1). The most common and abundant species across all transects were *M. chinense*. The fauna was concentrated mainly in two ant species, *M. chinense* and *Paratrechina flavipes*, which together comprised 90 % of total number of vials occupied ants. The average percent of vials occupied of ants in B transect was significantly different among transects (Kruskal-Wallis $X^2 = 7.58$, $df = 2$, $P = 0.0226$). In addition, the average percent of vials occupied of ants among experiment apparatus was no significantly different (Kruskal- Wallis $X^2 = 0.8043$, $df = 2$, $P = 0.6689$).

Table 1. Number of vials occupied [a] , and mean number per vial (in parentheses), of ant taxa at three treatment vials in the National Taipei University

Ant species	Female alate vial	Food vial	Control vial	Total
Monomorium chinense	17 (9)	45 (59)	4 (2)	66
Paratrechina flavipes	12 (4)	15 (28)	4 (2)	31
Pheidole ernesti	1 (2)	0	2 (1)	3
Crematogaster sbmuda formosae	0	2 (2)	0	2
Paratrechina kraepelini	0	2 (1)	0	2
Tapinoma melanocephalum	0	0	1 (1)	1
Paratrechina longicornis	0	0	1 (1)	1
Monomorium pharaonis	0	0	1 (1)	1
Campontus sp.	0	0	1 (1)	1

[a] n = 255 treatment vials.

3.2 The detection of *S. invicta* virus and social form

A total of 123 *S. invicta* colonies were collected from infected field sites in Taoyuan and Taipei Counties, 24 was determined as monogyne and 99 was designated as polygyne colony. SINV-1 and SINV-2 infection rates among different sites ranged from 0 % to 100 % with a mean of 46.3 % (SD = 33.2) and 0 % to 62.5 % with a mean of 12.1 % (SD = 20.3), respectively (Table 2).

Table 2. Results of *Solenopsis invicta* virus detection

Virus	Monogyne		Polygyne		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
SINV-1	4 (n = 24)	16.6	53 (n =99)	53.5	57 (n = 123)	46.3
SINV-2	9 (n = 24)	37.5	6 (n =99)	6.0	15 (n = 123)	12.1

3.3 Individual interference competition

The mortality of *P. fervens* increased as *S. invicta* had larger size (Figs. 5, 6, 7). When *S. invicta* minors combat with *P. fervens*, *S. invicta* caused serious high mortality of *P. fervens*, except for soldiers (Figs. 5, 6, 7). In other words, while *S. invicta* paired with *P. fervens* soldiers, the competitive ability of *S. invicta* decreased (Figs. 8, 9). In addition, virus infection didn't cause effects on outcome of individual competition between *S. invicta* and *P. fervens* ($Z = -0.03$, $df = 1$, $P = 0.9726$). In all cases, the mortality of *P. fervens* workers was approximately 100 % in any pair (Figs. 5, 6, 7). The workers of *P. fervens* were more aggressive than soldiers, which usually actively bite legs or antenna of *S. invicta*, however, such behavior couldn't successfully kill *S. invicta*. Instead, *S. invicta* was dismembered by *P. fervens* soldiers in most cases (Fig. 3). In the pair of *S. invicta* majors against *P. fervens* soldiers, the mortality of both was high. However, *S. invicta* performed strong competitive ability against *P. fervens* ($Z = 3.80$, $df = 1$, $P < 0.0001$), because *S. invicta* caused nearly 100 % mortality on *P. fervens* (Figs. 8, 9). Comparison of mortality of *P. fervens* caused by SINV-1 infected and healthy *S. invicta* showed no significant difference (Table 3).

When the results from individual interference confrontation between *S. invicta* and *M. chinense* was observed, the mortality of *M. chinense* was higher (> 85 %) at pairing with *S. invicta* major workers compared to mixed sizes and minors (< 25 %) (Table 4). On the contrary, only SINV-1 infected *S. invicta* mixed sizes and minors suffered beyond 60 % mortality. Furthermore, all *S. invicta* majors were alive after 1 hour of individual interference confrontation (Fig. 10). In fact, while mixed sizes of *S. invicta* combated with *M. chinense*, the dead individuals were belong to worker body size but not majors (personal observation). Additionally, *M. chinense* caused significantly higher mortality on SINV-1 infected *S. invicta* minors than healthy ones. At the end of the test,

81 % of SINV-1 infected *S. invicta* minors and 32 % of healthy *S. invicta* minors were dead, respectively (Fig. 10). That is to say that the competitive ability of *S. invicta* was decreased while their body size became smaller, especially for SINV-1 infected *S. invicta* (Fig. 11).

Fig. 3. The interaction between *Solenopsis invicta* minor and *Pheidole fervens* soldier. The *Pheidole fervens* major worker relies on its strong mandible to bite and dismember *Solenopsis invicta*. The fire ant rotates its gaster toward *Pheidole fervens* major worker and attempts to attack

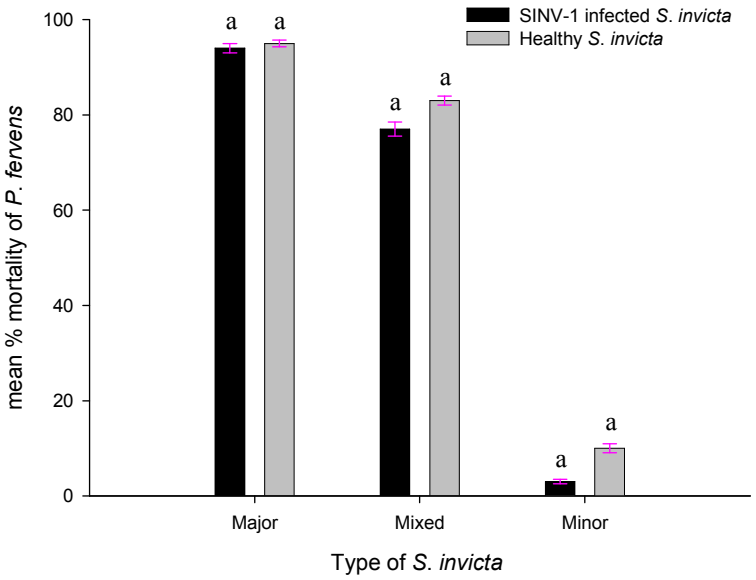

Fig. 4. The mean mortality of *Pheidole fervens* soldiers and *Solenopsis invicta* as a function of the *Solenopsis invicta* worker size in individual interference confrontations. The letters above error bars indicate that there are significant differences in mortality of *Pheidole fervens* for virus infected and healthy *Solenopsis invicta* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

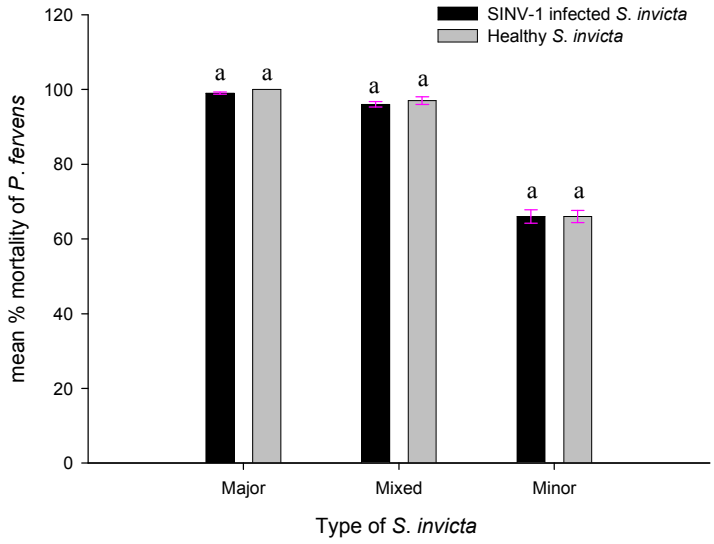

Fig. 5. The mean mortality of *Pheidole fervens* mixed sizes and *Solenopsis invicta* as a function of the *Solenopsis invicta* worker size in individual interference confrontations. The letters above error bars indicate that there are significant differences in mortality of *Pheidole fervens* for virus infected and healthy *Solenopsis invicta* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

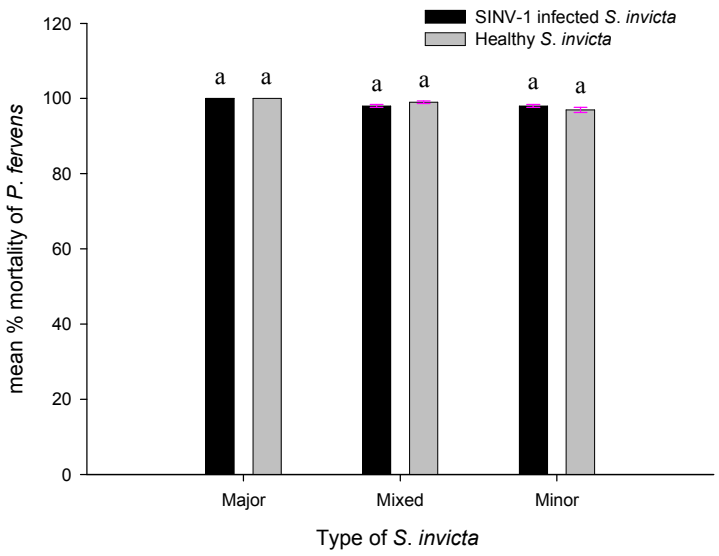

Fig. 6. The mean mortality of *Pheidole fervens* minors and *Solenopsis invicta* as a function of the *Solenopsis invicta* worker size in individual interference confrontations. The letters above error bars indicate that there are significant differences in mortality of *Pheidole fervens* for virus infected and healthy *Solenopsis invicta* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

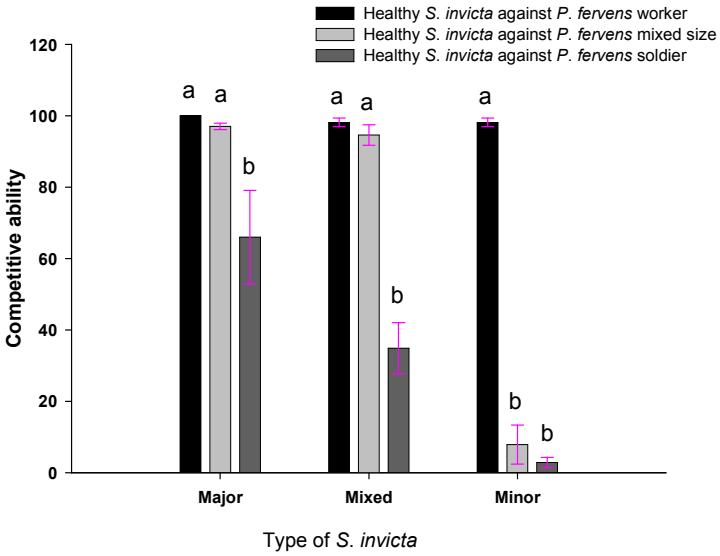

Fig. 7. The competitive ability of healthy *Solenopsis invicta* to against *Pheidole fervens* and as a function of the *Solenopsis invicta* worker size in individual interference confrontations. Letters above error bars indicate that there are significant differences in competitive ability of *Solenopsis invicta* for three categories of *Pheidole fervens* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

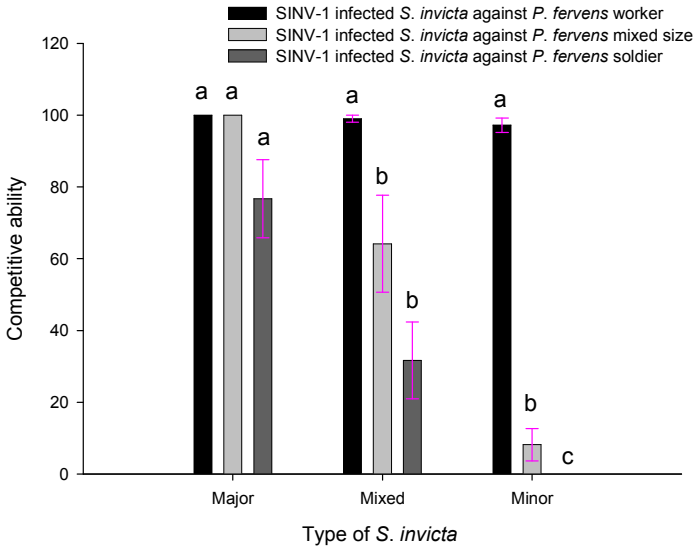

Fig. 8. The competitive ability of SINV-1 infected *Solenopsis invicta* to against *Pheidole fervens* and as a function of the *Solenopsis invicta* worker size in individual interference confrontations. Letters above error bars indicate that there are significant differences in competitive ability of *Solenopsis invicta* for three categories of *Pheidole fervens* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

Table 3. The mortality of *Pheidole fervens* against each size categories of healthy/virus infected *Solenopsis invicta*

	S. invicta majors (mean ± SE)			*S. invicta* mixed sizes (mean ± SE)			*S. invicta* minors (mean ± SE)		
	Healthy	Infected	*p*	Healthy	Infected	*p*	Healthy	Infected	*p*
P. fervens soldiers	94 ± 0.9 %	95 ± 0.7 %	1	77 ± 1.4 %	83 ± 0.9 %	0.54	3 ± 0.4 %	10 ± 0.9 %	0.09
P. fervens mixed sizes	99 ± 0.3 %	100 ± 0 %	1	96 ± 0.6 %	94 ± 1.0 %	0.88	66 ± 1.7 %	66 ± 1.6 %	0.96
P. fervens workers	100 ± 0 %	100 ± 0 %	1	98 ± 0.4 %	99 ± 0.3 %	1	98 ± 0.4 %	97 ± 0.6 %	1

Wilcoxon-Mann-Whitney test was used for tests of significance.
Significant difference at $\alpha < 0.05$ when compared the mortality of healthy and infected.

Table 4. The mean mortality of *Monomorium chinense* against each size categories of healthy/virus infected *Solenopsis invicta*

	S. invicta majors (mean ± SE)			*S. invicta* mixed sizes (mean ± SE)			*S. invicta* minors (mean ± SE)		
	Healthy	Infected	*p*	Healthy	Infected	*p*	Healthy	Infected	*p*
M. chinense workers	92.2 ± 1.1 %	87.4 ± 2.6 %	0.12	23.8 ± 2.4 %	18.9 ± 2.4 %	0.19	16 ± 2.7 %	17 ± 2.5 %	0.81

Wilcoxon-Mann-Whitney test was used for tests of significance.
Significant difference at $\alpha < 0.05$ when compared the mortality of healthy and infected

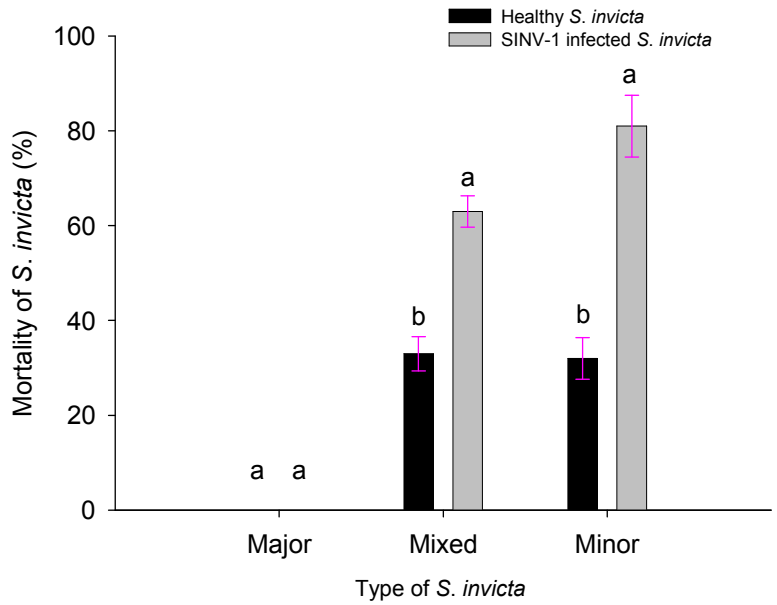

Fig. 9. The mean mortality of each size categories of healthy/virus infected *Solenopsis invicta* against *Monomorium chinense*. The letters above error bars indicate that there are significant differences in mortality of *Monomorium chinense* for virus infected and healthy *Solenopsis invicta* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

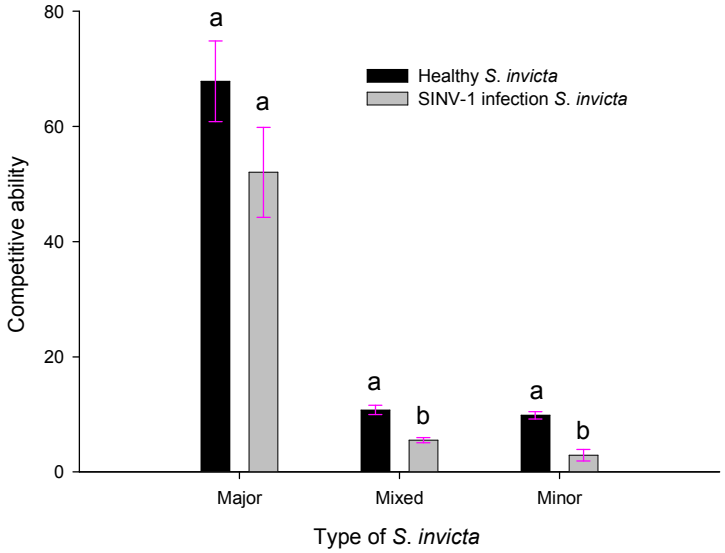

Fig. 10. The competitive ability of healthy and SINV-1 infected *Solenopsis invicta* to against *Monomorium chinense* and as a function of the *Solenopsis invicta* worker size in individual interference confrontations. Letters above error bars indicate that there are significant differences in competitive ability of *Solenopsis invicta* for virus infected and healthy *Solenopsis invicta* ($P < 0.05$, Wilcoxon-Mann-Whitney U test).

3.4 Colony interference competition

When setting up the trails, *P. fervens* generally discovered the food first and rapidly recruited additional nestmates (Figs. 12, 13, 14), but instead, *S. invicta* almost aggregated in the nest and seldom foraged resulted in low recruitment numbers, especially for SINV-1 infected *S. invicta* (personal observation). Subsequently, *P. fervens* quickly entered the nest container of *S. invicta* and attempted to attack *S. invicta* workers. When *P. fervens* first explored the central food arena and invaded *S. invicta* nesting space, *P. fervens* sustained the higher mortality during the first one day. On the other hand, when *M. chinense* competed with *S. invicta*, both ants performed varying patterns in discovering food time (Fig. 15, 16).

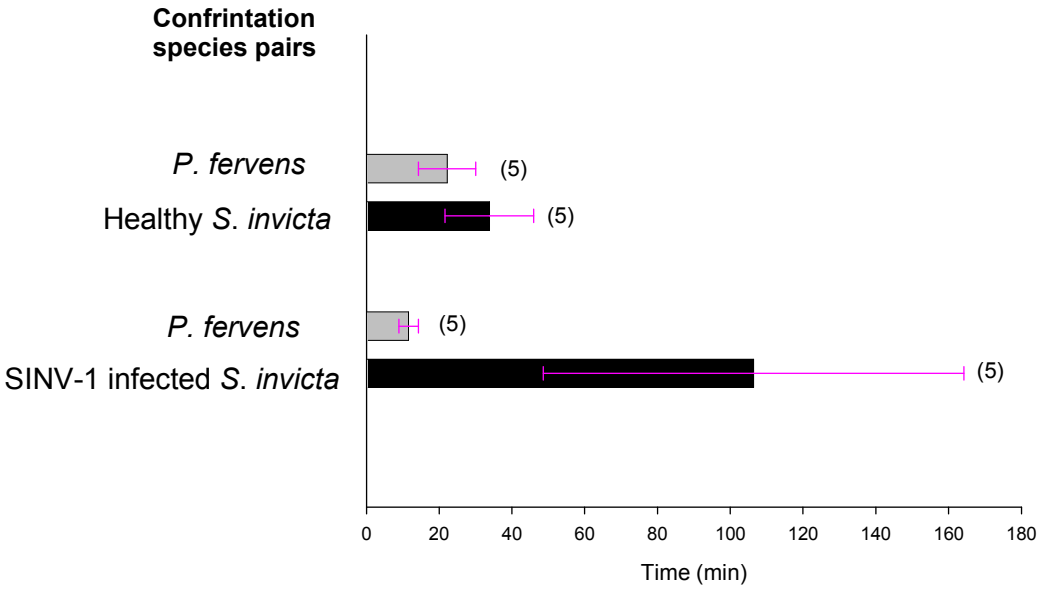

Fig. 11. The mean time for each pairing competitive ant species arrive at food arena. *Pheidole fervens* colony size is 20 workers, and *Solenopsis invicta* colony size is 20 workers. Number of arrive times are in parentheses. Each pair replicated five times.

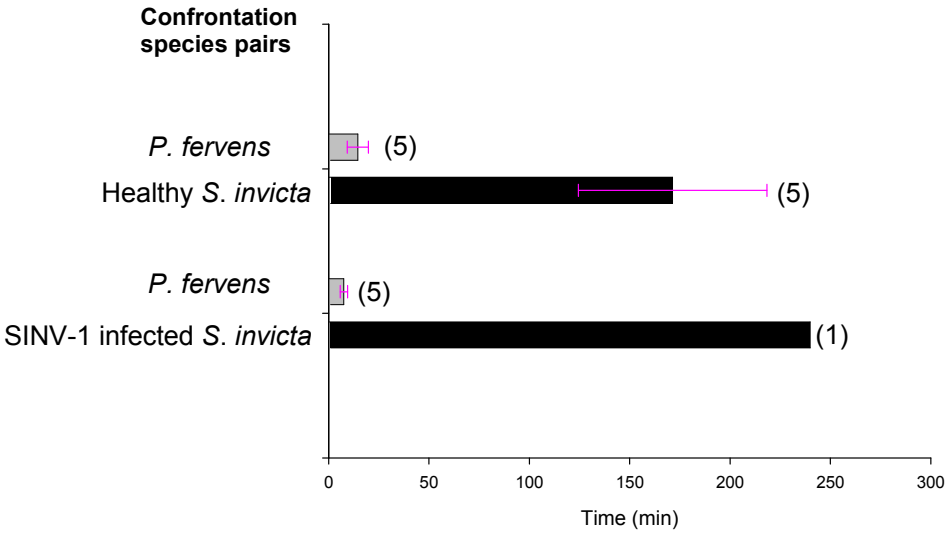

Fig. 12. The mean time for each pairing competitive ant species arrive at food arena. *Pheidole fervens* colony size is 100 workers, and *Solenopsis invicta* colony size is 20 workers. Number of arrive times are in parentheses. Each pair replicated five times.

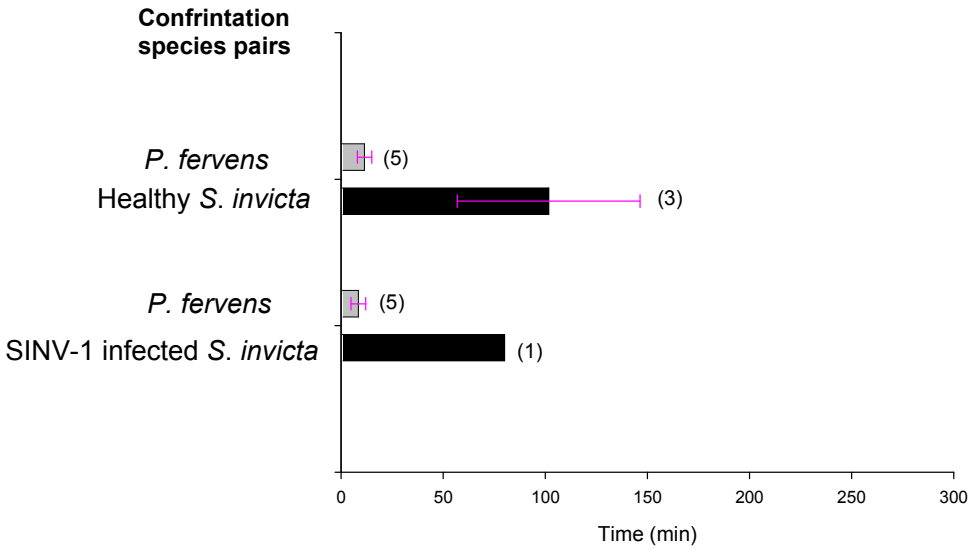

Fig. 13. The mean time for each pairing competitive ant species arrive at food arena. *Pheidole fervens* colony size is 200 workers, and *Solenopsis invicta* colony size is 20 workers. Number of arrive times are in parentheses. Each pair replicated five times.

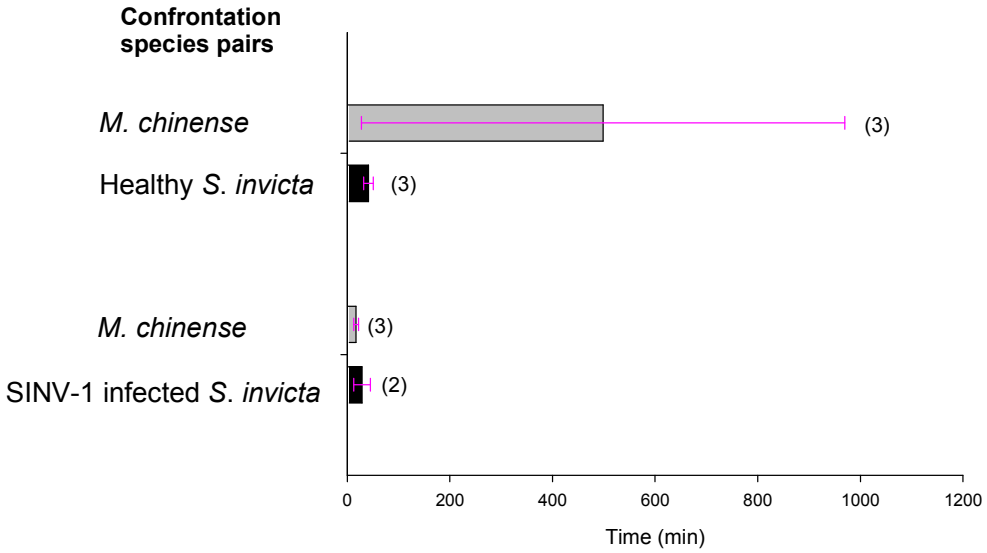

Fig. 14. The mean time for each pairing competitive ant species arrive at food arena. *Monomorium chinense* colony size is 150 workers, and *Solenopsis invicta* colony size is 20 workers. Number of arrive times are in parentheses. Each pair replicated three times.

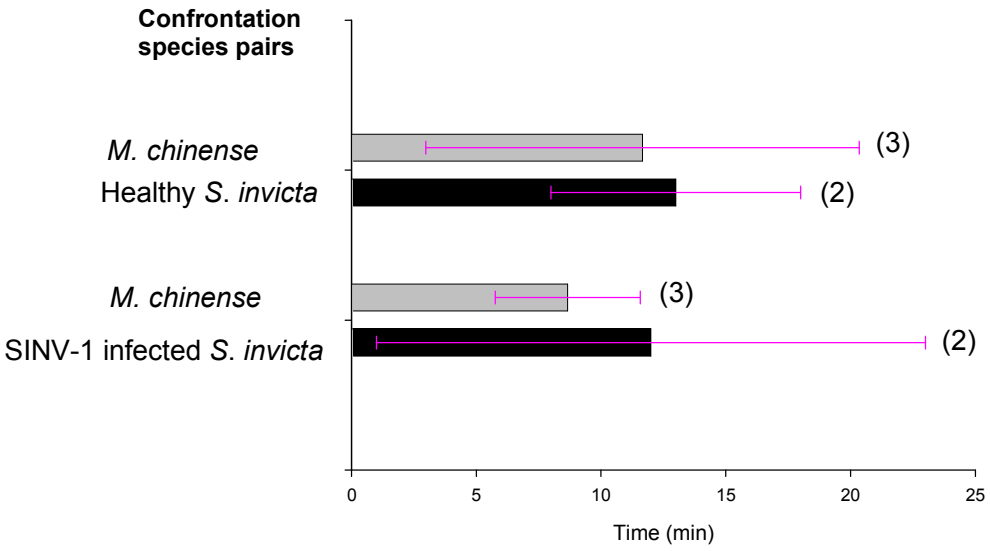

Fig. 15. The mean time for each pairing competitive ant species arrive at food arena. *Monomorium chinense* colony size is 750 workers, and *Solenopsis invicta* colony size is 20 workers. Number of arrive times are in parentheses. Each pair replicated five times.

In all observed interactions, it is necessary for *P. fervens* to kill individual *S. invicta* by groups of five or six *P. fervens* workers to bite antennae and legs of *S. invicta* worker and one or more *P. fervens* soldier dismembered gaster. Nevertheless, *S. invicta* were more aggressive than *P. fervens* when fighting and always caused painful stings to *P. fervens*, one *S. invicta* worker was able to kill more than three workers of *P. fervens* workers. In contrast, when *M. chinense* combated with *S. invicta*, *M. chinense* workers always attacked singly, raised abdomens, and tended to use stings and noxious chemicals against *S. invicta* workers (Fig. 16). *S. invicta* workers were generally killed by envenomization.

Fig. 16. A worker of *Monomorium chinense* attempts to attack a worker of *Solenopsis invicta*. A droplet of the venom is visible at the tip of the sting

When *S. invicta* was paired against 20 *P. fervens*, *S. invicta* defeat all *P. fervens* colonies. Moreover, in comparison with SINV-1 infected *S. invicta*, healthy *S. invicta* took less time to eliminate *P. fervens*, which generally only spent within one day ($Z = -2.17$, $df = 1$, $P = 0.0286$) (Table 5). On the contrary, when *P. fervens* colony size increased to 100 workers, *P. fervens* invaded the nest of *S. invicta* and killed the queen of *S. invicta* in most trail experiments but only two of *P. fervens* colonies were terminated by healthy *S. invicta* after 20 days (Table 5). In this case, the time for healthy and SINV-1 infected *S. invicta* to eliminate *P. fervens* were no significant difference ($Z = -0.30$, $df = 1$, $P = 0.75$). Next, *P. fervens* were successful invaders of *S. invicta* colony size of 20 workers while *P. fervens* colony size up to 200 workers, regardless of virus infection ($Z = 0$, $df = 1$, $P = 1$) (Table 5).

Similarly, when 750 *M. chinense* was paired with 20 *S. invicta*, *M. chinense* performed invading ability against *S. invicta*. The mean times for *M. chinense* to kill SINV-1 infected *S. invicta* queens were significant difference when compare healthy and infected colonies ($Z = -2.10$, $df = 1$, $P = 0.0317$) (Table 6). *M. chinense* took about average 8 days to kill the SINV-1 infected *S. invicta* queen as well as within 2 days to kill healthy *S. invicta* queen. All *S. invicta* queens, included healthy and SINV-1 infected, were killed by *M. chinense* (Table 6). However, while the colony size of *M. chinense* was decreased to 150 workers, *M. chinense* took significant longer time to

eliminate *S. invicta* colony compared to 750 *M. chinense* workers (Z= 2.09, *df* = 1, *P* = 0.0179). There was no significant difference in the ability of *M. chinense* to kill the SINV-1 infected *S. invicta* queen when comparing healthy *S. invicta* (Z= −1.74, *df* = 1, *P* = 0.1413) (Table 6).

.

Table 5. Time required for queen to be killed by opposite competitive species in competition of *Pheidole fervens* and *Solenopsis invicta* (n = total trail numbers of virus infection condition test. Number of trails are in parentheses)

Confrontation ratio	n	*S. invicta* queen killed by *P. fervens* (mean ± SE, days)			*P. fervens* queen killed by *S. invicta* (mean ± SE, days)		
		No virus infection	SINV-1 infection	*p*	No virus infection	SINV-1 infection	*p*
20 *S. invicta* v.s. 20 *P. fervens*	5	—[a]	—[a]	—	0.82 ± 0.27 (5)	12.75 ± 1.44 (5)	0.02
20 *S. invicta* v.s. 100 *P. fervens*	5	13 ± 3.51 (3)	13.8 ± 1.99 (3)	0.75	21.25 ± 0.25 (2)[b]	—	—
20 *S. invicta* v.s. 200 *P. fervens*	5	4.75 ± 1.43 (5)	4.88 ± 1.39 (5)	1	—[c]	—[c]	—

Significant at $\alpha < 0.05$ by Wilcoxon-Mann-Whitney method.

[a] *S. invicta* posed all threat to *P. fervens*.

[b] In two trails, *P. fervens* queen were never killed by *S. invicta*.

[c] *S. invicta* never posed a threat to *P. fervens*

Table 6. Time required for queen to be killed by opposite competitive species in competition of *Monomorium chinense* and *Solenopsis invicta* (n = total trail numbers of virus infection condition test. Number of trails are in parentheses)

Confrontation ratio	n	*S. invicta* queen killed by *M. chinense* (mean ± SE, days)			*M. chinense* queen killed by *S. invicta* (mean ± SE, days)		
		No virus infection	SINV-1 infection	*p*	No virus infection	SINV-1 infection	*p*
20 *S. invicta* v.s. 150 *M. chinense*	3	13.33 ± 0.88 (3)	24.33 ± 2.4 (3)	0.14	—[a]	—[a]	0.02
20 *S. invicta* v.s. 750 *M. chinense*	5	7.8 ± 1.82 (5)	1.61 ± 0.68 (5)	0.03	—[a]	—[a]	—

Significant at $\alpha < 0.05$ by Wilcoxon-Mann-Whitney method.

[a] *M. chinense* queen were never killed by *S. invicta*.

Comparison of time required to kill the queen of SINV-1 infected *S. invicta*, 100 *P. fervens* took longer time than 750 *M. chinense* (Z= −2.08, *df* = 1, *P* = 0.03) (Fig. 17). However, there was no significant differences between 100 *P. fervens* and 750 *M. chinense* regarding time required to terminate healthy *S. invicta* (Z= −0.44, *df* = 1, *P* = 0.6).

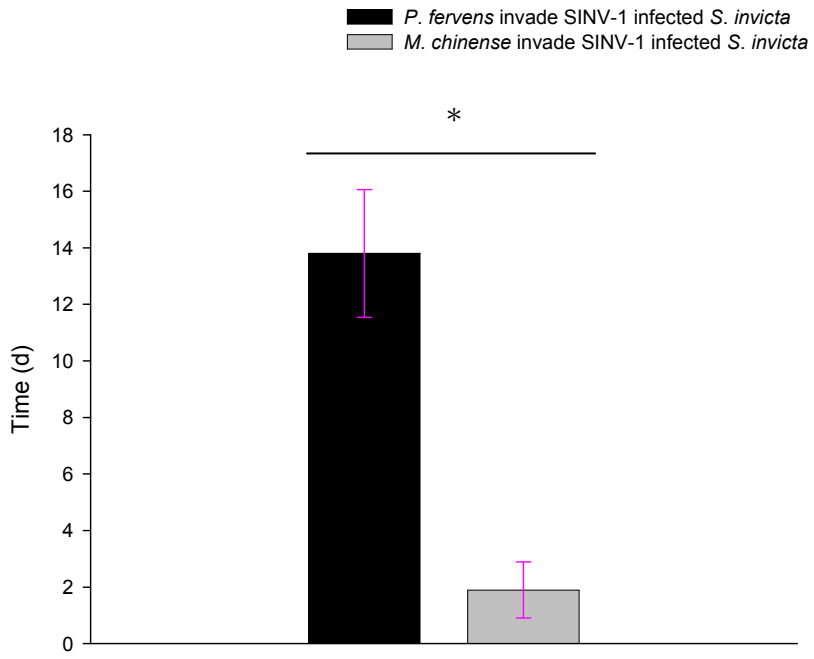

Fig. 17. Time required for 100 *Pheidole fervens* and 750 *Monomorium chinense* to kill the queen of SINV-1 infected *S. invicta*. Significance at $\alpha < 0.05$ by Wilcoxon-Mann-Whitney test method.

In comparing with recruitment numbers of healthy and SINV-1 infected *S. invicta* competed with 100 *P. fervens*, healthy *S. invicta* performed more active foraging activity and recruited larger number workers on the food (Z= 3.91, *df* = 1, *P* = 0.00004) (Fig. 19). Similarly, while *S. invicta* competed with 750 *M. chinense*, healthy *S. invicta* recruited more workers than SINV-1 infected ones (Z= −3.10, *df* = 1, *P* = 0.0012) (Fig. 22). Generally, healthy *S. invicta* started to occupy the food item after one hour of the experiment. Additionally, the recruitment numbers of 20 healthy and 20 SINV-1 infected *S. invicta* in control experiment, which opposite competitive native ants were absented, were significant over than experimental setup to compete 100 *P. fervens* (Healthy condition: Z= −3.50, *df* = 1, *P* = 0.0002; SINV-1 infected condition: Z= −3.95, *df* = 1, *P* = 0.00002) (Figs. 23, 24). Likewise, *S. invicta* were paired with 750 *M. chinense* showed the similar recruitment pattern (Healthy condition: Z= −3.58, *df* = 1, *P* = 0.0001; SINV-1 infected condition: Z= −3.89, *df* = 1, *P* = 0.00003) (Figs. 25, 26). In control experiment, no matter of colony size, healthy *S. invicta* recruited more workers than SINV-1 infected *S. invicta* on the food during the time passed (colony size 20: Z= 2.07, *df* = 1, *P* = 0.0357; colony size 50: Z= −2.02, *df* = 1, *P* = 0.0408; colony size 100: Z= −2.02, *df* = 1, *P* = 0.0218) (Figs. 27, 28).

However, whether *S. invicta* are healthy or SINV-1 infected, *S. invicta* showed no foraging activity in competition with 200 *P. fervens* (Fig. 20). On the other hand, there

are no significant differences in recruitment numbers between healthy and SINV-1 infected *S. invicta* on competition with 20 *P. fervens* and 150 *M. chinense* (*P. fervens* condition: Z= 0.03, *df* = 1, *P* = 0.0001; *M. chinense* condition: Z= −0.88, *df* = 1, *P* = 0.1872) (Figs. 18, 21).

When 20 *S. invicta* were paired against 20, 100, 200 *P. fervens*, the average cumulative mortality was 35 ± 2.2 (day 1), 65 ± 3 (day 8), 62.5 ± 15.3 % (day 1) for 20 healthy *S. invicta* and 12 ± 5.6, 79 ± 4, 66.25 ± 12.8 % for 20 SINV-1 infected *S. invicta* (colony size 20: Z= −2.34, *df* = 1, *P* = 0.0189; colony size 100: Z= 1.43, *df* = 1, *P* = 0.1556; colony size 200: Z= −0.14, *df* = 1, *P* = 0.8839) (Figs. 29, 30, 31).

The average cumulative mortality was 48.3 ± 4.4 (day 8), 75 ± 0.6 % (day 2) for 20 healthy *S. invicta* and 68.3 ± 8.8, 91 ± 0.6 % for 20 SINV-1 infected *S. invicta* (colony size 150: Z= −0.43, *df* = 1, *P* = 0.6625; colony size 750: Z= 2.18, *df* = 1, *P* = 0.0291) in competition with 150, 750 *M. chinense* (Figs. 32, 33).

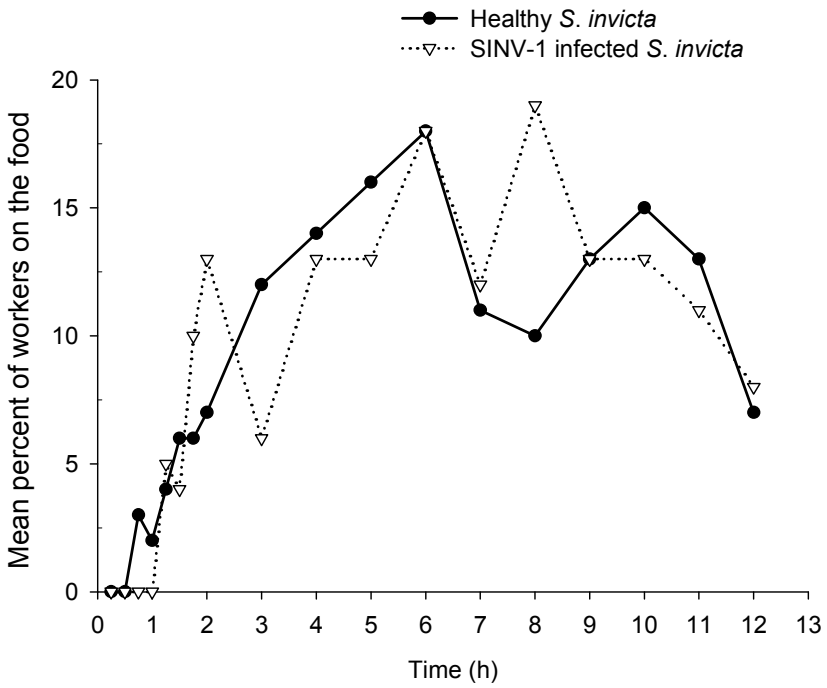

Fig. 18. Mean percent of individual healthy and SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Pheidole fervens* (20 workers) on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

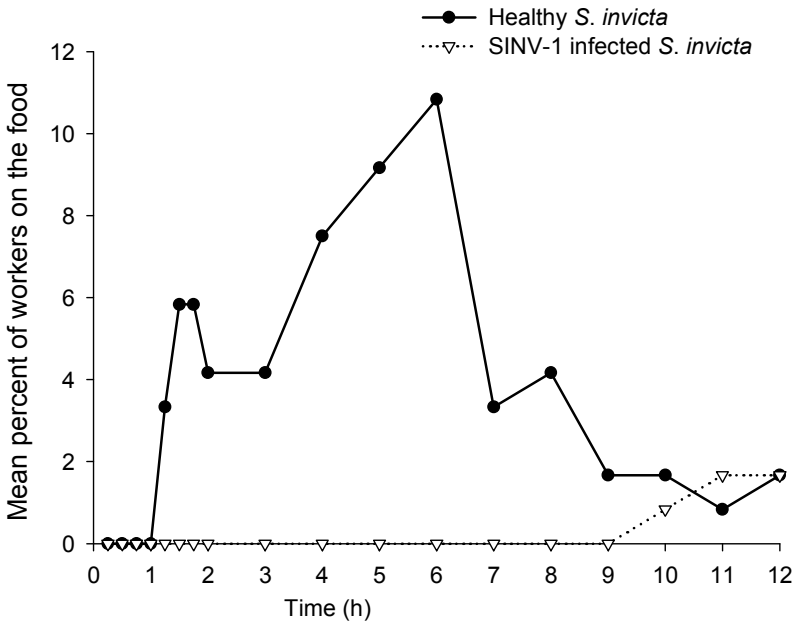

Fig. 19. Mean percent of individual healthy and SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Pheidole fervens* (100 workers) on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

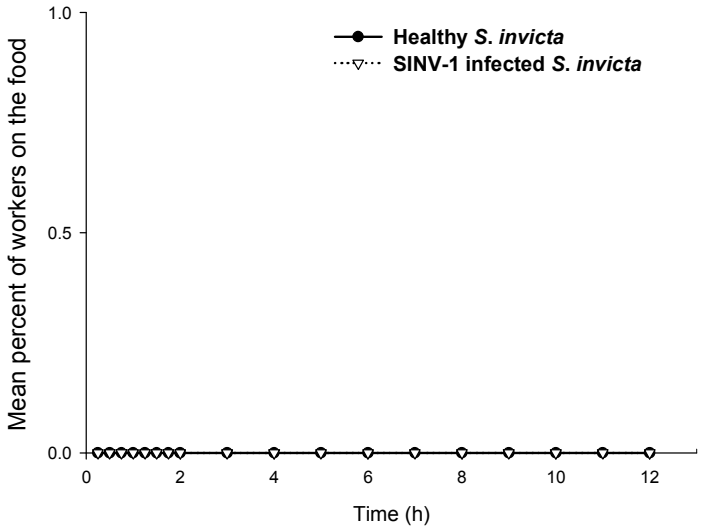

Fig. 20. Mean percent of individual healthy and SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Pheidole fervens* (200 workers) on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

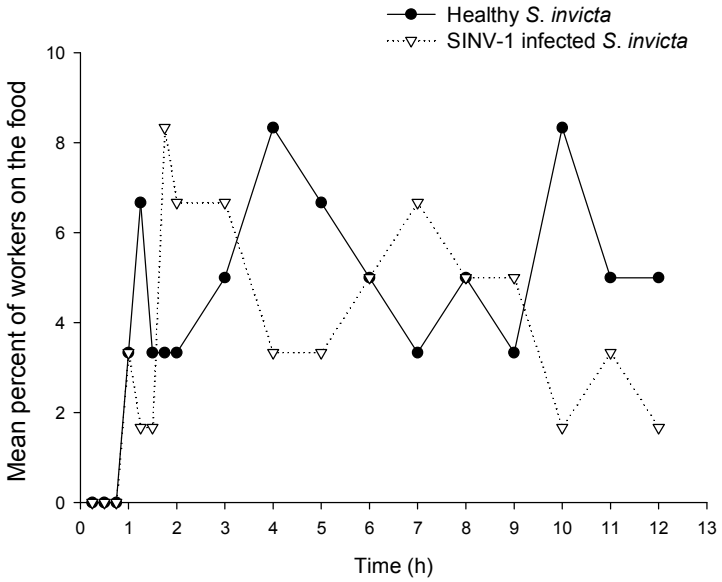

Fig. 21. Mean percent of individual healthy and SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Monomorium chinense* (150 workers) on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

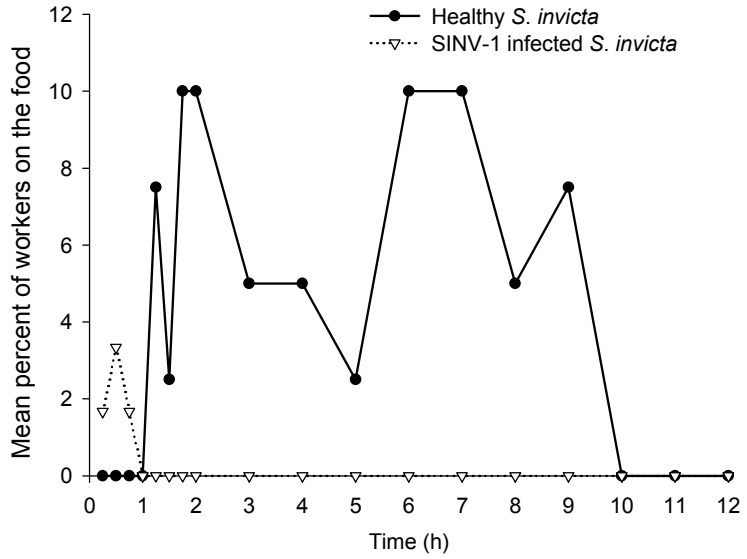

Fig. 22. Mean percent of individual healthy and SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Monomorium chinense* (750 workers) on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

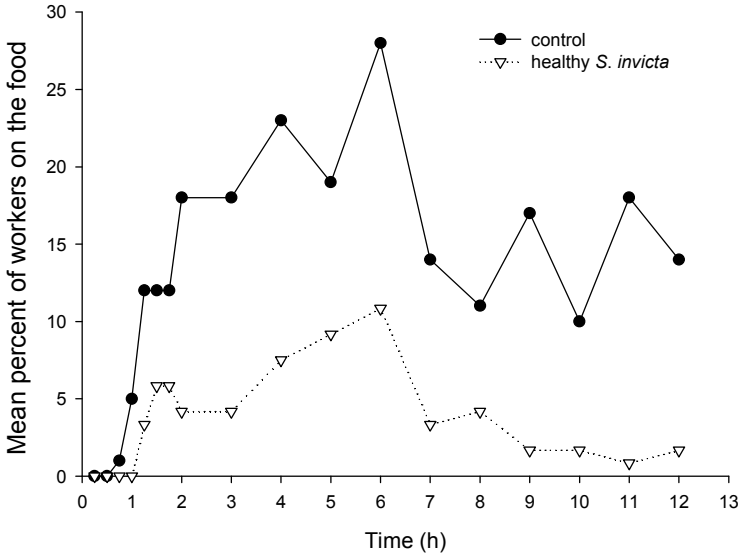

Fig. 23. Mean percent of individual healthy *Solenopsis invicta* (20 workers) which competed with *Pheidole fervens* (100 workers) and control experiment which *Pheidole fervens* was absent on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

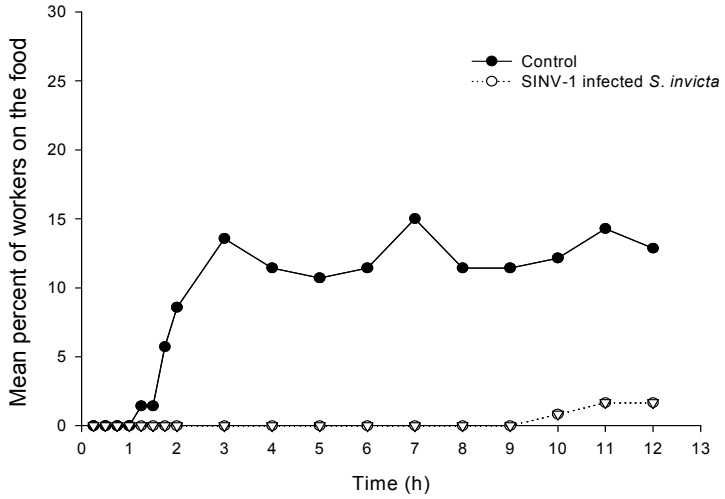

Fig. 24. Mean percent of individual SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Pheidole fervens* (100 workers) and control experiment which *Pheidole fervens* was absent on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

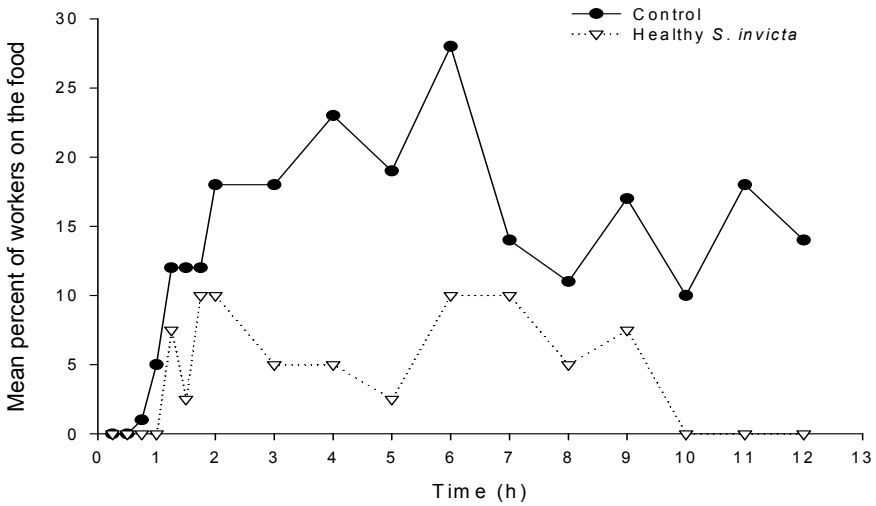

Fig. 25. Mean percent of individual healthy infected *Solenopsis invicta* (20 workers) which competed with *Monomorium chinense* (750 workers) and control experiment which *Monomorium chinense* was absent on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

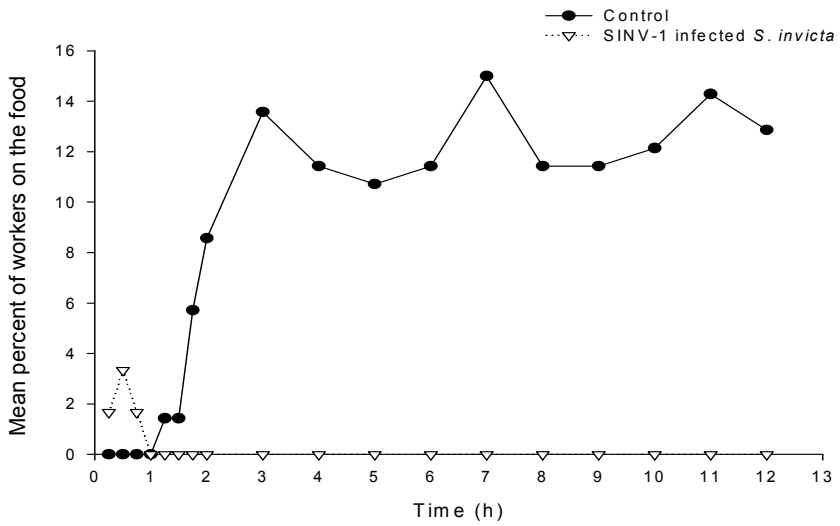

Fig. 26. Mean percent of individual SINV-1 infected *Solenopsis invicta* (20 workers) which competed with *Monomorium chinense* (750 workers) and control experiment which *Monomorium chinense* was absent on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

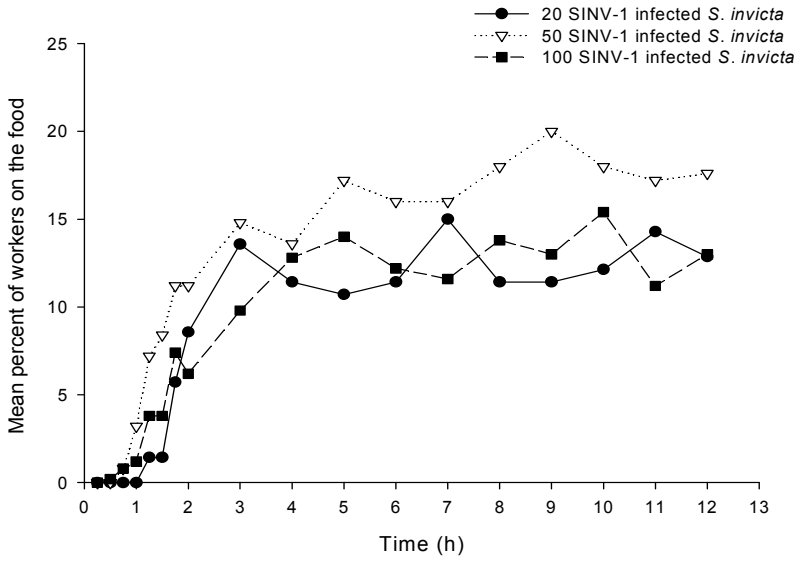

Fig. 27. Mean percent of control SINV-1 infected *Solenopsis invicta* workers with three colony sizes on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

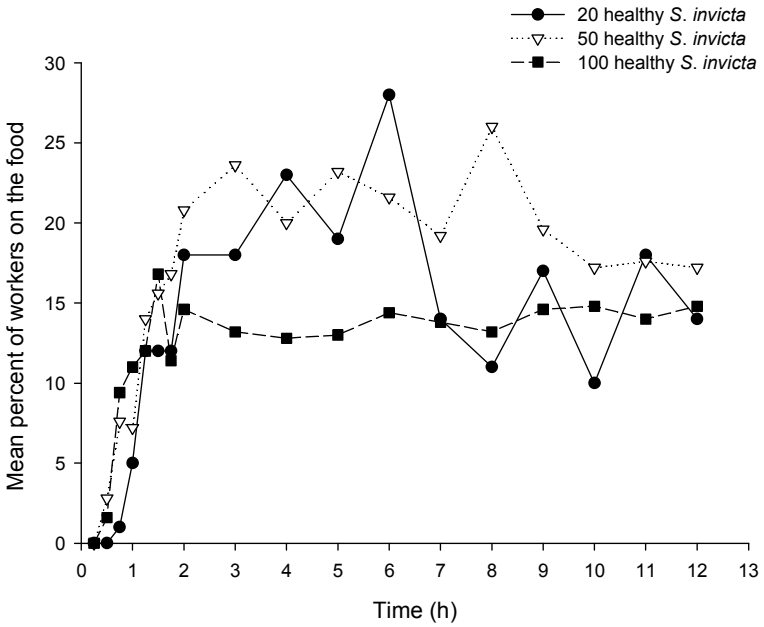

Fig. 28. Mean percent of control healthy *Solenopsis invicta* workers with three colony sizes on food at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours.

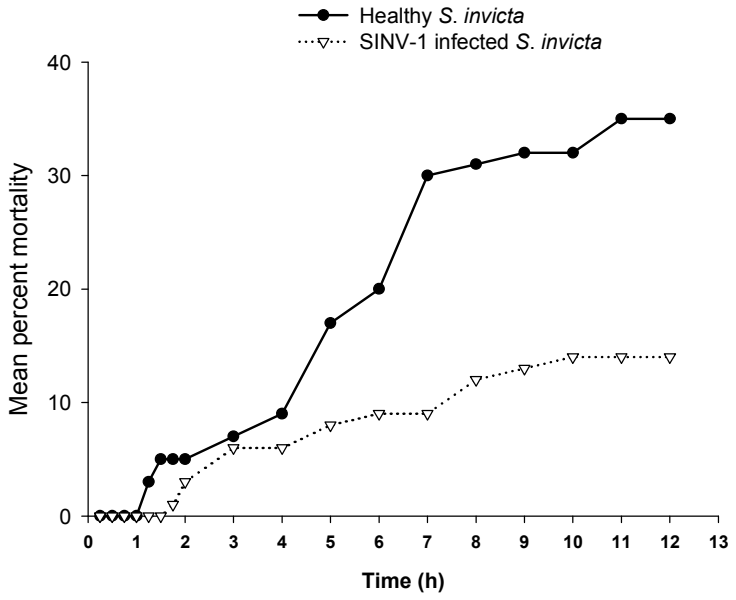

Fig. 29. Mean percent mortality of 20 *Solenopsis invicta* workers against 20 *Pheidole fervens* at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours and follow by once every 12 hours.

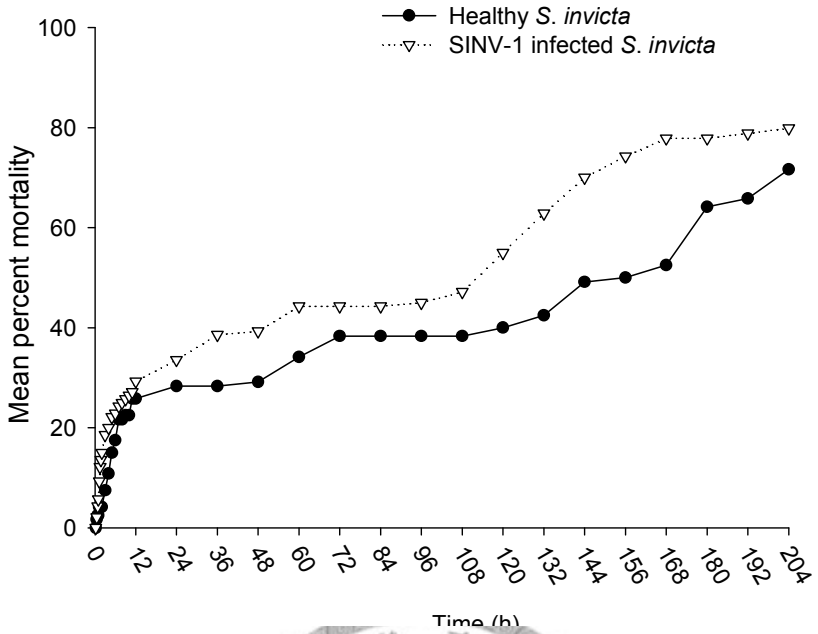

Fig. 30. Mean percent mortality of 20 *Solenopsis invicta* workers against 100 *Pheidole fervens* at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours and follow by once every 12 hours.

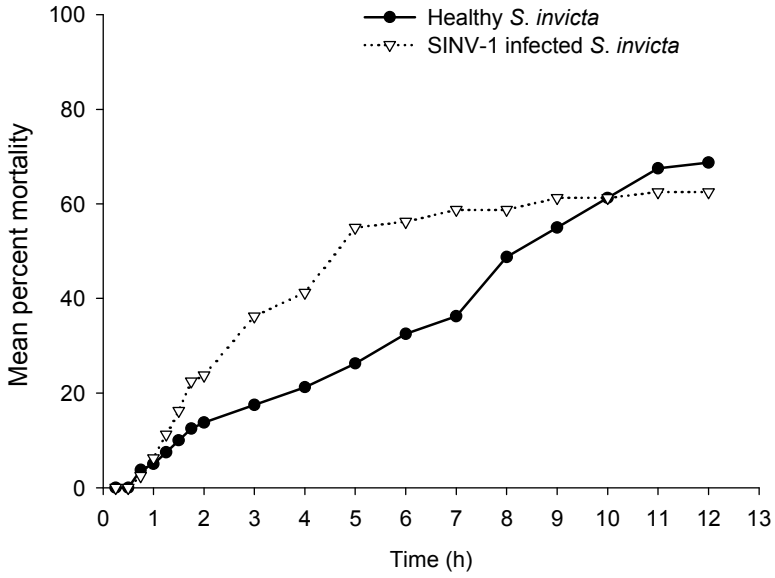

Fig. 31. Mean percent mortality of 20 *Solenopsis invicta* workers against 200 *Pheidole fervens* at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours and follow by once every 12 hours.

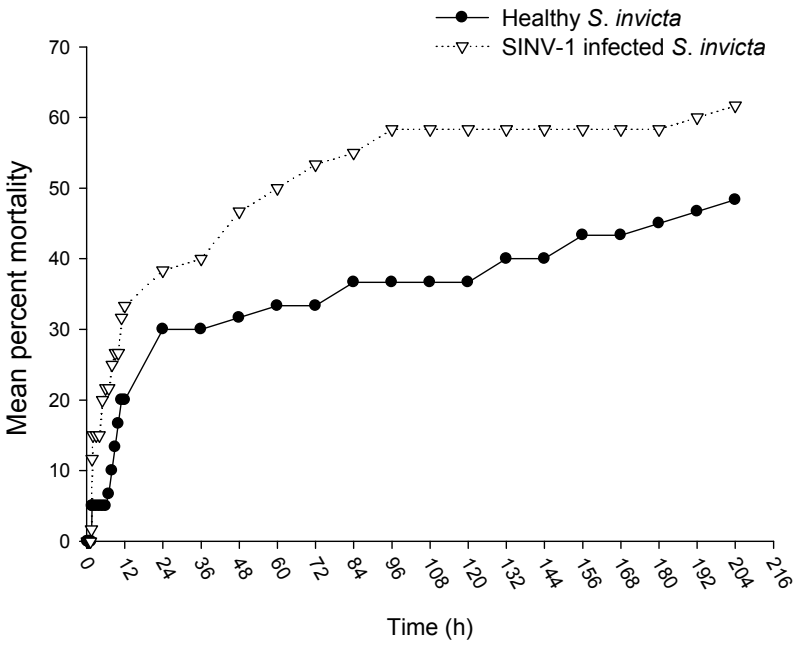

Fig. 32. Mean percent mortality of 20 *Solenopsis invicta* workers against 150 *Monomorium chinense* at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours and follow by once every 12 hours.

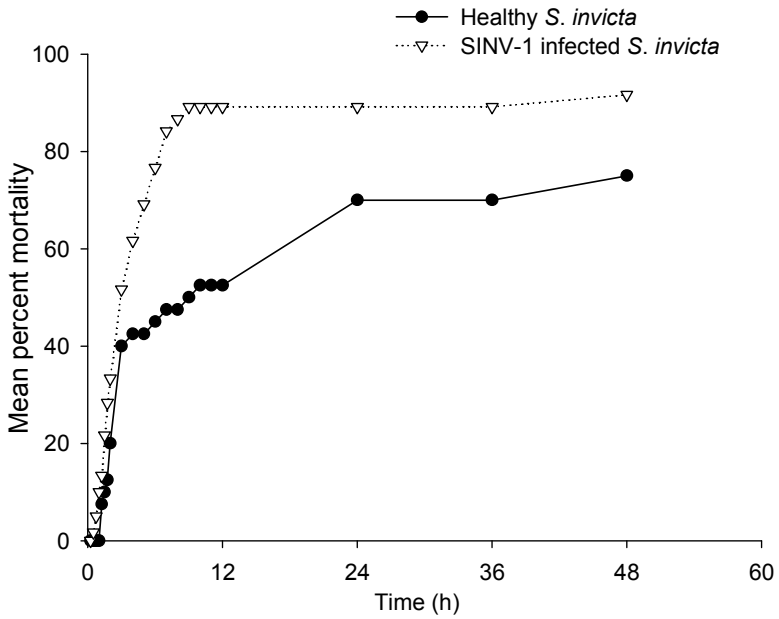

Fig. 33. Mean percent mortality of 20 *Solenopsis invicta* workers against 750 *Monomorium chinense* at 15-min intervals for first 2 hours and every hour thereafter for a total 12 hours and follow by once every 12 hours.

4. Discussion

4.1 Potential competitive native ant species

The major reason why only nine ant species have been captured during the study might be that the entrance of plastic vials is too small to some ant species with larger body size to enter into the vials. Moreover, the other explanation might be derived from the fact that diversity of the local ant species in the campus of National Taipei University has been significantly decreased after the fire ant eradication programs (Tsai, 2008). The third possibility is the principle of employed experimental apparatus. Baits were believed less effective methods to estimate biodiversity and only attracted specific feeding ant species (King and Porter, 2005). The survey by set-up experimental apparatus might be somehow similar to bait methods so that it is possible that few ant species could be attracted.

The native ants entered into experiment apparatus at a random manner instead of attacking or preying on *S. invicta* female alates in particular. Once native ants were presented in the vial, they generally attempted to attack the queen. Although few dead female alates were observed in the vials, the mortality of queens might not be directly associated with native ants. The experiment apparatus might be restricted the activities of native ants to recruit more nestmates, resulting in decreasing attack efficacy, especially small, monomorphic ant species, such as *M. chinense*. In those vials, three

ant species found near the dead queens were, *M. chinense*, *Crematogaster sbmuda formosae*, and *Paratrechina flavipes*. At the same time, some dead individuals of *M. chinense* were observed biting the legs of queens, and some individuals raised their gaster vertically against the queen, whereas other individuals appeared to be stinging the queen. But the other two ant species didn't show any attacking behavior when the vials were recaptured.

According to previous study in central Texas by specially designed vials which allowed potential ant predators free to enter and exit, during that study, twelve ant species were observed as predators on *S. invicta* founder queens. Those predator ant species included *Pheidole porcula*, *P. dentata*, *C. clara*, *M. minimum*, and others. And *P. porcula*, and *M. minimum* occupied 80 % of the total number of individuals (Nichols and Sites, 1991). In another study, during the monitoring program in the campus of National Taipei University, *M. chinense* was the most abundant ant species and *P. fervens* was one of the sub-dominance species (Tsai, 2008). In addiction, highly efficient interference competition ant species which owned superior interference and/or exploitation competition ability may be most adversely affected by toxic baits (Buczkowski and Bennett, 2008). After the bait treatments, the population densities of *P. fervens* soon declined sharply, indicating that *P. fervens* could be recognized as the most dominant ant species followed by *S. invicta* (Tsai, 2008). It is suggested that *P. fervens*

is likely one of candidate management agents against *S. invicta*. Combined all the features mentioned above, both *M. chinense* and *P. fervens* were being selected to test competition relationship with *S. invicta* in this study.

4.2 Interference competition mechanisms

4.2.1 Agonistic interference competition

All ant species in this study use a mass-recruitment foraging strategy as suggested by previous studies. (Adams and Traniello, 1981; Beckers *et al*., 1989; Jones and Phillips, 1990). With mass-recruitment, the scouts return to the nest and recruit larger nestmates to the food resources (Beckers *et al*., 1989). As a result, one could expect these ant species are experienced to perform interference competition with other ant species, especially with the existence of food resource. Interference ability plays an important role in competition among three ant species.

The body size is indeed an important factor on interspecific competition; larger sizes have higher possibility to defeat the smaller ones (Persson, 1985; Morrison, 2000; Kabashima *et al*., 2007). Compared to *P. fervens* soldiers, the workers were more susceptible to *S. invicta*, regardless of size of *S. invicta*. Although *P. fervens* workers were able to fight with *S. invicta*, *P. fervens* workers have almost no chance to kill off *S. invicta* workers due to their poor attack effectiveness and lack of chemical defense (Kugler, 1979). Accordingly, groups of *P. fervens* workers cooperatively grip antennae

and legs of *S. invicta* workers, providing a chance for *P. fervens* soldiers to dismember gaster of *S. invicta*. By fighting in groups and organizing to kill off individual *S. invicta*, numerical advantage of *P. fervens* has been created, and possibilities to win battles with *S. invicta* have also increased.

Actually, the division of worker in the genus of *Pheidole* is obvious by their dimorphic morphology. The small-headed minor workers are characterized on conducting the foraging and taking care of most of the quotidian tasks of the colony, and big-headed major workers (soldiers) function in seed milling, food storage, and defense, as well as participation within nest activity (Wilson, 1984; Sempo and Detrain, 2004). Besides, the attack assemblage of the big-headed ant species (*Pheidole* spp.) belongs to group defense and has been organized for the enemy specification in the alarm-recruitment system that some scouts directly attack enemies, the others withdrew to nest to request recruitment to defend their colony (Wilson,1976).

The numerical advantage was necessary for *P. fervens* to compete with agonists and their soldier castes played an important role affecting the outcome of aggressive competition among ants. However, in the great majority of *Pheidole* nest, only 8-20 % individuals in the nest are soldiers (Wilson, 1976). For this reason, in equal worker numbers competition test, *P. fervens* were not able to defend their colony and

eliminated from the competition with *S. invicta*. In other words, once *P. fervens* soldiers were dead in a large amount during competition, this colony may collapsed quickly and lost the defense ability.

4.2.2 Chemical interference competition

The chemical interference may alter the outcome of competition (Adams and Traniello, 1981; Andersen *et al*., 1991), as evident by this study that *M. chinense* was good at chemical defense and killed the *S. invicta* even encountered with asymmetrical size. However, no references have documented any support of that chemical defense actually generates the toxic effects or contact insecticide activity. On the other hand, according to previous researches, the venom secreted by poison gland from the sting has the repellency against other ants (Adams and Traniello, 1981; Andersen *et al*., 1991). In order to compete to acquire food, *Monomorium* species can apply the venom alkaloids such like Dialkylpyrrolidines (Blum, 1992) to repel other more aggressive species of ants from occupying the food (Jones *et al*., 1982; Andersen *et al*., 1991). Therefore, it can be hypothesized that *M. chinense* indeed secreted the venom from stings and attacked the *S. invicta*. While *S. invicta* were stung by *M. chinense*, *S. invicta* showed the seizure-like behavior and died afterward.

Such defensive alkaloids (Dialkylpiperidines) are also known from species of genera *Solenopsis* (Blum, 1992). Similarly, *S. invicta* also can secrete the

toxic venom from the sting apparatus, and the venom consists of alkaloids and small amount of venom protein, which is used to kill the competitor and prey by injection (Tschinkel, 2006). At the same time, unlike *S. invicta* minors which suffered high mortality against *M. chinense*, *S. invicta* major workers seem to be more tolerant of chemical defense by *M. chinense*. But the further study such as that using natural venom collected from *M. chinense* to determine the required dose to kill 50% of *S. invicta* between minors and majors should be performed. However, this situation may be caused by that *M. chinense* was difficult to attack *S. invicta* majors or easily affected by venom produced from *S. invicta* majors.

There is a variation in venom volume in *S. invicta*; major workers contain larger amount of volume of venom than minor works do (Tschinkel, 2006). Accordingly, *S. invicta* major workers always exhibit much higher survival rate than minors, and resulted in the higher mortality of native ants in the individual pairing between different castes. In other words, our results demonstrated that size advantages and chemical defenses simultaneously are crucial to defeat opposite competitors even on unequal numbers.

4.3 The effects of SINV-1 on *Solenopsis invicta*

The response behavior of ants to microbial pathogen included grooming, secretion of antibiotics, nest hygiene, avoidance, and dispersal (Oi and Pereira, 1993). Changes in

host behavior are hypothesized as a kind of adaptation for either host ants or parasite (Poulin, 1995). The parasites may alter their host response to environmental stimuli and likely to facilitate their transmission (Poulin, 1994). Data from this study suggested that recruitment numbers of SINV-1-infected *S. invicta* has been reduced and that infected individuals altered their response to recruitment intensity and abundance of competitive native ants and showed adaptive fairly low foraging behavior.

Once native ants recruited larger amount of workers on the food or invaded into SINV-1 infected *S. invicta* nest container, SINV-1 infected *S. invicta* stayed in the nest and did not attempted to forage. This behavior might be one of self defending mechanisms that allow the weakened individuals avoid the interference competition with competitors. Moreover, even in the control experiments which competitive ant species was absent, SINV-1 infected *S. invicta* recruited fewer workers on the food than healthy *S. invica* do. As a result, virus infection seems to affect the forage behavior of *S. invicta*. Additionally, compared to the scenario SINV-1 infected *S. invicta* competed with 100 *P. fervens*, more recruited individuals of SINV-1 infected *S. invicta* were found when competed with 20 *P. fervens*. This pattern suggested that SINV-1 infected *S. invicta* appeared to be able to determine relative competitive intensity or competitor abundance, and changed their foraging frequency.

Considering the effect of virus infection on the interspecific competition of

the *S. invicta*, SINV-1 infected *S. invicta* minors were more susceptible to *M. chinense* regardless of individual level or colony competition. *M. chinense* caused higher mortality of SINV-1 infected *S. invicta* minors than healthy ones, which means that might be a synergistic effect of virus and chemical defense of *M. chinense*. Tentcheva *et al*. (2004) surveyed the virus in honey bee, *Apis mellifera* L., and suggested that the environmental factors play an important role in disease outbreaks of bee colony. Furthermore, such virus without any perceptible symptoms may require the existence of other pathogens to trigger mass replication that generates negative effects on the host (Tentcheva *et al*., 2004; Valles *et al*., 2004). Similarly, pathogens or parasitoids probably reduce the competitive asymmetries between *S. invicta* and its competitors (LeBrum, 2005; Keck *et al*., 2005). Keck *et al*. (2005) found that one microsporidium species, *Thelohania solenopsae* may harmfully affect the competition ability of *S. invicta* against *M. minimum* that may attributed by loss of defensive abilities to prevent *M. minimum* from invading.

Based on the results of this study, virus infection seems to have effect on competition between *S. invicta* and *M. chinense*. Chemical interference by *M. chinense* might be a more likely influencing factor on mortality of infected *S. invicta*. However, research about the relationships between SINV-1 and venom of *M. chinense* should be further conducted. In contrast, the physical attack by *P. fervens* makes the similarly

confrontation consequence on either SINV-1 infected or healthy *S. invicta*. As a result, virus infection seems could not induce attack behavior plasticity on *S. invicta*, both of SINV-1 infected and healthy *S. invicta* performed aggressive response against attack of *P. fervens* and always caused high percentage mortality on *P. fervens*.

Nevertheless, in colony interference test, healthy *S. invicta* discovered food as more actively than SINV-1 infected ones, which give rise to increase the encountering probabilities with *P. fervens*. In two trials (20 *S. invicta* v.s. 100 *P. fervens*), therefore healthy *S. invicta* workers therefore quickly displaced *P. fervens* at food arena by their strong competitive ability, and eventually kill off most *P. fervens* workers and queens. In addiction, while 20 *S. invicta* competed with 20 *P. fervens*, healthy *S. invicta* only spent one day to eliminate the *P. fervens* colony compared to more than 10 days for SINV-1 infected *S. invicta*. The healthy *S. invicta* soon recruited workers even sooner on food and invaded into the nest of *P. fervens*. But SINV-1 infected *S. invicta* seldom invaded into the nest of *P. fervens*, and that less aggressive behavior shift might reduce the competition intensity.

On the other hand, while *S. invicta* competed with 750 *M. chinense*, all *S. invicta* colonies were eliminated by *M. chinense*. Undoubtedly, *M. chinense* had the ability to kill the *S. invicta* and works even more effectively when encountered with SINV-1 infected *S. invicta*. However, the numerical factor on competition should not be

neglected. Owing to the small body size of *M. chinense*, the equal biomass was selected for the experiments. Due to that experimental design, *M. chinense* had a over-dominate numerical advantage and further increased the chance to defeat *S. invicta*. Similarly, 150 *M. chinense* could overall invaded the nest of *S. invicta*, but it took more times than 750 *M. chinense*. At this pair of competition, both *S. invicta* and *M. chinense* perform less foraging activity and reduced the frequencies of encountering. *S. invicta* had less mortality and increased the survival time resulted from low foraging behavior.

Forager recruitment is common in the genus of *Monomorium* (Andersen *et al*., 1991), hence *Monomorium* species are always in large amount workers to encounter other ant species in natural ecosystems. Moreover, *Monomorium* species were also the most abundant ant species in many communities, in this case, *M. chinense* were more likely to fight with *S. invicta* that might restrict its further spread. Meanwhile, Alder and Silverman (2005) indicated another *Monomorium* species, *M. minimum*, did not feed on liquid fipronil baits, but another study demonstrated that hydramethylon baits made adverse effects on the density of *M. minimum* (Vogt *et al*., 2005). The ant population monitoring program in the campus of National Taipei University showed that the population density of *M. chinense* was not affected by the pyriproxyfen broadcast (Tsai, 2008), suggesting that use of native ants as an alternative strategy might work without effects of toxic bait.

4.4 Biotic resistance

Undoubtedly, the incipient *S. invicta* colonies were likely annihilated by native ant species. The findings of this study support the general theory that native ants perform biotic resistance to affect the survival and establishment of invasive species (Wilson, 1971; Whitcomb *et al*., 1973; O'neal, 1974; Nickerson *et al*., 1975; Hölldobler and Wilson, 1990; Rao and Vinson, 2004; Walters and Mackay, 2005; Buczkowski and Bennett, 2008). Moreover, numerical advantages of other native ant species over incipient *S. invicta* colonies may be important in the role of biotic resistance.

During this study, the competitive arena can not completely mimic the natural environments. As a result, experimental set-up may increase encounter and interaction probabilities between agonist species. In this case, Sagata and Lester (2008) manipulated propagule size of Argentine ant *Linepithema humile* to examine the effect of propagule size on invasion success of the Argentine ant, their results showed that small popagules of Argentine ant were quickly eliminated in laboratory, but in field studies these small colonies persisted for up to 2 months. Judging from the above, we can not only rely on the native ants as control strategy, control strategies should involve with other biological control agents or bait broadcast to ensure the success of eradication of *S. invicta*.

According to the aforementioned, other potential biological control agents should be included in integrated pest management of *S. invicta* given no researchers showed the SINV-1 virus correlated with the mortality of *S. invicta*. Data from this study suggests that SINV-1 virus probably affect the foraging behavior and invading ability and resulted in decreasing the competition ability of *S. invicta*. Additionally, SINV-1 infected *S. invicta* suffered higher mortality against the *M. chinense* implying the both *M. chinense* and SINV-1 will reduce the reinfestations of *S. invicta* into management areas and subsequently prevent outlier sensitive areas for the invasions of monogyne colonies.

5. Conclusion

From the IPM point of view, it's important that utilizing critical baits treatments and/or integrated with self-sustained biological control methods to manage *S. invicta* populations. Through the interspecific competition, native ants proved as a major biological resistance and control agents against *S. invicta*. In addition, the SINV-1 virus transmission also decreases the aggressiveness of *S. invicta* against native ants, especially *M. chinense*. Therefore, new technologies could be developed for the rearing and increasing the density of native ant species through enhancement the native ants at sensitive outlier areas. In this way, it can prevent not only the expansion of *S. invicta* but also prevent their recolonization. If native ants are conserved in areas which are already eradicated or sensitive to *S. invicta* colonization to inhabit or reintroduce that could generate the bio-resistance to compete the *S. invicta*. At the same time, virus infection would decrease the competitive abilities of *S. invicta*. However, future studies in laboratory and field test need to be done to identify the impacts of virus on *S. invicta*.

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Appendix

Appendix 1. The RdRp sequence alignment of SINV-1

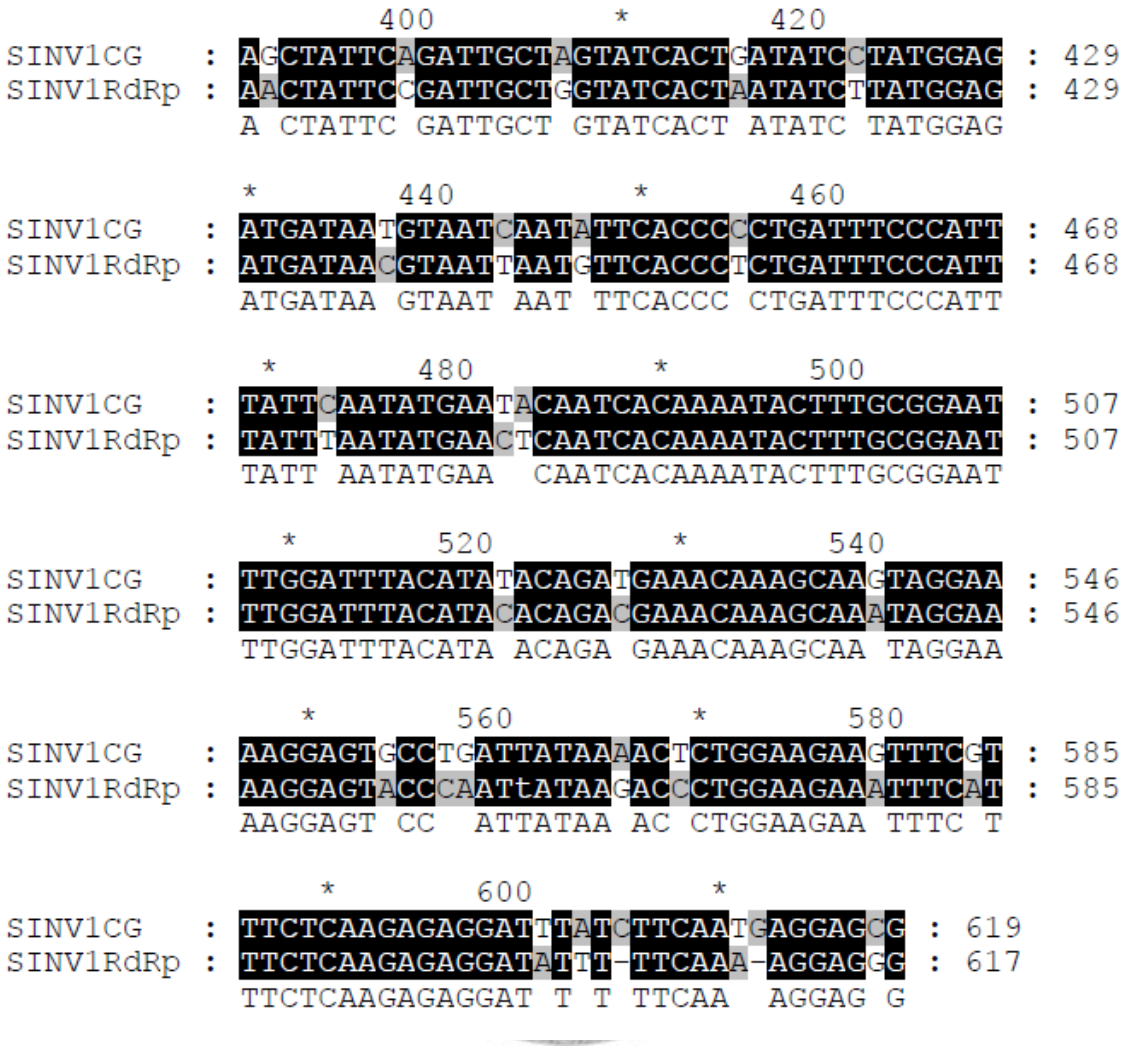
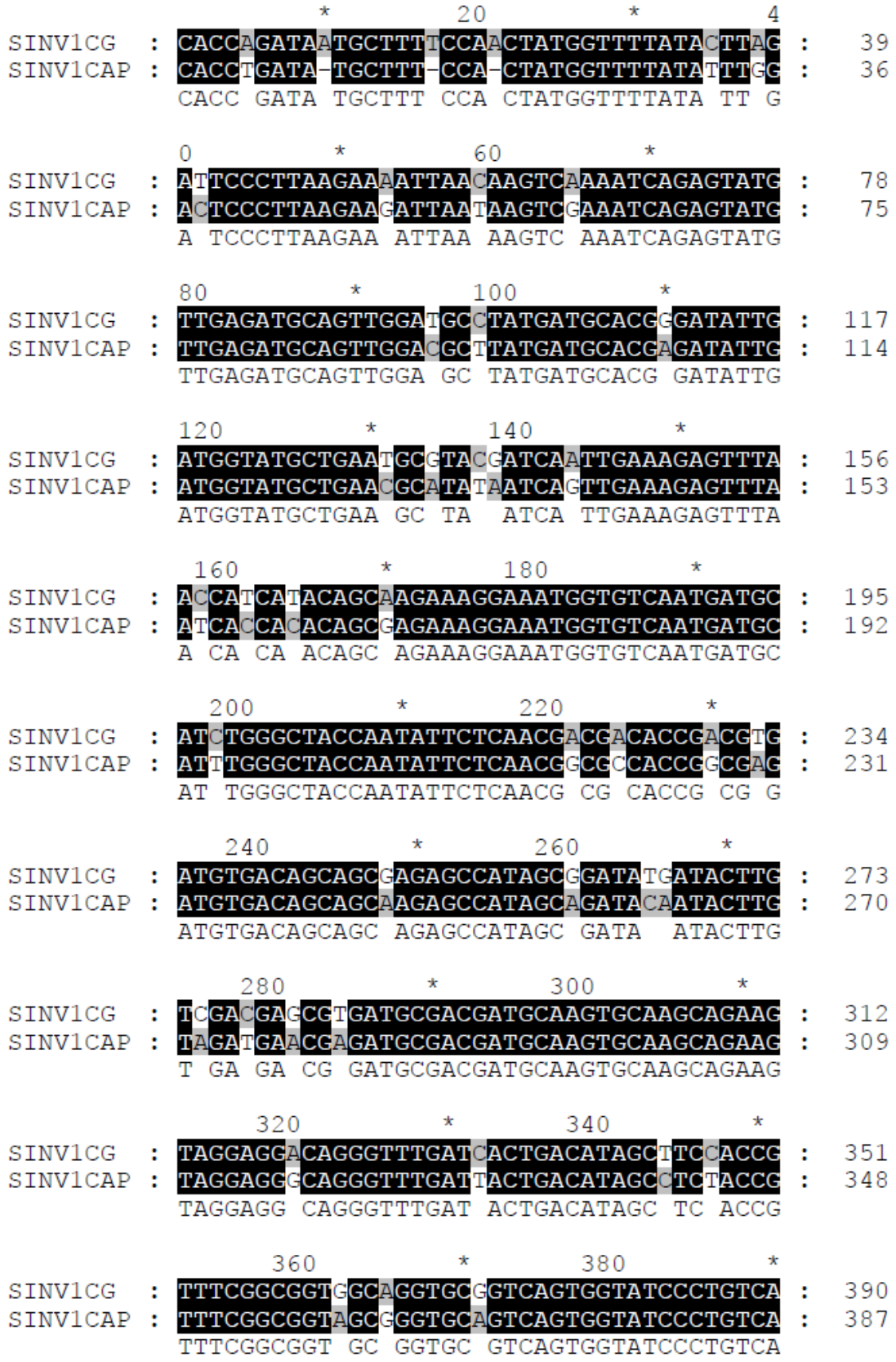


Fig. 1. The alignments of nucleotide sequence of RdRp gene of SINV-1 from GenBank Accession No. AY634314 (SINV1CG) and Taiwan (SINV1RdRp).

Appendix 2. The capsid protein sequence alignment of SINV-1



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                400         *       420
SINV1CG  :  TACGTGAAATAGCATCTACCGTTGGTTGGGTTTCTGACA  :  429
SINV1CAP :  TAGGTGAAATTGCATCCACAGTGGGTTGGGTTTCTGACA  :  426
            TA GTGAAAT GCATC AC GT GGTTGGGTTTCTGACA

            *       440         *       460
SINV1CG  :  TAGTTGGAGGAATTTCCTCTATCTTTGGATGGTCTCGAC  :  468
SINV1CAP :  TAGTTGGAGGAATTTCCTCTATCTTtGGATGGTCCCGAC  :  465
            TAGTTGGAGGAATTTCCTCTATCTTTGGATGGTC CGAC

             *       480         *       500
SINV1CG  :  CAAATGATATGGAGAAAGTGACATCTTTGGCTAACGTCC  :  507
SINV1CAP :  CGAACGACATGGAGAAAGTGACATCccTGgCAAACGTtC  :  504
            C AA GA ATGGAGAAAGTGACATC  TGGC AACGT C

              *       520         *       540
SINV1CG  :  CCGGCAAGTATTATTCCCATGTAAAAGCGATAGATAATA  :  546
SINV1CAP :  cTGgCAaGTATtATtCCCATGTGaAAGCAGTAGATAATA  :  543
            C GGCAAGTATTATTCCCATGT AAAGC  TAGATAATA

               *       560         *       580
SINV1CG  :  GTGTAGCTTTAGCTTTGAGTAATGAGAACGAGCTTCTCC  :  585
SINV1CAP :  GCGTAGCTTtAGCTTTAAGTaATGAAAATGAACTtCTCC  :  582
            G GTAGCTTTAGCTTT AGTAATGA AA GA CTTCTCC

                *       600         *       620
SINV1CG  :  CACTTAGCGACATCTTTCCCTCAGCGGTAGATGAGATGG  :  624
SINV1CAP :  CGCtTAGTGACATCTTTcCTTCAGCAGTAGATGAGATGG  :  621
            C CTTAG GACATCTTTCC TCAGC GTAGATGAGATGG

                 *       640         *       660
SINV1CG  :  ACTTGGCATATGTGTGTGCTAATCCTGGAGTGAAGGAAG  :  663
SINV1CAP :  ATTtGGCATACGTGTGCGCGAATcCTGGAGTGAAGGAGG  :  660
            A TTGGCATA GTGTG GC AATCCTGGAGTGAAGGA G

                  *       680         *       700
SINV1CG  :  TCATTACGCGGTCGAAAACGGACCCYATGAATAGAACTT  :  702
SINV1CAP :  TCATTACGTGGGCAAAGACAGACCCTATGAATAAGACAC  :  699
            TCATTACG GG C AA AC GACCC ATGAATA  AC

                *       720         *       740
SINV1CG  :  TAGCTTTAATGGAAGTGGGATTACCTAGTTTTAATAGAT  :  741
SINV1CAP :  TAGCCTTAATGGAAGTAGGGTTACCTAGTTTTAATAGAT  :  738
            TAGC TTAATGGAAGT GG TTACCTAGTTTTAATAGAT

                  *       760         *       780
SINV1CG  :  ACCAAGATAAGGCAATAGATTGTGATAGTGAACCTACCC  :  780
SINV1CAP :  ATCAAGATAAGGCTATCACGTGTGATAGCGAACCTACAC  :  777
            A CAAGATAAGGC AT    TGTGATAG GAACCTAC C
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SINV1CG : CATATAATATCTGTAACAAAGRTTTGATCAAACCAAATG : 819
SINV1CAP : CATACAACTTGTGTGACAAGAAAGTGCTGGAACCCAGCG : 816
SINV1CG : GGAACATCATTTTGAGCCCTGGAGATCTGGTGCAGATGA : 858
SINV1CAP : GAAGAGAAGTGATAAAACCTGGAGACTTAATTCAATTAA : 855
SINV1CG : AGGGCAGCTTGGCTGCGACAATTTTGGATACTGTTCCTT : 897
SINV1CAP : AagGAGATATGGCTGCAaCaATATTAGATACAGTGCcTT : 894
SINV1CG : GTGAATATGTGTCCCAATTGTTTCAGTATTGGCGTGCTA : 936
SINV1CAP : GTGAaTATATTtCACaACTATTTCAGTATTgGgCGAGCcA : 933
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SINV1CAP : CTATTTGTTTtAaGATTTcAGTtGTtAaGACAGggTTCc : 972
SINV1CG : ATACAGGACGTTTAGAAATTTTCTTTGACCCGGGTGAGT : 1014
SINV1CAP : ATACTGgTCGATtAGAAaTCTTTttCGACCcAggGGAGT : 1011
SINV1CG : ATCTAACGAATCCTAAGGCGGATTGGCATAATTATGTTG : 1053
SINV1CAP : ATCTGACTAaTCcAAaGTCTGATtGgCATAaTTATGTtG : 1050
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SINV1CAP : AT-TtACGggCTtATGACaaAGTGGATACTGCAAATTCA : 1088
SINV1CG : TACAAATATATTTTAGATTTAACAAATGATTCAGAAATT : 1130
SINV1CAP : TACAAGTATATTTTAGACTTAACTAATGATTCAGAGATA : 1127
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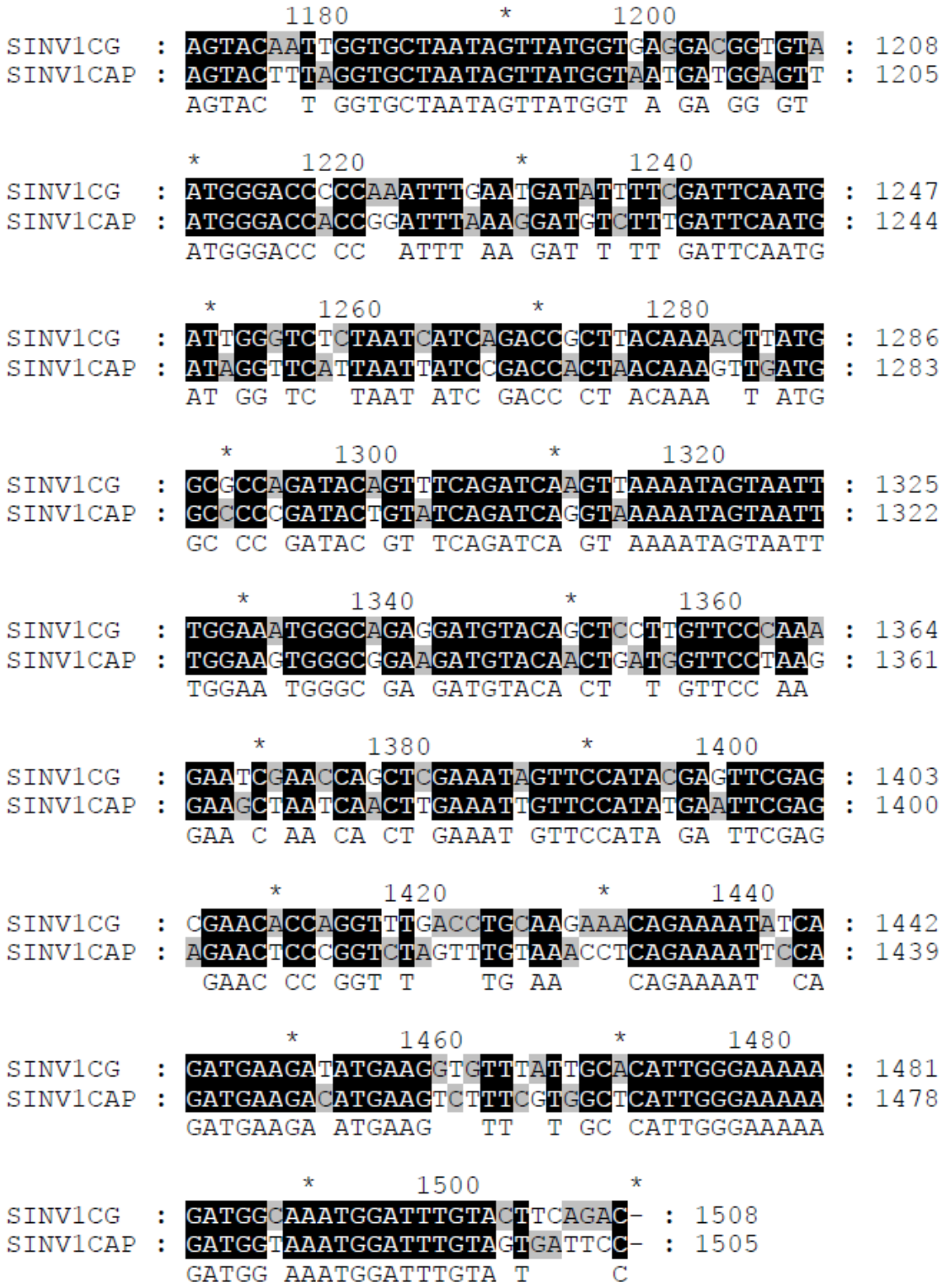

Fig. 2. The alignments of nucleotide sequence of capsid protein region of SINV-1 from GenBank Accession No. AY634314 (SINV1CG) and Taiwan (SINV1CAP).