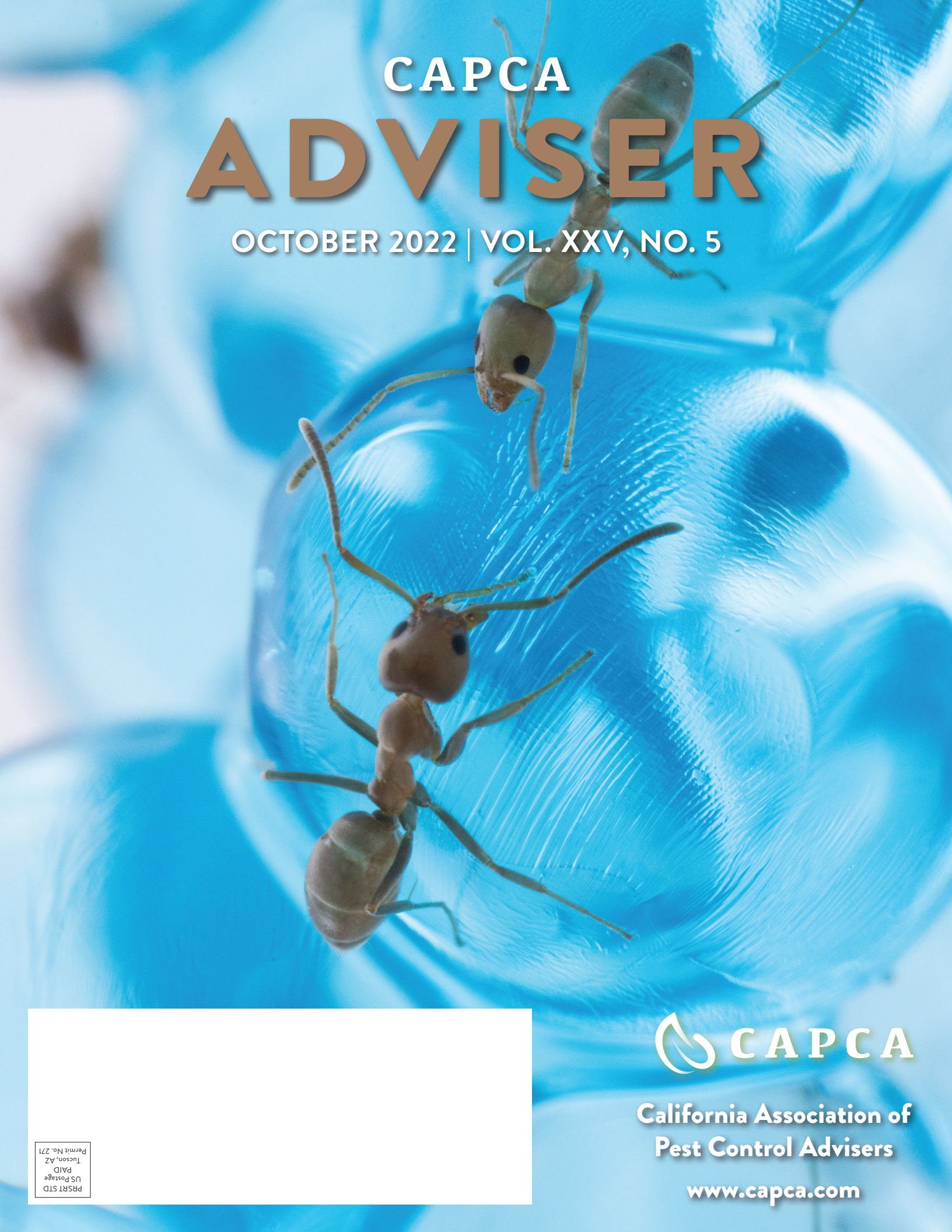

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Spinosad-laced hydrogel baits effectively control Argentine ants in California citrus

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Why is Argentine Ant control necessary?

Argentine ants (AA), *Linepithema humile*, form strong relationships with honeydew producing sap sucking pests (SSPs) in citrus and other crops (e.g., grapes) (Fig. 1). AA protect SSPs from their natural enemies (i.e., predators and parasitoids; [NEs]) and in return for this protection AA are rewarded with honeydew, a sugary waste product that results from sap feeding (Milosavljević et al. 2017) (Fig. 1). Additionally, AA move SSPs to new parts of the orchard thereby driving pest infestations, and increased SSP populations provide more honeydew to AA which in turn increases ant populations. AA tend a diverse variety of SSPs in citrus (e.g., mealybugs, and brown soft scale), including, Asian citrus psyllid (ACP), *Diaphorina citri*, the vector of *Candidatus* Liberibacter asiaticus (CLas), a bacterium that causes huanglongbing (HLB), a lethal citrus disease (McCalla 2019; Milosavljević et al. 2017, 2021). In southern California citrus, AA protects more than 85 percent of SSP colonies and over 55 percent of ACP colonies from NEs (Tena et al. 2013). The consequence of ants protecting SSPs from NEs are increased costs of pest control. Therefore, AA control is a critical component of integrated pest management (IPM) programs for SSPs in citrus, especially programs that rely heavily upon biological control.

The loss of chlorpyrifos in California drives a new approach for Argentine ant control

Chlorpyrifos, a contact insecticide, is the only product registered for AA control in citrus, and its use has now been banned in California because it poses significant risks to human health (CDPR 2019). In response to this ban, the California Department of Pesticide Regulations (CDPR) funded work to develop an alternative ant control technology. The approach our team took was the

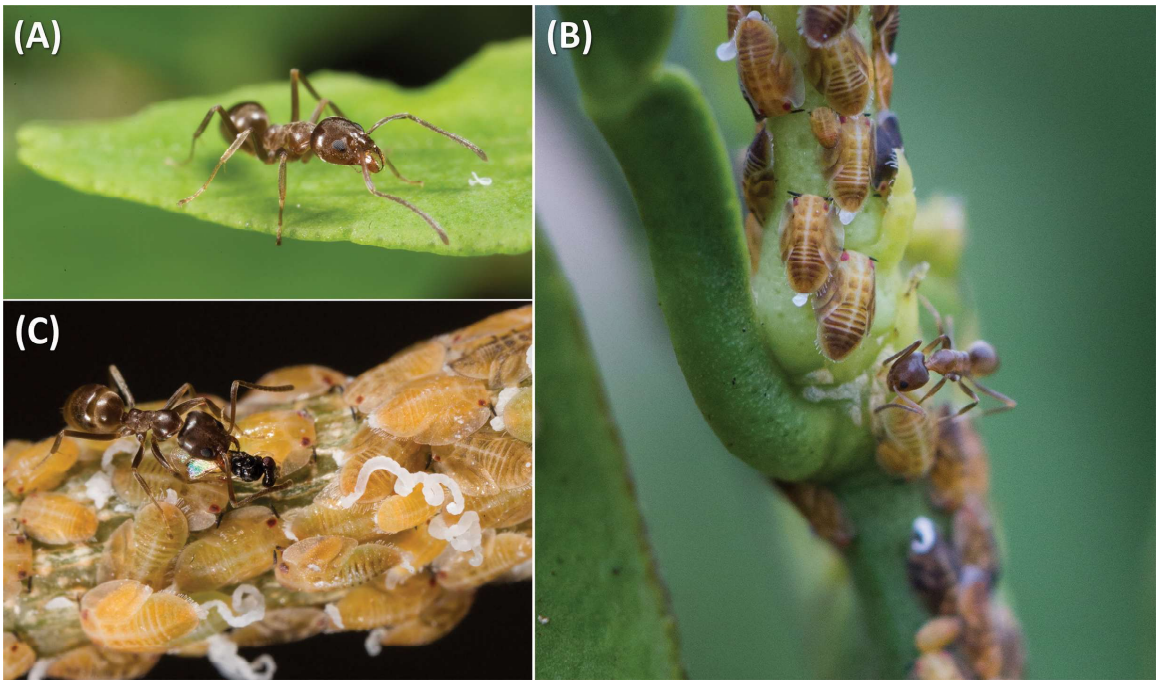

FIG. 1. (A) An Argentine ant foraging on a citrus leaf. (B) Argentine ants tend an Asian citrus psyllid colony to collect sugary honeydew; (C) Argentine ant attacking *Tamarixia radiata*, a key natural enemy of Asian citrus psyllid. *Photos: Mike Lewis, Center of Invasive Species Research, UC Riverside*

development of biodegradable alginate hydrogel beads (HGBs) (Schall et al. 2018). These beads are impregnated with ant trail pheromone and infused with a 25% sucrose solution laced with ultra-low concentrations of insecticide (0.0001%). HGBs are easily made from inexpensive commercially available materials, and they require no field maintenance as they biodegrade (Fig. 2). HGBs can be easily distributed under trees where rapid AA recruitment occurs. Foraging ants imbibe the toxic sucrose water, return it to the nest to feed brood and queen(s) which kills them, and this results in colony collapse. Consequently, HGBs may provide an alternative to plastic bait dispensers (Fig. 3) that are used in liquid baiting programs for AA control (McCalla et al. 2020). Rapid (< 5 days) long term (> 3 months) control of AA results from repeated HGB applications (~3-4 applications ~3 weeks apart over summer) and this can result in long-term reductions of SSPs in citrus. Populations of SSPs collapse when ants are controlled because NE efficacy increases markedly as AA are no longer present to harass and attack NEs (Schall & Hoddle 2017; McCalla 2019).

In pursuit of alternative insecticides for Argentine ant control

Using CDPR funding our lab demonstrated that thiamethoxam, a neonicotinoid insecticide, when delivered to AA using HGBs is highly efficacious (McCalla et al. 2020). However, there are increasing concerns over using neonicotinoids for pest control. Consequently, we investigated the efficacy of alternative chemistries for use in HGB's for AA control. A very promising insecticide, spinosad, an organically registered product, was identified from laboratory trials for incorporation into HGBs (Milosavljević & Hoddle 2021). This insecticide has been moved into the field evaluation pipeline for comparison to thiamethoxam, the "gold standard" against which we measure the efficacy of different products for AA control. Field trials in three commercial citrus orchards were run over July through December 2021. Experiments compared the efficacy of HGBs loaded with low (0.0001%) and high (0.001%) concentrations of organically approved spinosad, low (0.0001%) and high (0.001%) concentrations of thiamethoxam, and control treatments lacking insecticide treatments. Insecticide treatment groups received a series of three HGB applications each spaced 3 weeks apart at a rate of 250 g of hydrogel per tree. HGBs were hand-distributed (it is possible to mechanically distribute HGBs under trees) on recently irrigated soil (< 48 h) (Fig. 4). Timed visual estimations of the number of AA workers ascending and descending each tree trunk were used to measure treatment effects on densities of foraging AA. Preliminary statistical analyses have indicated that AA densities in plots treated with insecticide-laced HGBs were around three-to-four

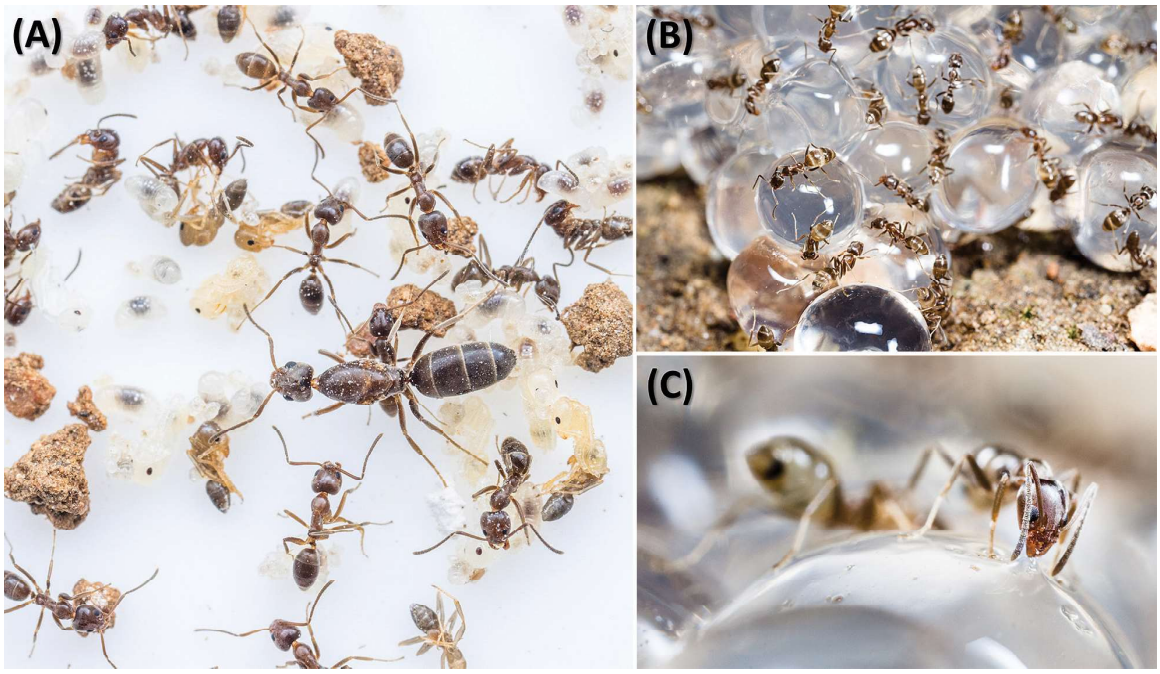

FIG. 2. (A) Argentine ant colony in the lab. Alongside the large queen are workers, pupae, and grub-like larvae. (B) Argentine ants imbibing sugar water laced with an ultra-low concentration of insecticide contained with biodegradable hydrogel beads. (C) Close-up of Argentine ants feeding from an alginate hydrogel bead filled with toxic liquid bait. *Photos: Mike Lewis, Center of Invasive Species Research, UC Riverside*

FIG. 3. Liquid baiting with plastic bait dispensers necessitates initial investment in bait dispensers and continuous labor costs for servicing. *Photos: Mike Lewis, Center of Invasive Species Research, UC Riverside*

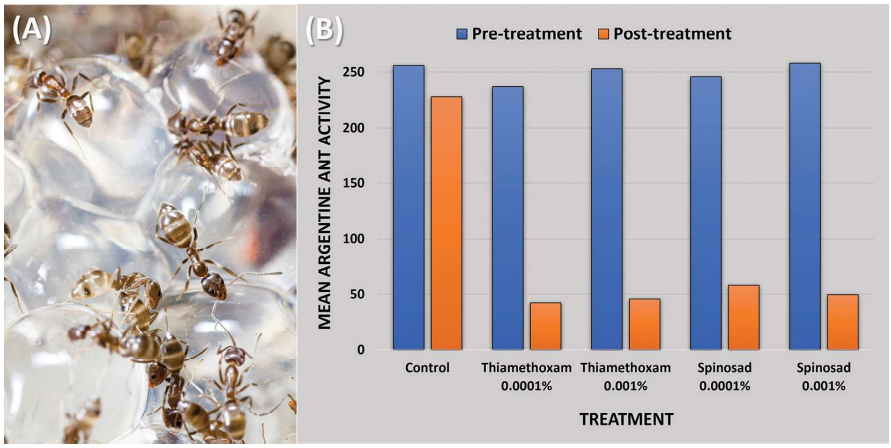

FIG. 4. (A) Argentine ants feeding on hydrogels soon after deployment. (B) Comparison of mean Argentine ant activities as estimated visually (number of ants traversing tree trunks per 1-min observation) on hydrogel-treated and control citrus trees across all sites on baseline sampling dates (blue bars) and following three HGB applications (orange bars). *Photos: Mike Lewis & Ivan Milosavljević, Center of Invasive Species Research, UC Riverside*

times lower than control plots lacking insecticides (Fig. 4). Importantly, the impacts of low and high concentrations of spinosad and thiamethoxam on foraging AA were statistically similar. This finding suggests that the 0.0001% concentration of spinosad provides significant suppression of AA populations, and its efficacy is similar to our "gold standard," thiamethoxam, for AA control.

We conclude that HGBs have significant benefits compared to current AA treatment options in citrus. Insecticide delivery to AA in HGBs provides a level of control that is highly competitive with commercially available products and applications are highly targeted to ants foraging on the soil which preserves NEs that attack SSPs in the canopy. Organic growers may be able to benefit from the use of HGBs to control AA as our work has demonstrated that an organically approved spinosad formulation provides effective ant control. More work is needed to optimize the use of HGBs for AA control. We are currently determining the minimum amounts of HGB and frequency of applications needed to control AA in commercial citrus.

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