Herbal Plants as Alternatives for the Management of the Red Imported Fire Ant, Solenopsis Invicta (Hymenoptera: Formicidae)

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Research Article

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Abstract

The red imported fire ant (RIFA) is one of the most detrimental invasive species, threatening native ecosystems, human health, and economic activities worldwide. In the quarantine zone of Taiwan, RIFA re-infestation frequently occurs despite the intensive application of synthetic pesticides, making its control costly and ineffective. Thus, there is an urgent need to identify effective, eco-friendly, and sustainable alternatives for controlling RIFA populations. In this study, we examined the efficacy and feasibility of planting herbal species for RIFA control. Five herbal species, *Tagetes lemmonii*, *Armoracia rusticana*, *Cymbopogon citratus*, *Cymbopogon nardus*, and *Chrysopogon zizanioides,* were planted in a RIFA-infested field with local weeds as controls. Bait and pitfall traps and RIFA intruded plants were used to compare the ant activity in the control fields and those containing herbal plants. The RIFA repellent activity of the five herbal plants and their basal soil was further evaluated through digging bioassays. Generally, the field surveys showed more ants and intruded plants in the control than in the herbal groups; however, the significance varied based on the trap type and plant species. The bioassays demonstrated the significant repellency of the aboveground parts of *T. lemmonii*, *C. nardus,* and *C. citratus,* and the belowground parts of *T. lemmonii*, *C. citratus*, and *V. zizanioides* against RIFA. The basal soil of *T. lemmonii*, *C. citratus,* and *C. nardus* also exhibited deterrent activity toward RIFA. Our results demonstrated that herbal plants are eco-friendly, sustainable alternatives for controlling and preventing RIFA infestation in severe infested and non-infested areas.

Key Message

We evaluated the efficacy and feasibility of five herbal species as eco-friendly and sustainable repellents for RIFA control.

The numbers of trapped RIFA workers and RIFA-intruded plants were generally higher in the control than in the herbal groups.

RIFA workers were repelled by either aboveground or belowground parts of the herbal plants.

Live herbal plants are eco-friendly, sustainable alternatives for controlling and preventing RIFA infestation.

Introduction

The red imported fire ant (RIFA) (*Solenopsis invicta*) is one of the most damaging pests worldwide. The workers attack *en masse* when their nest is intruded or accidentally trampled. The sting presents as a typical wheal-and-flask reaction with a sterile pustule, and the affected area is sensitive to pain and itches persistently for several weeks. Severe attacks of RIFA may cause skin inflammation, secondary infection, anaphylactic shock, and occasionally death. In the infested area of the United States, up to 30%–60% of residents experience RIFA stings every year (Pereira and Bolton 2005; Wylie and Janssen-May 2017). More than 1% of the victims were hypersensitive and required medical care (Pereira and Bolton 2005).

Red imported fire ant attacks occur both outdoors and indoors (deShazo and Banks 1994; deShazo et al. 1999), and individuals who cannot instantly respond to these attacks, such as infants and immobilized patients, are especially susceptible. In Australia, the total estimated medical cost due to RIFA attacks in 2011 amounted to AUD 114 million (ABS 2011). It is also predicted that the developing Pacific Island Countries and Territories will incur medical expenditures of up to USD 35.1 million if invaded by RIFA (Gruber et al. 2021).

Red imported fire ant infestation also has adverse impacts on agriculture, animal industries, public facilities, and the natural environment (Wylie and Janssen-May 2017). In the United States of America, different degrees of yield loss due to RIFA infestation have been reported in more than 50 food crops, including citrus, soybeans, potatoes, sunflowers, cucumbers, eggplants, and grain crops (Wylie and Janssen-May 2017). In the developing western Pacific, the potential loss of cropping and apiculture was projected to account for 44% of the total socioeconomic impacts caused by RIFA (Gruber et al. 2021). This pest species can also deplete or contaminate livestock food sources, resulting in malnourished cattle or chickens. The mucous tissues around the eyes and nostrils of domestic animals are susceptible to RIFA stings, which can then swell and lead to blindness or suffocation (Barr and Drees 1996). Overall, the invasion of RIFA could reduce production in the livestock sector.

Infestation with RIFA negatively impacts native ecosystems and wildlife (Epperson and Allen 2010; Vinson 2013). These invasive ants prey on invertebrates and attack pipping young of reptile, ground-nesting birds, and the altricial young of small mammals, consequently reducing newborn survival (Allen et al. 2004; Wojcik et al. 2001). Additionally, RIFA competes with local species for food resources, alter animal behavior, hinder animal growth, and threaten the survival of several rare species, such as the Stock Island tree snail (*Orthalicus reses*), Schaus' swallowtail (*Papilio aristodemus ponceanus* Schaus), and several cave-dwelling species (Elliott 1993; Wojcik et al. 2001). Therefore, numerous native species have been eliminated, while the abundance of the other (usually non-native) species has been maintained, causing a dramatic loss of biodiversity (Epperson and Allen 2010; Wojcik et al. 2001).

The management of RIFA largely depends on synthetic insecticides, including pyrethroid (cypermethrin and cyhalothrin), nicotinoid (imidacloprid), indoxacarb, and growth regulators (pyriproxyfen, methoprene, or diflubenzuron). While these chemicals are practical and versatile, we cannot afford to ignore their known weaknesses. For example, non-selective pesticides can kill beneficial non-target species (Goulson et al. 2015). Moreover, the repetitive application is necessary for short-lived insecticides to maintain their effect, which causes heavy financial burdens for large-scale control schemes. However, long-lasting insecticides may reach a lethal amount in species with higher trophic levels through bioaccumulation (Fu et al. 2018); the local extinction of these keynote species may break down the entire ecosystem. For reducing the use of synthetic pesticides in pest management projects, it is important to search for more sustainable approaches, such as the phorid fly borne pathogen, *Kneallhazia solenopsae* (Oi and Williams 2003; Oi et al. 2008), competitive ant species, *Monomorium minimum* (Wang and Chen 2015) and cost-effective aerial surveillance systems (Spring et al. 2017). Among these, plant-based repellents have long been the most attractive alternatives to synthetic insecticides for RIFA management (Chen and Oi 2020).

However, most of these repellents are volatile and thus require specialized formulation to extend the effective period. In contrast, live herbal plants can persistently produce and release bioactive phytochemicals. Nevertheless, potential repellent species against RIFA currently underexplored (but see Sternberg et al. 2006).

Although the Taiwanese government has invested a huge sum of money in the eradication of RIFA since its discovery in 2003, its control is still far from successful. The RIFA-infested area in Taiwan has recently expanded southward and northward from Taoyuan and Hsinchu, occupying many abandoned fields and replotted land. The eradication of RIFA in these areas is unfeasible because of their size and budget limitations. Alternatively, it is possible to create an environment that discourages RIFA recolonization by planting herbal plants, thus reducing the infested area or minimizing the impact of RIFA. As such, we aim to study the effect of herbal plants on RIFA activities, as well as investigate the repellence of aboveground and belowground plant organs and field-collected soil.

Materials And Methods

Study site and plants

A field experiment was conducted in a RIFA-infested replotted land in Banqiao District, New Taipei, Taiwan (3.42 ha, 25.034020°N, 121.469732°E). The study area was dominated by polygynous RIFA populations and grasslands under regular mowing. The vegetation was dominated by grasses (e.g., *Imperata cylindrica, Paspalum urvillei, and Setaria viridis*) and dicotyledonous weeds (e.g., *Bidens pilosa* var. *radiata and Mimosa pudica*). *Cyperus rotundus, Sesbania cannabina, and Eleusine indica were also frequently detected.* The density of active RIFA nests was 1,160 ± 480 (mean ± SD) per hectare. Arthropods such as millipedes, spiders, springtails, pillbugs, crickets, and cockroaches are frequently caught by pitfall traps.

Based on the results of a literature search and our preliminary bioassay using plant essential oils and field-collected soils, we selected five herbal species: Lemmon's marigold (*Tagetes lemmonii*), horseradish (*Armoracia rusticana*), citronella (*Cymbopogon nardu*s), lemongrass (*Cymbopogon citratus*), and vetiver (*Chrysopogon zizanioides*). The dominant grass species (*I. cylindrica, P. urvillei, and S. viridis*) were planted *indiscriminately* as the control plant for *citronella grass, lemongrass, and vetiver, and Bidens pilosa* for marigold and horseradish.

Within the replotted land, we designed an experimental region of approximately 380 m^2 (18.5 × 20.5 m), which was plowed to reset the RIFA population and grow the herbal plants. The area was subsequently divided into five plots: three large plots containing 12 subunits (six replicates × two pairs of herbal and control subunits) and two small plots consisting of six subunits (three replicates × two pairs of herbal and control subunits) (Fig. 1). The control and herbal plants were planted in subunits arranged in a chessboard pattern to minimize variations due to uncertain environmental factors, such as soil moisture. From July 9–31, 2020, the potted plants of Lemmon's marigold, horseradish, and vetiver were transplanted into the large plots, and rooted suckers of citronella grass and lemongrass were planted in the small plots. The control plants were collected on-site and planted as well. Within each plot, herbal and control plants were planted in a 7 × 7 matrix with an interval of 30 cm (5 × 5 and 40 cm intervals for horseradish and the respective control plant because the size of horseradish seedlings are larger than that of other herbal plants). A total of 1,376 plants were planted. The plots were watered twice daily until late October when the fine roots should have been well established, after which they were only watered when leaves were wilting. For the first two months, weeds large enough to cover the herbal plants were removed manually.

To increase the survival of horseradish, we sheltered the plants with commercial plastic shading nets (approximately 50% natural irradiance) for the first month. We also applied extended-release, broad-effect fertilizers (NPK 20.10.10, Zuid-Chemie B.V., Sas van Gent, Netherlands) when horseradish showed symptoms of nutritional deficiencies. *Bacillus thuringiensis aizawai* strain NB-200 (Valent BioSciences LLC) was used to control the infestation of *Pieris rapae caterpillars*.

Monitoring RIFA activity

The RIFA population was monitored biweekly using both bait and pitfall traps. The traps were set and collected from 10:00 to 16:00 on non-rainy days. The bait trap method was slightly modified from the version used in Bao et al. (2011). For each experimental subunit, an uncapped 50 mL centrifuge tube containing a half piece (approximately 0.8 g) of Pringles® original flavor potato chips was placed horizontally on the ground near the center of each subunit for 1 h. The tubes were then capped and stored in a refrigerator overnight to kill the ants before counting them. The pitfall trap was composed of a 50 mL uncapped centrifuge tube containing 15 mL 75% ethanol buried vertically in the soil near the center of each subunit, with the tube rim aligned with the ground level. After 24 h, the number of ants caught by each pitfall trap was counted. We observed that the workers frequently dig the soil around the base of the plants, forming small mounds (Fig. S1), which is considered an indicator of ant activity. As such, we monitored the base of every living plant biweekly, disturbed the mounds slightly with a thin iron wire to check for the presence of fire ants, and recorded the number of intruded plants.

Digging bioassay

Nests of the RIFA were collected from the same area outside the experimental site and were cultured in plastic buckets rimmed with Fluon (DuPont, Wilmington, DE, USA) at ambient temperature under a 16:8 (Light:Dark) photoperiod, and fed potato chips and cockroaches; water was provided *ad libitum*.

The repellence of the herbal plants and the plant-cultivated soil were tested by digging bioassay developed by Chen et al. (2019; 2021) with slight modifications. The test unit was assembled with four 2.5 mL glass vials and a Petri dish (14.5 cm in diameter). The caps of the glass vials were glued onto the bottom of the Patri dish, and an entry hole (3 mm in diameter) was drilled through the contacting part of the dishes and caps. The inner wall of each Petri dish was coated with a thin layer of Fluon (DuPont, Wilmington, DE, USA) to prevent ants from escaping. Two types of tests were performed in the digging bioassays: two-choice tests and multiple-choice tests. For each two-choice test, two of the glass vials were filled with sands (a control and a herbal); the other two vials remained empty, and the entry hole was sealed. For multiple-choice test, each of the four vials was filled with differentially treated sands. For each test, 50 RIFA workers were introduced into the dish provided with moist cotton in a 0.5 mL centrifuge tube. The test unit was kept in a dark environment for 24 h, where the workers tended to dig and remove the sand and stay in the vials. The vials were weighed before and after the experiments to calculate the sand weight removed by the ants. The number of ants residing in the vials was counted as another indicator of the ants' affinity to the sand. The tests were repeated five to six times.

Repellent properties of plant tissues

To investigate the origin of repellent activity of herbal plants, we examined the repellent activity of fresh and dried plant tissues using digging bioassays. The fresh tissues sampled from the root and rhizome (belowground), leaf, and leaf sheath (aboveground) of the herbal and control plants were ground into a paste and mixed thoroughly with sea sand (grain size approximately 300–350 μm, Scharlau, SPAIN) and 6% water to make 1.5% (tissue weight/sand weight) sea sand mixtures; these mixtures were then subjected to two-choice digging bioassays. The tissue samples were oven-dried at 60 °C for 3 d, ground into powder, mixed with sea sand and 6% water to make a 0.7% mixture (tissue weight/sand weight) to be used in two-choice digging bioassays.

Fresh tissues with significant repellence against RIFA were subjected to further investigation for dose-responsive relationships using the multiple-choice bioassay. Three concentration levels (low, medium, and high) were tested (Table S1).

Repellent properties of herbal plants-cultivated soil

The active ingredients of plants may be released into the soil through excretion, leaching, or tissue decomposition, making the soil more repellent to RIFA over time. For citronella grass and lemongrass, the soil was taken from the base of two plants in each subunit (n = 6). For marigold, horseradish, and vetiver, the soil was taken from the base of one plant in each subunit (n = 6). Soil moisture ranged from 14% to 22% (volumetric water content). The soil was sampled to match the moisture of the herbal and control subunits so that the difference was within 2%. The soil was sifted through a 1.2 mm pore size sieve and subjected to the repellent test using two-choice digging assays.

Statistical Analysis

All statistical analyses were performed with R software, version 4.0.3 (R Core Team 2020). Data manipulation and visualization were performed using the mega-package tidyverse (Wickham 2017; Wickham et al. 2019).

We observed an apparent linear trend in the number of captured ants in trap data. Therefore, we used log-linked generalized additive models (GAMs) to account for the seasonal pattern to analyze better the differential repellent activity of herbal and control plants against RIFA. A negative binomial distribution was assumed instead of a Poisson distribution because overdispersion was detected for all models. For each GAM, we considered one predictor (TREAT) and two covariates (TIME and POSITION). TREAT labeled the ants captured in the herbal or control subunits. TIME entered the model as a non-parametrically smoothed function *s*(TIME). POSITION was coded from 1 to 3 according to the distance between the subunit and the edge of the experimental region (Fig. S2). By including POSITION, we attempted to partial-out the edge effect, i.e., the invasion starting from the border of the experimental region. The deviance explained by the models was 42%–82%, except for those of *A. rusticana* bait trap data (22.6%, Table S2). We also fitted mixed-effect models (GAMM) with the experimental subunit as a random variable and specified temporal autocorrelation across repeated measures on the same subunits. None of these models performed better than the fixed effect models (i.e., GAM). We then re-examined the interaction term and POSITION to determine the final model for analyzing the TREAT effect (Table S2). Akaike information criterion (AIC) and likelihood ratio (LR) tests were used as criteria for comparison of nested models. When the model performance did not differ significantly, a simpler model with fewer model terms was selected. We tentatively removed one to three extreme values and re-ran the models. These results were consistent with those of the original models. The GAM and GAMM were built with the *gam and gamm* functions and diagnosed using the gam.check function of the mgcv package (Wood 2017).

The data obtained for intruded plants contained an excessive number of zeros. A portion of this number (true zeros) represented the plants suitable for but unexploited by the RIFA, while the others (false zeros) may be due to constraints, such as cold seasons, dry weather, and underground ant activities. Therefore, we utilized zero-augmented models, i.e., zero-altered (ZA) and zero-inflated (ZI) models, to deal with the excessive zeros in the intruded plant data. The ZA is a two-part model: first, the data are considered zeros versus non-zeros and are dealt with by a binomial model; second, the non-zero count data are modeled using a zero-truncated Poisson or a negative binomial model. In contrast, ZI is a mixture model that treats zeros as coming from two sources: the binomial and count processes (for details, see Zuur et al. (2009)). We chose the best modeling approach based on AIC and LR and used the selected model to analyze the intruded plant data. For the initial zero-augmented model, we considered one predictor variable (TREAT) and two covariates (TIME and POSITION). The explanatory variables were specified separately for the binomial and count processes. We then included the interactive effects, excluded insignificant model terms one by one, and re-evaluated the models. The final model components for each experimental plot are listed in Table S3. The ZI and ZA models were built with the zeroinfl and hurdle functions, and the model fit was diagnosed using Q-Q plot and rootogram with the countreg package (Kleiber and Zeileis 2016; Zeileis and Kleiber 2020; Zeileis et al. 2008).

The sand weight moved by RIFA workers and the number of residing ants were used as indicators for sample repellency. The results of the two-choice bioassays were analyzed using one-sided paired *t*-tests at a 95% confidence level. We hypothesized that sands treated with herbal plants would show higher repellence against RIFA than the control sands. Similarly, the soil collected from the herbal subunits may have a higher repellence than the control ones.

The results of the multiple-choice bioassay were examined with an analysis of variance (ANOVA) followed by Tukey's honest significant difference tests using the *HSD.test* function of the *agricolae* package (de Mendiburu 2020). The dependent variables (sand weight and the number of residing ants) were arcsine-square root transformed before the analyses. The residuals were checked using Q−Q plots, Shapiro-Wilk tests, and Kolmogorov-Smirnov tests for normality. We found no evidence suggesting the non-normality of the residuals, except for the medium concentration level of lemongrass leaves and the low concentration level of vetiver root. We further analyzed the data using non-parametric Kruskal-Wallis rank sum tests followed by an *ad hoc* Dunn's test with the rstatix package (Kassambara 2021). The results of the non-parametric tests agree with those of the parametric results, with slight differences. Here, we presented the results of parametric tests while included the non-parametric tests in the supplementary materials.

Results

Ant activity in fields with herbal plants

Generalized additive models were used to analyze the number of ants captured in the studied plots. The smooth term *s*(TIME) was significant ($P < 0.05$) for all experimental plots, indicating a pronounced seasonal pattern. As shown in Fig. 2, the number of captured ants peaked in October and subsequently decreased, flattened until late February, and rebounded after March. The covariate POSITION was only retained in the bait trap data of *A. rusticana, V. zizanioides,* and the pitfall trap data of *C. nardus*. The interactive effect of POSITION and TREATMENT was retained in the bait trap data of *V. zizanioides* and the pitfall trap data of *C. nardus* (Table S2). In general, the analysis showed that more ants were captured in the control than in the herbal subunits (Figs. 2 and 3, Table 1). In the bait trap data, this difference was significant for *T. lemmonii* (P =0.002) and *V. zizanioides* (P = 0.019), marginally significant for *C. citratus* (P = 0.076), but insignificant for *A. rusticana* (P = 0.130) and *C. nardus* (P = 0.934). In the pitfall trap data, this difference was significant for all species, except for *T. lemmonii* (P = 0.531). For *C. nardus*, this difference was marginally significant (P = 0.082), whereas the interactive effect was significant (P = 0.015), indicating that the difference was compounded by the edge effect. The estimated number of ants was higher in the control subunits than in the herbal subunits for POSITION 3 but lower for POSITION 1, although the number of data points might be insufficient to provide a definitive conclusion (Fig. 3).

Number of plants intruded by RIFA

The zero-inflated data of RIFA-intruded plants were analyzed with ZI and ZA models (Table 2, Fig. 4). Generally, the models estimated a higher number of intruded plants for the control subunits (Fig. 4). The difference between the herbal and control groups was significant for all species, except for *V. zizanioides* (Table 2). The edge effect (POSITION) was detected in *T. lemmonii, A. rusticana,* and *V. zizanioides.* For *T. lemmonii,* the difference was larger in POSITION 1 (outer) than in POSITION 2 (inner), while the reverse was true for *A. rusticana.* For *V. zizanioides,* the number was higher in control subunits from September to November, and the difference was reduced in February and March. Overall, the results showed different degrees of repellent activity in herbal plants.

Organ-specific repellence of herbal plants toward RIFA

The organ-specific repellent activity was tested using a digging bioassay. The ants were expected to avoid digging sea sands mixed with the effective tissue extracts and reside in the control vials (Fig. 5, Table S4). For the fresh samples, this pattern was observed in the aboveground organs of *T. lemmonii* and *Cymbopogon* species and the belowground organs of *T. lemmonii*, *C. citratus*, and *V. zizanioides.* The results of the dried samples were generally consistent with or showed less pronounced differences than those of the fresh samples. In contrast, the dried tissues of *A. rusticana* had an effective repellent activity, while the fresh ones did not.

The fresh tissues of the effective plant organs were further investigated for minimum effective concentration using the multiple-choice bioassay (Figs. 6, S3, and S4, and Tables S5 and S6). The results showed different degrees of repellence against RIFA depending on the species and tissues. The minimum effective concentration of *T. lemmonii* was approximately 0.2% (tissue weight/sand weight) for leaves and lower than 0.1% for roots. The fresh roots of horseradish did not completely suppress ant activity until the dose increased up to 10%. For *C. citratus* leaves, the minimum dosage was approximately 0.2%–0.3%; for their roots, 0.5%–1.0% dramatically suppressed the digging activity, while 0.1%–0.2% substantially reduced the number of residing ants. Although the *C. nardus* leaves exhibited very similar results to lemongrass, their roots were not further tested owing to a lack of repellence compared to the control plants (Fig. 5). The minimum effective concentration of the vetiver root was below 0.1%.

Repellent activity of the soil collected from the base of plants

We examined soil collected from the experimental subunits. The soil water content was comparable between the control (19.4% ± 3.2% SD) and herbal subunits (19.7% ± 3.6 SD). Compared to the control subunits, the soil collected from herbal subunits generally showed repellence in terms of the soil weight moved by ants, except for vetivers. However, this difference was only significant in *T. lemmonii* and *C. citratus* soils (Fig. 7). The number of residing ants was only statistically significant in the *C. nardus* soil.

Discussion

In searching for more eco-friendly surrogates for synthetic chemicals, phytochemicals and other bio-derived compounds have been intensively studied (Campolo et al. 2018; Geetha and Roy 2014; Huang et al. 2019; Hyldgaard et al. 2012; Kimutai et al. 2017; Lee 2018; McAllister and Adams 2010; Mendoza-García et al. 2014, 2019; Panella et al. 2005; Pinheiro et al. 2013; Regnault-Roger et al. 2012; Tawatsin et al. 2006; Wang et al. 2012, 2014; Wiltz et al. 2007). However, these compounds are usually volatile or easily degradable, and therefore require specialized formulations to extend their release duration (Golden et al. 2018). In contrast, live repellent plants can persistently generate and release bioactive components. Sternberg et al. (2006) suggested using the old world bluestem (*Bothriochloa bladhii*) to replace other grass species and thus reduce the number of RIFA colonies. Zhang et al. (2017) also found that the 0–10 cm soil layer in the rhizosphere of *Viburnum odoratissimum* exhibited excellent insecticidal and repellent effects against RIFA, likely due to methyl salicylate leaching from fallen leaves. Deterring RIFA with repellent plants seems to be a convincing but underexplored research field. In this study, we examined five potential repellent plant species with active phytochemicals against RIFA and discussed the possible routes through which the active ingredients could be released into the soil.

Tagetes lemmonii (Lemmon's marigold)

Tagetes species, also known as marigolds, are frequently used for nematode control in crops (Krueger et al. 2010; Wang et al. 2007). Marigolds have secretory ducts and cavities distributed in the tissues of roots, stems, leaves, and the corolla of flowers in the inflorescence (Poli et al. 1995; Simon et al. 2002) Additionally, the shoots of many species are covered with secretory glandular trichomes. The leaves of Lemmon's marigold contain numerous phytochemicals with insecticidal and repellent activity against a broad range of insects, such as 4-ethyl-4-methyl-1-hexene, β-ocimene, dihydrotagetone, (E)-tagetone, and (E)-tagetenone (Tucker and Maciarello 1996; Mendoza-García et al. 2014, 2019). The roots of marigolds also release bioactive compounds against nematodes present in the rhizosphere. Among these, α-terthienyl might be one of the most toxic photoreactive phytochemicals. Under ultraviolet light, these secondary metabolites target and attack DNA, cell membranes, membrane proteins, and several enzymes of insect pests. These processes involve the inhibition of superoxide dismutase and the promotion of superoxide anion radical generation (Nivsarkar et al. 2001). In addition to nematodes, α-terthienyl also has phototoxicity against numerous insect pests (Nivsarkar et al. 2001), including RIFA (Liu et al. 2011). With a 30 min exposure to ultraviolet light, this compound has the potential to enervate the ants and seriously disrupt their behavior; with more than 90 min of exposure, it can knock down 90% of RIFA workers (Liu et al. 2011). Based on these results, Liu et al. (2011) proposed the use of α-terthienyl as bait at dusk. The phototoxin could be transported and spread throughout the ant colony, possibly via trophallaxis and allogrooming. Subsequently, the poison would take effect when the ants were exposed to sunlight after sunrise.

In the present study, we confirmed that both leaf and root extracts of Lemmon's marigold showed repellent activity against RIFA (Fig. 5 and Table S4). We also demonstrated that, compared to control plants, Lemmon's marigold reduced RIFA activity in terms of the number of captured ants and intruded plants within eight months. However, it is uncertain which phytochemicals are responsible for repellence against RIFA. Moreover, the release pathway into soils and the soil's contents of bioactive compounds remain unknown and require further investigation.

Armoracia rusticana (horseradish)

In Europe, horseradish has been cultivated for over 2,000 years for its fleshy, pungent roots. These plants produce few seeds and regenerate through root cuttings and rhizomes (Sampliner and Miller 2009). The presence of horseradish is closely related to human activity, and wild populations are unlikely to exist nowadays. Horseradish root has traditionally been used as a condiment, preservative, and folk medicine.

The leaves were used to wrap up rice, meat, onions, and condiments into a traditional Romanian dish, sarmale. Horseradish tissue contains sinigrin and myrosinase, which are physically separated at the cellular or subcellular levels. When the plant tissue is injured, the reservoirs of these two compounds break. Subsequently, sinigrin is hydrolyzed by myrosinase into allyl isothiocyanate (AITC) (Yu et al. 2001). This chemical process is known as the “mustard oil bomb”. AITC exhibits antimicrobial and antifungal activities (Hyldgaard et al. 2012; Manyes et al. 2015; Romeo et al. 2018), as well as high toxicity against a broad range of arthropods, including the maize weevil (*Sitophilus zeamais*), lesser grain borer (*Rhyzopertha dominica*), *Tribolium ferrugineum*, book louse (*Liposcelis entomophila*) (Wu et al. 2009), and *Dermatophagoides farinae* (Wu et al. 2009; Yun et al. 2012). Hashimoto et al. (2019) demonstrated that microencapsulated AITC could completely deter RIFA and Du et al. (2020) revealed that AITC exhibited contact and fumigation toxicity against RIFA.

In the present study, the fresh tissue of horseradish roots could only repel RIFA at very high concentrations (Fig. 6). As mentioned previously, AITC is only generated upon tissue injury and is very unstable. Therefore, it is difficult to obtain an effective amount in the collected soil within a short experimental period (Fig. 7). In contrast to the fresh tissue, the dried tissue exhibited significant deterrence against RIFA (Fig. 5). This may be because the sinigrin hydrolysis was arrested in the dried samples until water was added immediately before the sand mixture was filled into the glass vials. Our field survey only partially supported the repellency of horseradish. We cannot recommend horseradish as a repellent plant against RIFA because the plants were frequently infested with *Pieris rapae* and *Phyllotreta striolata* and showed signs of nutrition deficiency, which requires intensive management with and pesticides and fertilizers.

Cymbopogon nardus (citronella)

In the 1950s Taiwan, citronella was an important cash crop cultivated for its essential oil. Citronella oil exhibits antibiotic and antifungal properties (Nakahara et al. 2003; Wei and Wee 2013), as well as repellent and insecticidal activities against a broad range of pests, including mosquitoes, black flies, fleas, ticks, red flour beetles, thrips, and green peach aphids (Clemente et al. 2010; Geetha and Roy 2014; Kalita et al. 2013; Pinheiro et al. 2013; Regnault-Roger et al. 2012; Sharma et al. 2019). Wiltz et al. (2007) demonstrated that the essential oils of basil, citronella, lemon, peppermint, and tea tree had apparent deterrence against RIFA, but only citronella oil could cause significant mortality within 24 h. Our digging assay confirmed the effectiveness of the citronella leaf extracts (Fig. 5). The signature components of citronella oil are citronellal and citral (including the two geometric isomers, neral, and geranial). Other bioactive ingredients include citronellol, geraniol, linalool, limonene, camphor, eucalyptol, eugenol, and α- and β-pinene (Nakahara et al. 2003; Wei and Wee 2013). These phytochemicals are initially stored in the adaxial epidermal and subepidermal cells of the leaves and might be released upon tissue injury (Lai and Tsai 1975). However, many of these active components are volatile and may evaporate before leaching into the soil. This is probably the reason that the repellency of citronella could only be detected by the number of residing ants and not by the sand weight moved by ants in the soil digging bioassay (Fig 7 and Table S7).

Nevertheless, as a preliminary experiment, we tested soil samples from five *C. nardus* growing sites in middle Taiwan. For each site, the soil samples were collected at different distances (0, 30, 60, and 90 cm) from a citronella plant that grew more than three years. The soil samples were tested with a multiple-choice digging bioassay, and the results suggested significant deterrence of the basal soil (0 cm) against RIFA, compared to the soil samples at farther distances (30, 60, and 90 cm) (Fig. S5, Table S8). In addition, our field survey showed a lower number of captured ants in the citronella subunits than in the control subunits. We deduced that the soil repellency might increase over time due to the accumulation of leaf litter, which may entrap the essential oil and gradually release it through tissue decomposition. Although citronella could not repel RIFA in this short-term experiment, its long-term potential deserves further evaluation in future studies.

Cymbopogon citratus (Lemongrass)

Lemongrass, a closely related species of citronella, is native to Asia, Southeast Asia, Africa, and the Americas and was introduced in temperate and tropical regions of the world, including Taiwan. Its fragrant leaves are famously used for condiments and in medicine (Lawal et al. 2017). Lemongrass oil contains 70%–80% citral and 10%–15% myrcene (Andrade et al. 2009; Bossou et al. 2013), and other minor bioactive components, such as linalool, geraniol, β-ocimene, citronellal, and α-terpineol (Andrade et al. 2009; Bossou et al. 2013; Lawal et al. 2017). Citral is also a common mandibular secretion that pertains to the behavior of Hymenoptera (including bees and ants) (Tengö and Bergström 1976). The stingless bee (*Trigona subterranea*) is attracted to a low level of citral but is repelled or alarmed by a high level of this terpene (Blum et al. 1970). The robber bee (*Lestrimelitta limao)* uses citral-dominated mandibular gland secretions to disorient and rob food resources from the colonies of *Melipona* and *Trigona* (Blum et al. 1970). Mandibular secretions of smaller yellow ants (*Lasius claviger*, formerly *Acanthomyops claviger*), composed of citral and citronellal (1:9), are employed as a defense substance (Chadha et al. 1962). The leafcutter ant (*Atta sexdens*) releases citral as an alarm pheromone. As such, citral was formulated as a repellent to control pest ants (Oi and Williams 1999). The fumigating activity of citral against RIFA has also been demonstrated (Xiao et al. 2020).

The leaf repellence of lemongrass was very similar to that of citronella, based on the results of the digging bioassay (Fig. 5 and 6). In contrast to citronella root, the belowground parts of lemongrass showed clear deterrence against RIFA workers. This means that the repellence of the basal soil of lemongrass (Fig. 7) could be due to the active ingredients released from the roots and leaf litter. The major components of the crude extracts of rhizomes were selina-6-en-4-ol (27.8%), citral (14.6%), α-cadinol (8.2%), neointermediol (7.2%), eudesm-7(11)-en-4-ol (5.3%), and α-muurolol (5%). With the exception of citral, these phytochemicals are currently underexplored as potential biopesticides.

Chrysopogon zizanioides (Vetiver)

Vetiver, or khus, is a fast-growing and resilient species that is native to northern India. It is an excellent hedge plant that can stabilize the soil and thus reduce its erosion (National Research Council 1993). Traditionally, dried vetiver roots have been used to deter clothes moths, head lice, and bedbugs. Its root extract, that is, vetiver oil, can repel Formosan subterranean termites (Zhu et al. 2001) and reduce oviposition, inhibit egg hatching, kill larvae, and deter adults of *Anopheles stephensi*. In addition, Henderson et al. (2005) verified that vetiver oil is also repellent and toxic to ticks, cockroaches, and RIFA. Vetiver oil contains at least 300 chemicals which include insect-repellent compounds, such as α-, β-vetivone, bicyclovetivenol, khusimone, nootkatone, zizanol, zizanal, and epizizanal (Jain et al. 1982; Zhu et al. 2001). Among these chemicals, nootkatone alone can effectively repel pests with a long residual time (4–8 weeks) (Henderson et al. 2005; Panella et al. 2005). In our study, the digging bioassay using root extracts and the ant activity survey confirmed this potential repellent efficacy (Fig. 6). However, the soil digging bioassay did not show promising results (Fig. 7). It is likely that the bioactive compounds are not actively excreted into soils but released only upon injury or decomposition of root tissues. Thus, the phytochemicals cannot reach an effective level to deter RIFA within a short experimental period.

Conclusion

In this study, we investigated five herbal plant species that produce insecticidal or deterring phytochemicals against RIFA. Both field survey and digging bioassay suggested that Lemmon's marigold and lemongrass are potential repellent plants for the passive control of RIFA in a short period of eight months, although follow-up observation is necessary to justify the long-term efficacy of citronella. Nevertheless, some considerations associated with the plantation of repellent plants should be noted. First, planting repellent plants cannot immediately kill RIFA. Therefore, repellent plants are not appropriate for highly sensitive areas, such as kindergartens, schools, and healthcare centers, where eradication is urgent. However, it could be applied in a non-infested and recovered area near the quarantine zone to reduce the chance of RIFA colonization and recolonization. We also suggest planting repellent plants in abandoned fields and replotted lands, where RIFA infestation was severe and re-invaded frequently, such that immediate eradication is considered unlikely. In these areas, repellent plants could reduce the population size and activity of RIFA, thereby reducing the quarantine zone of RIFA and alleviating infestation severity to a manageable scale in a long run.

Second, in contrast to synthetic insecticides, the initial cost of herbal plants can be intimidating. However, if the herbal plants are carefully chosen, the investment will decrease sharply, as the repelling effects will be long-lasting. To maximize cost-effectiveness, in addition to their repellent activity, the chosen plants should be robust, resilient, and able to outcompete common weeds. After seedling establishment, they should not require additional care, such as irrigation, fertilization, weeding, and pest control. Plant propagation should be simple so that the planting area can expand in the following years at a low cost. Added values of the chosen plants can increase the landowners' willingness to pay for them. For example, citronella, lemongrass, vetiver, and marigold can be used for essential oil distillation, and the stalks of lemongrass and the roots and rhizomes of horseradish are popular food condiments.

Third, plants introduced without careful evaluation could become invasive species that might negatively impact the local population of native species through competitive exclusion, niche displacement, and introgression hybridization (Mooney and Cleland 2001). Therefore, native species should be preferred, introduced species should be carefully evaluated, and invasive species should be avoided. Finally, an omnipotent, ideal repellent plant does not exist; however, potentially qualified plants, as well as the pros and cons of their use, should be researched and appropriately integrated into comprehensive programs for better RIFA control.

Declarations

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Authors' contributions CCT performed experiments, data curation, formal analysis, and wrote the first draft. SHH and SRL coordinated the field studies. RNH designed the studies, reviewed the manuscript and funding acquisition. All of the authors read and approved the manuscript.

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References

1. ABS (2011) Category 3236.0: Household and Family Projections Australia 2011 to 2036. Australian Bureau of Statistics, Canberra
2. Allen CR, Epperson DM, Garmestani AS (2004) Red imported fire ant impacts on wildlife: A decade of research. Am Midl Nat 152:88–103. https://doi.org/10.1674/0003-0031(2004)152[0088:RIFAIO]2.0.CO;2
3. Andrade EHA, Zoghbi M, das GB, Lima M da P (2009) Chemical composition of the essential oils of *Cymbopogon citratus* (DC.) Stapf cultivated in North of Brazil. J Essent Oil-Bear Plants 12:41–45. https://doi.org/10.1080/0972060X.2009.10643689
4. Bao SZ, Kafle L, Shih CJ (2011) A new baited trap for monitoring *Solenopsis invicta* (Formicidae: Hymenoptera) in Taiwan. Appl Entomol Zool 46:165–169. https://doi.org/10.1007/s13355-011-0028-z
5. Barr CL, Drees BM (1996) Texas Cattle Producers Survey: Impact of Red Imported Fire Ants on the Texas Cattle Industry: Final Report. Texas Agricultural Extension Service, College Station, Texas

6. Blum MS, Crewe RM, Kerr WE et al (1970) Citral in stingless bees: Isolation and functions in trail-laying and robbing. J Insect Physiol 16:1637–1648. https://doi.org/10.1016/0022-1910(70)90263-5
7. Bossou AD, Mangelinckx S, Yedomonhan H et al (2013) Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles). Parasites Vectors 6:337. https://doi.org/10.1186/1756-3305-6-337
8. Campolo O, Giunti G, Russo A et al (2018) Essential oils in stored product insect pest control. J Food Qual 2018:1–18. https://doi.org/10.1155/2018/6906105
9. Chadha MS, Eisner T, Monro A, Meinwald J (1962) Defence mechanisms of arthropods-VII. Citronellal and citral in the mandibular gland secretion of the ant *Acanthomyops claviger* (Roger). J Insect Physiol 8:175–179. https://doi.org/10.1016/0022-1910(62)90087-2
10. Chen J (2021) Evidence of social facilitation and inhibition in digging behavior of red imported fire ants, *Solenopsis invicta*. Insect Sci 28:566–570. https://doi.org/10.1111/1744-7917.12781
11. Chen J, Oi DH (2020) Naturally occurring compounds/materials as alternatives to synthetic chemical insecticides for use in fire ant management. Insects 11:1–18. https://doi.org/10.3390/insects11110758
12. Chen S, Chen H, Xu Y (2019) Safe chemical repellents to prevent the spread of invasive ants. Pest Manag Sci 75:821–827. https://doi.org/10.1002/ps.5184
13. Clemente MA, de Oliveira Monteiro CM, Scoralik MG et al (2010) Acaricidal activity of the essential oils from *Eucalyptus citriodora* and *Cymbopogon nardus* on larvae of *Amblyomma cajennense* (Acari: Ixodidae) and *Anocentor nitens* (Acari: Ixodidae). Parasitol Res 107:987–992. https://doi.org/10.1007/s00436-010-1965-0
14. de Mendiburu F (2020) agricolae: Statistical procedures for agricultural research. R package version 1.3-3. https://CRAN.R-project.org/package=agricolae
15. deShazo RD, Banks WA (1994) Medical consequences of multiple fire ant stings occurring indoors. J Allergy Clin Immunol 93:847–850. https://doi.org/10.1016/0091-6749(94)90376-X
16. deShazo RD, Williams DF, Moak ES (1999) Fire ant attacks on residents in health care facilities: A report of two cases. Ann Intern Med 131:424–429
17. Du Y, Grodowitz MJ, Chen J (2020) Insecticidal and enzyme inhibitory activities of isothiocyanates against red imported fire ants, *Solenopsis invicta*. Biomol 10:1–10. https://doi.org/10.3390/biom10050716
18. Elliott WR (1994) Fire ants and endangered cave invertebrates: A control and ecological study. Revised Final Report. Endangered Resources Branch, Resource Protection Division, Texas Parks and Wild-life Department, Austin
19. Epperson DM, Allen CR (2010) Red imported fire ant impacts on upland arthropods in Southern Mississippi. Am Midl Nat 163:54–63. https://doi.org/10.1674/0003-0031-163.1.54
20. Fu L, Lu X, Tan J et al (2018) Bioaccumulation and human health risks of OCPs and PCBs in freshwater products of Northeast China. Environ Pollut 242:1527–1534. https://doi.org/10.1016/j.envpol.2018.08.046

21. Geetha R, Roy A (2014) Essential oil repellents – A short review. Int J Drug Dev Res 6:20–27
22. Golden G, Quinn E, Shaaya E et al (2018) Coarse and nano emulsions for effective delivery of the natural pest control agent pulegone for stored grain protection. Pest Manag Sci 74:820–827. https://doi.org/10.1002/ps.4787
23. Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957. https://doi.org/10.1126/science.1255957
24. Gruber MAM, Janssen-May S, Santoro D et al (2021) Predicting socio-economic and biodiversity impacts of invasive species: Red Imported Fire Ant in the developing western Pacific. Ecol Manag Restor 22:89–99. https://doi.org/10.1111/emr.12457
25. Hashimoto Y, Yoshimura M, Huang RN (2019) Wasabi versus red imported fire ants: preliminary test of repellency of microencapsulated allyl isothiocyanate against *Solenopsis invicta* (Hymenoptera: Formicidae) using bait traps in Taiwan. Appl Entomol Zool 54:193–196. https://doi.org/10.1007/s13355-019-00613-5
26. Henderson G, Heumann DO, Laine RA et al (2005) Extracts of vetiver oil as repellent and toxicant to ants, ticks, and cockroaches. 1–13
27. Huang HT, Lin CC, Kuo TC et al (2019) Phytochemical composition and larvicidal activity of essential oils from herbal plants. Planta 250:59–68. https://doi.org/10.1007/s00425-019-03147-w
28. Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:12. https://doi.org/10.3389/fmicb.2012.00012
29. Jain SC, Nowicki S, Eisner T, Meinwald J (1982) Insect repellents from vetiver oil: I. zizanal and epizizanal. Tetrahedron Lett 23:4639–4642. https://doi.org/10.1016/S0040-4039(00)85675-0
30. Kalita B, Bora S, Sharma AK (2013) Plant essential oils as mosquito repellent – A review. Int J Res Dev Pharm Life Sci 3:741–747
31. Kassambara A (2021) rstatix: Pipe-friendly framework for basic statistical tests. R package version 0.7.0
32. Kimutai A, Ngeiywa M, Mulaa M et al (2017) Repellent effects of the essential oils of *Cymbopogon citratus* and *Tagetes minuta* on the sandfly, *Phlebotomus duboscqi*. BMC Res Notes 10:1–9. https://doi.org/10.1186/s13104-017-2396-0
33. Kleiber C, Zeileis A (2016) Visualizing count data regressions using rootograms. Am Stat 70:296–303. https://doi.org/10.1080/00031305.2016.1173590
34. Krueger R, Dover KE, McSorley R, Wang K-H (2010) Marigolds (*Tagetes* spp.) for nematode management. IFAS Extension ENY -056:1–7
35. Lai K, Tsai Y-Z (1975) Studies on the morphogenesis of citronella grass (*Cymbopogon nardus rendle* var. *maha pengiri*) Leaf. J Agr Assoc China 91:32–43

36. Lawal OA, Ogundajo AL, Avoseh NO, Ogunwande IA (2017) Cymbopogon citratus. In: KUETE V (ed) Medicinal Spices and Vegetables from Africa – Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases. Elsevier Inc., London, pp 397–423
37. Lee MY (2018) Essential oils as repellents against arthropods. BioMed Res Int 2018:6860271. https://doi.org/10.1155/2018/6860271
38. Liu N, Cheng D, Xu H et al (2011) Behavioral and lethal effects of α-terthienyl on the red Imported fire ant (RIFA). Sci Agric Sin 44:4815–4822. https://doi.org/10.3864/j.issn.0578-1752.2011.23.007
39. Manyes L, Luciano FB, Mañes J, Meca G (2015) In vitro antifungal activity of allyl isothiocyanate (AITC) against *Aspergillus parasiticus* and *Penicillium expansum* and evaluation of the AITC estimated daily intake. Food Chem Toxicol 83:293–299. https://doi.org/10.1016/j.fct.2015.06.011
40. McAllister JC, Adams MF (2010) Mode of action for natural products isolated from essential oils of two trees is different from available mosquito adulticides. J Med Entomol 47:1123–1126. https://doi.org/10.1603/ME10098
41. Mendoza-García EE, Ortega-Arenas LD, Serrato-Cruz MA et al (2014) Effects of *Tagetes coronopifolia* and *T. lemmonii* (Asteraceae) essential oils in nymphs of *Diaphorina citri* (Hemiptera: Psyllidae) Permalink. J Citrus Pathol 1:173
42. Mendoza-García EE, Ortega-Arenas LD, Serrato-Cruz M et al (2019) Chemical composition, toxicity, and repellence of plant essential oils against *Diaphorina citri* (Hemiptera: Liviidae). Chil J Agr Res 79:636–647. https://doi.org/10.4067/S0718-58392019000400636
43. Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. P Natl Acad Sci USA 98:5446–5451. https://doi.org/10.1073/pnas.091093398
44. Nakahara K, Alzoreky NS, Yoshihashi T et al (2003) Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella Grass). JPN Agric Res Q 37:249–252. https://doi.org/10.6090/jarq.37.249
45. National Research Council (1993) Vetiver grass: a thin green line against erosion. The National Academies Press, Washington, DC
46. Nivsarkar M, Cherian B, Padh H (2001) Alpha-terthienyl: A plant-derived new generation insecticide. Curr Sci 81:667–672
47. Oi DH, Williams DF (1999) An Ant Repellent. 14
48. Oi DH, Williams DF (2003) *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in reproductives of red imported fire ants (Hymenoptera: Formicidae) and its implication for intercolony transmission. Environ Entomol 32:1171–1176. https://doi.org/10.1603/0046-225X-32.5.1171
49. Oi DH, Williams DF, Pereira RM et al (2008) Combining biological and chemical controls for the management of red imported fire ants (Hymenoptera: Formicidae). Am Entomol 54:46–55. https://doi.org/10.1093/ae/54.1.46
50. Panella NA, Dolan MC, Karchesy JJ et al (2005) Use of novel compounds for pest control: Insecticidal and acaricidal activity of essential oil components from heartwood of Alaska Yellow Cedar. J Med Entomol 42:352–358. https://doi.org/10.1093/jmedent/42.3.352

51. Pereira RM, Bolton H (2005) Imported fire ants and their management. In: Proceedings of 1st Quail Management Shortcourse. Arcadia, Florida, pp 49–67
52. Pinheiro PF, Queiroz VT de, Rondelli VM et al (2013) Insecticidal activity of citronella grass essential oil on *Frankliniella schultzei* and *Myzus persicae*. Ciênc e Agrotecnologia 37:138–144. https://doi.org/10.1590/s1413-70542013000200004
53. Poli F, Sacchetti G, Bruni A (1995) Distribution of internal secretory structures in *Tagetes patula* (Asteraceae). Nordic J Bot 15:197–205. https://doi.org/10.1111/j.1756-1051.1995.tb00143.x
54. R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
55. Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: Low-risk products in a high-stakes world. Annu Rev Entomol 57:405–424. https://doi.org/10.1146/annurev-ento-120710-100554
56. Romeo L, Iori R, Rollin P et al (2018) Isothiocyanates: An overview of their antimicrobial activity against human infections. Molecules 23:1–18. https://doi.org/10.3390/molecules23030624
57. Sampliner D, Miller A (2009) Ethnobotany of horseradish (*Armoracia rusticana*, Brassicaceae) and its wild relatives (*Armoracia* spp.): Reproductive biology and local uses in their native ranges. Econ Bot 63:303–313. https://doi.org/10.1007/s12231-009-9088-1
58. Sharma R, Rao R, Kumar S et al (2019) Therapeutic potential of citronella essential oil: A Review. Curr Drug Discov Technol 16:330–339. https://doi.org/10.2174/1570163815666180718095041 PMID – 30019646
59. Simon P, Katinas L, Arambarri A (2002) Secretory structures in *Tagetes minuta* [Asteraceae, Helenieae]. Bol Soc Argent Bot 37:181–191
60. Spring D, Croft L, Kompas T (2017) Look before you treat: increasing the cost effectiveness of eradication programs with aerial surveillance. Biol Invasions 19:521–535. https://doi.org/10.1007/s10530-016-1292-1
61. Sternberg T, Perry G, Britton C (2006) Grass repellency to the red imported fire ant. Rangel Ecol Manag 59:330–333. https://doi.org/10.2111/05-151R1.1
62. Tawatsin A, Asavadachanukorn P, Thavara U et al (2006) Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). SE Asian J Trop Med 37:915–931
63. Tengö J, Bergström G (1976) Comparative analyses of lemon-smelling secretions from heads of Andrena F. (Hymenoptera, Apoidea) bees. Comp Biochem Phys B 55:179–188. https://doi.org/10.1016/0305-0491(76)90227-3
64. Tucker AO, Maciarello MJ (1996) Volatile leaf oil of *Tagetes lemmonii* gray. J Essent Oil Res 8:417–418. https://doi.org/10.1080/10412905.1996.9700652
65. Vinson SB (2013) Impact of the invasion of the imported fire ant. Insect Sci 20:439–455. https://doi.org/10.1111/j.1744-7917.2012.01572.x

66. Wang J, Qiu X, Zeng L, Xu T (2014) Interference of plant essential oils on the foraging behavior of *Solenopsis invicta* (Hymenoptera: Formicidae). Fla Entomol 97:454–460
67. Wang J, Zhang H, LingZeng, Xu Y-J (2012) Repellent effect of five plant essential oil on the Red Imported Fire Ants, *Solenopsis invictia*. Sociobiology 59:695–701
68. Wang K-H, Hooks CR, Ploeg A (2007) Protecting crops from nematode pests: using marigold as an alternative to chemical nematicides. Plant Dis PD -35:1–6
69. Wang L, Chen J (2015) Fatty amines from little black ants, *Monomorium minimum*, and their biological activities against red imported fire ants, *Solenopsis invicta*. J Chem Ecol 41:708–715. https://doi.org/10.1007/s10886-015-0609-3
70. Wei LS, Wee W (2013) Chemical composition and antimicrobial activity of *Cymbopogon nardus* citronella essential oil against systemic bacteria of aquatic animals. Iran J Microbiol 5:147–152
71. Wickham H (2017) tidyverse: easily install and load the "Tidyverse." R package version 1.2.1
72. Wickham H, Averick M, Bryan J et al (2019) Welcome to the tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686
73. Wiltz BA, Suiter DR, Gardner WA (2007) Deterrency and toxicity of essential oils to argentine and red imported fire ants (Hymenoptera: Formicidae). J Entomol Sci 42:239–249. https://doi.org/10.18474/0749-8004-42.2.239
74. Wojcik DP, Allen CR, Brenner RJ et al (2001) Red imported fire ants: Impact on biodiversity. Am Entomol 47:16–23. https://doi.org/10.1093/ae/47.1.16
75. Wood SN (2017) Generalized Additive Models: An Introduction with R, 2nd edn. Chapman and Hall/CRC, Boca Raton
76. Wu H, Zhang GA, Zeng S, Lin KC (2009) Extraction of allyl isothiocyanate from horseradish (*Armoracia rusticana*) and its fumigant insecticidal activity on four stored-product pests of paddy. Pest Manag Sci 65:1003–1008. https://doi.org/10.1002/ps.1786
77. Wylie FR, Janssen-May S (2017) Red imported fire ant in Australia: What if we lose the war? Ecol Manag Restor 18:32–44. https://doi.org/10.1111/emr.12238
78. Xiao CX, Tan YT, Wang FF et al (2020) The fumigating activity of *Litsea cubeba* oil and citral on *Solenopsis invicta*. Sociobiology 67:41–47. https://doi.org/10.13102/sociobiology.v67i1.4481
79. Yu EY, Pickering IJ, George GN, Prince RC (2001) In situ observation of the generation of isothiocyanates from sinigrin in horseradish and wasabi. Biochimi Biophys Acta 1527:156–160. https://doi.org/10.1016/S0304-4165(01)00161-1
80. Yun YK, Kim HK, Kim JR et al (2012) Contact and fumigant toxicity of *Armoracia rusticana* essential oil, allyl isothiocyanate and related compounds to *Dermatophagoides farinae*. Pest Manag Sci 68:788–794. https://doi.org/10.1002/ps.2327
81. Zeileis A, Kleiber C (2020) countreg: Count data regression. R package version 0.2-1
82. Zeileis A, Kleiber C, Jackman S (2008) Regression models for count data in R. J Stat Softw 27:1–25. https://doi.org/10.18637/jss.v027.i08

83. Zhang Y, Fu JT, Huang CL et al (2017) Insecticidal activity of the soil in the rhizosphere of *Viburnum odoratissimum* against *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology 64:1–6. https://doi.org/10.13102/sociobiology.v64i1.1067
84. Zhu BCR, Henderson G, Chen F et al (2001) Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. J Chem Ecol 27:1617–1625. https://doi.org/10.1023/A:1010410325174
85. Zuur AF, Ieno EN, Walker N et al (2009) Mixed Effects Models and Extensions in Ecology with R. Springer Science + Business Media, LLC, New York

Tables

Table 1. Parametric parts of generalized additive models fitting the trap data

Species	Term	Estimate	SD	*z*	*P*
Bait trap					
T. lemmonii	TREAT	-0.69	0.22	-3.16	**0.002**
A. rusticana	TREAT	-0.41	0.27	-1.52	0.130
	POSITION	-0.73	0.27	-2.65	0**.008**
C. nardus	TREAT	0.03	0.35	0.08	0.934
C. citratus	TREAT	-0.56	0.32	-1.77	0.076
V. zizanioides	TREAT	-1.65	0.70	-2.34	**0.019**
	POSITION	-1.13	0.35	-3.28	**0.001**
	TREAT×POSITION	0.92	0.47	1.94	0.052
Pitfall trap					
T. lemmonii	TREAT	-0.10	0.16	-0.63	0.531
A. rusticana	TREAT	-0.45	0.18	-2.52	**0.012**
C. nardus	TREAT	1.08	0.62	1.74	0.082
	POSITION	0.40	0.20	2.06	**0.040**
	TREAT×POSITION	-0.70	0.29	-2.43	**0.015**
C. citratus	TREAT	-0.64	0.20	-3.14	**0.002**
V. zizanioides	TREAT	-0.49	0.18	-2.73	**0.006**

Significant test results are in boldface.

The nonparametric smooth term s(TIME) for all selected models is significant ($P < 0.05$); only parametric parts were reported.

Table 2. Summary of the zero-augmented models for the intruded plant data

Species	Term	Estimate	SD	*z*	*P*
Count process					
T. lemmonii	TREAT	-3.97	1.03	-3.86	**<0.001**
	POSITION	-0.88	0.19	-4.63	**<0.001**
A. rusticana	TREAT	-0.15	0.29	-0.53	0.594
	POSITION	1.07	0.19	5.52	**<0.001**
C. nardus	TREAT	-4.18	0.90	-4.66	**<0.001**
	TIME	-0.06	0.01	-5.70	**<0.001**
	POSITION	1.47	0.49	3.02	**0.003**
C. citratus	TREAT	-1.10	0.52	-2.13	**0.033**
	TIME	-0.05	0.01	-4.14	**<0.001**
V. zizanioides	TREAT	-0.49	0.51	-0.95	0.344
	TIME	0.005	0.005	0.97	0.330
	POSITION	0.97	0.28	3.43	**<0.001**
	TIME×TREAT	-0.02	0.01	-2.15	**0.032**
Binomial process					
T. lemmonii	TREAT	-5.26	0.69	-7.65	**<0.001**
A. rusticana	TREAT	5.12	1.33	3.86	**<0.001**
	POSITION	3.90	1.48	2.63	**0.008**
C. nardus	TREAT	-5.51	3.50	-1.57	0.116
	TIME	-0.11	0.07	-1.67	0.100
	POSITION	3.38	1.94	1.74	0.082
C. citratus	TREAT	2.84	228.28	0.01	0.990
	TIME	-2.23	23.44	-0.10	0.924
V. zizanioides	TREAT	19.90	105.40	0.19	0.850
	TIME	0.08	0.029	2.71	**0.007**
	POSITION	2.42	1.98	1.23	0.220
	TREAT×TIME	-1.28	9.58	-0.13	0.893

Significant test results are in boldface

Figures

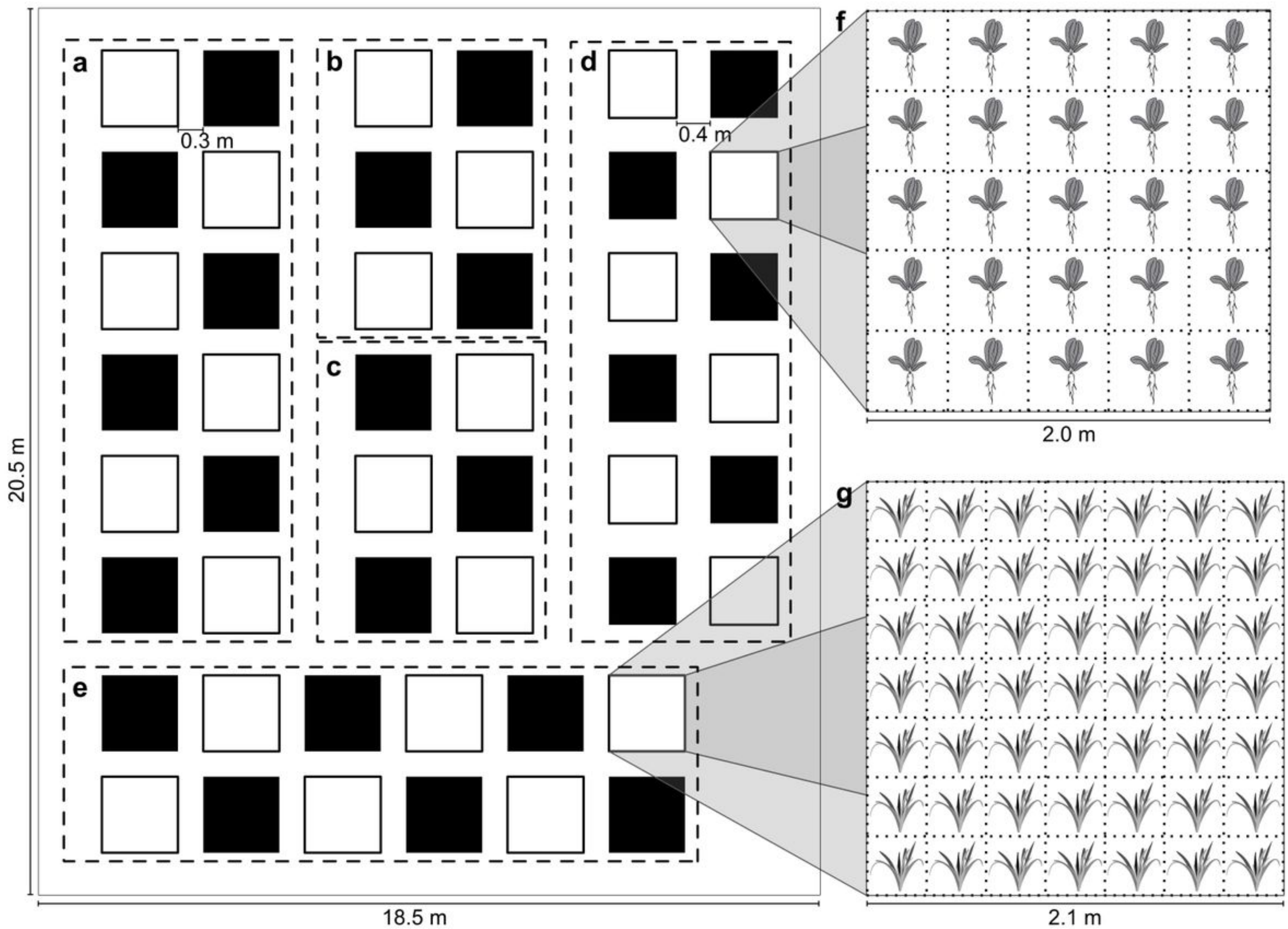

Figure 1

Experimental design, showing plots for Tagetes lemmonii (a), Cymbopogon nardus (b), C. citratus (c), Armoracia rusticana (d), and Chrysopogon zizanioides (e). A. rusticana was planted in 5 × 5 matrices (f), while the other species were planted in 7 × 7 matrices (g). Empty squares indicate subunits for planting herbal species and filled squares for control plants

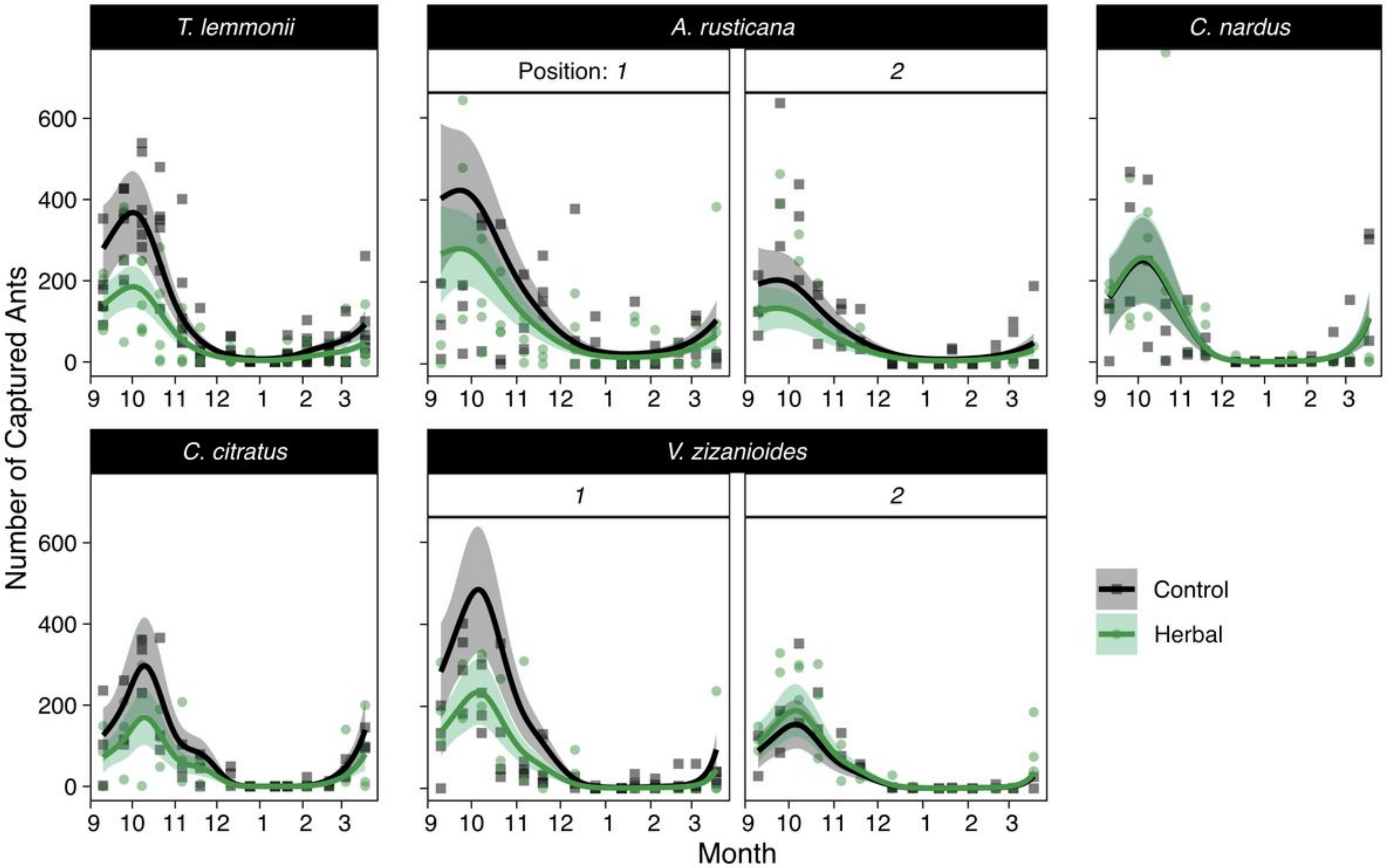

Figure 2

Generalized additive mixed model estimations of bait trap data from the studied field. One extreme value larger than 800 was not drawn. The color envelopes indicate the standard error of estimates (solid lines). Datapoints with different position codes were plotted separately if the edge effect (POSITION) was included in the models (Table S2)

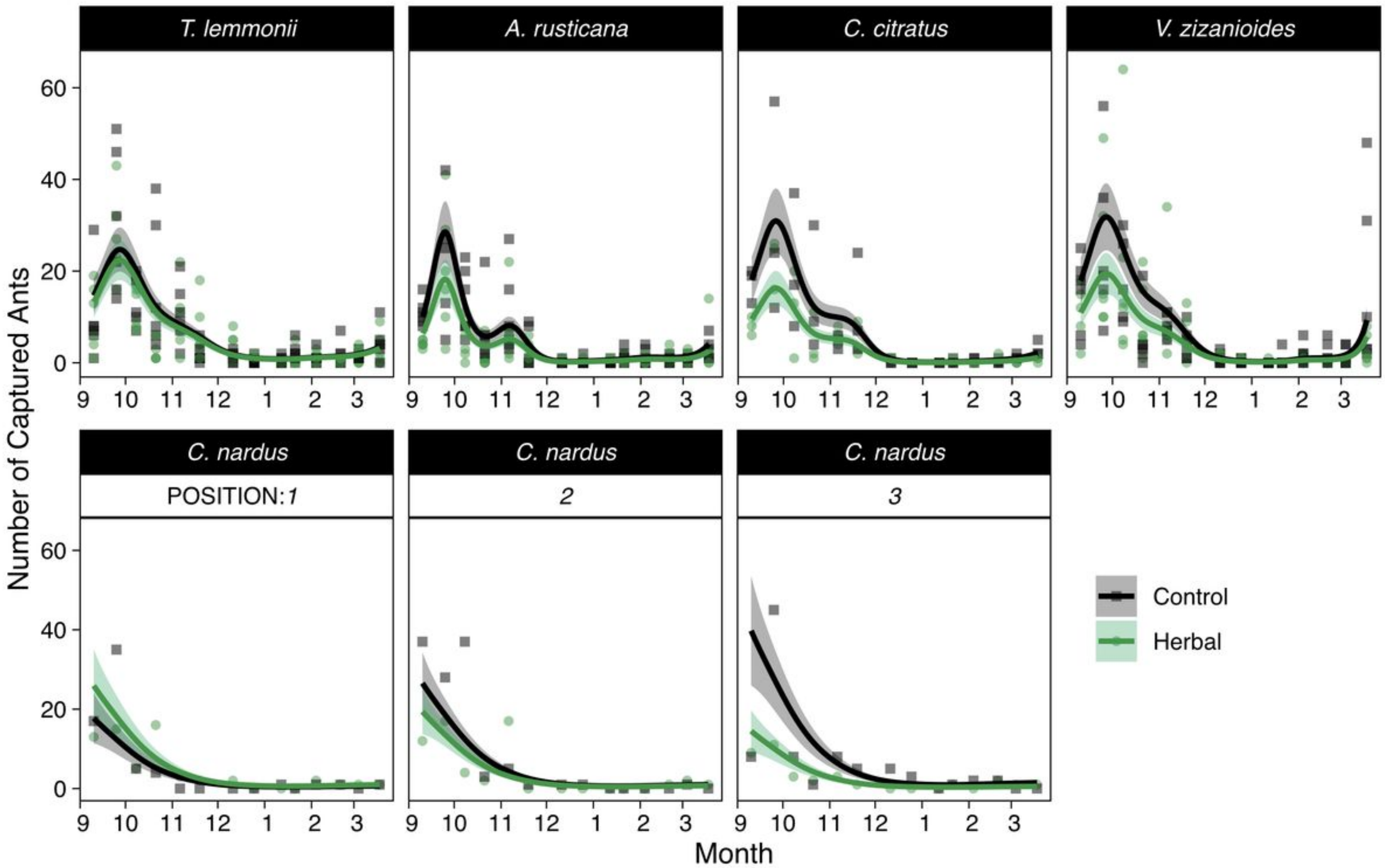

Figure 3

Generalized additive mixed model estimations of pitfall trap data from the studied field. Three extreme values larger than 80 were not drawn. The color envelopes indicate the standard error of estimates (solid lines). Datapoints with different position codes were plotted separately if the edge effect (POSITION) was included in the models (Table S2)

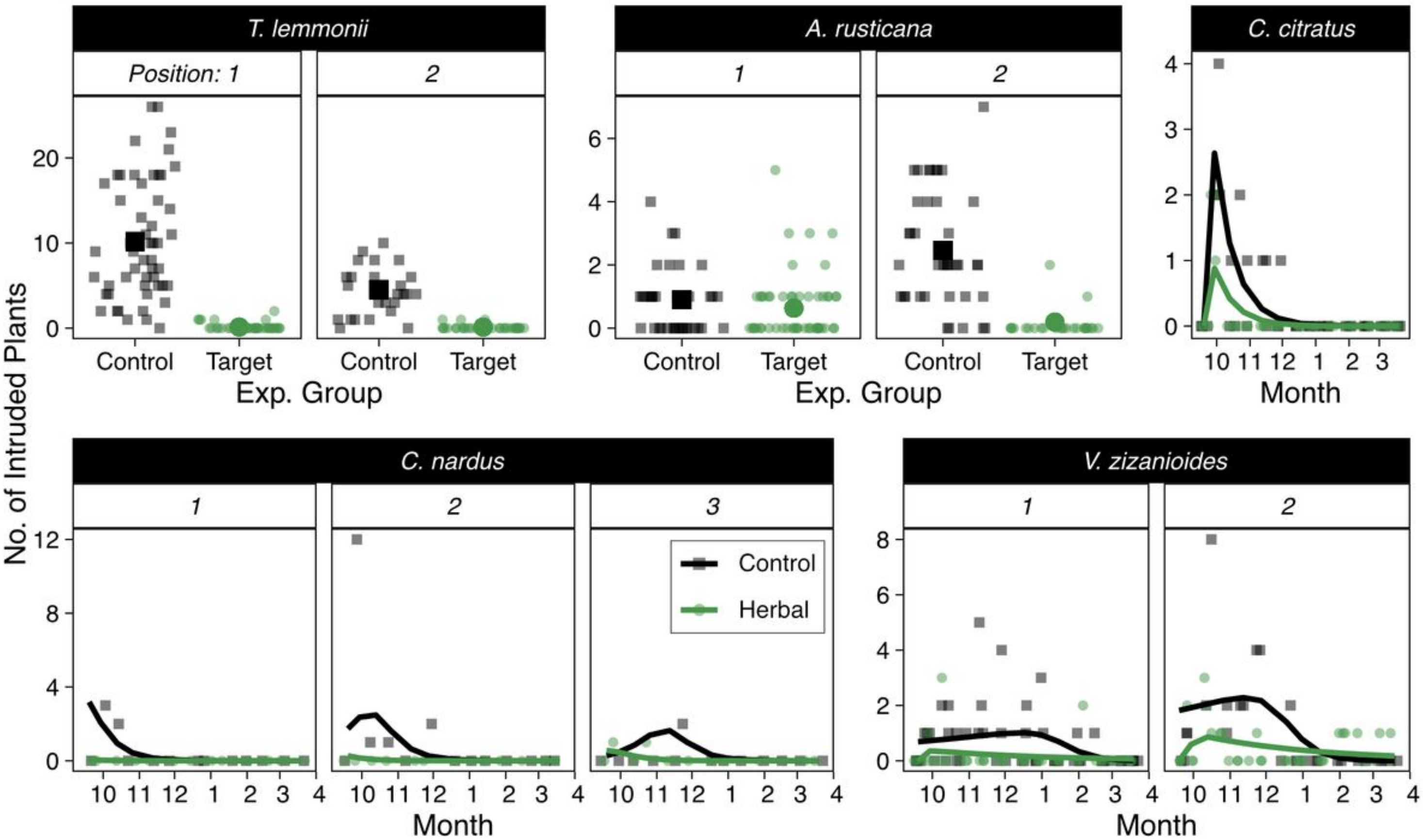

Figure 4

Zero-augmented model estimation of the number of intruded plant in the studied field. The estimated number of intruded plants (large points and solid lines) tends to be higher in the control than in the herbal experimental groups. The number of intruded plants was plotted against observation dates if temporal variation (TIME) was included in the model. Datapoints with different position codes were plotted separately if the edge effect (POSITION) was included. The selected model components are listed in Table S3. Data points (small points) are horizontally jittered to avoid overlapping

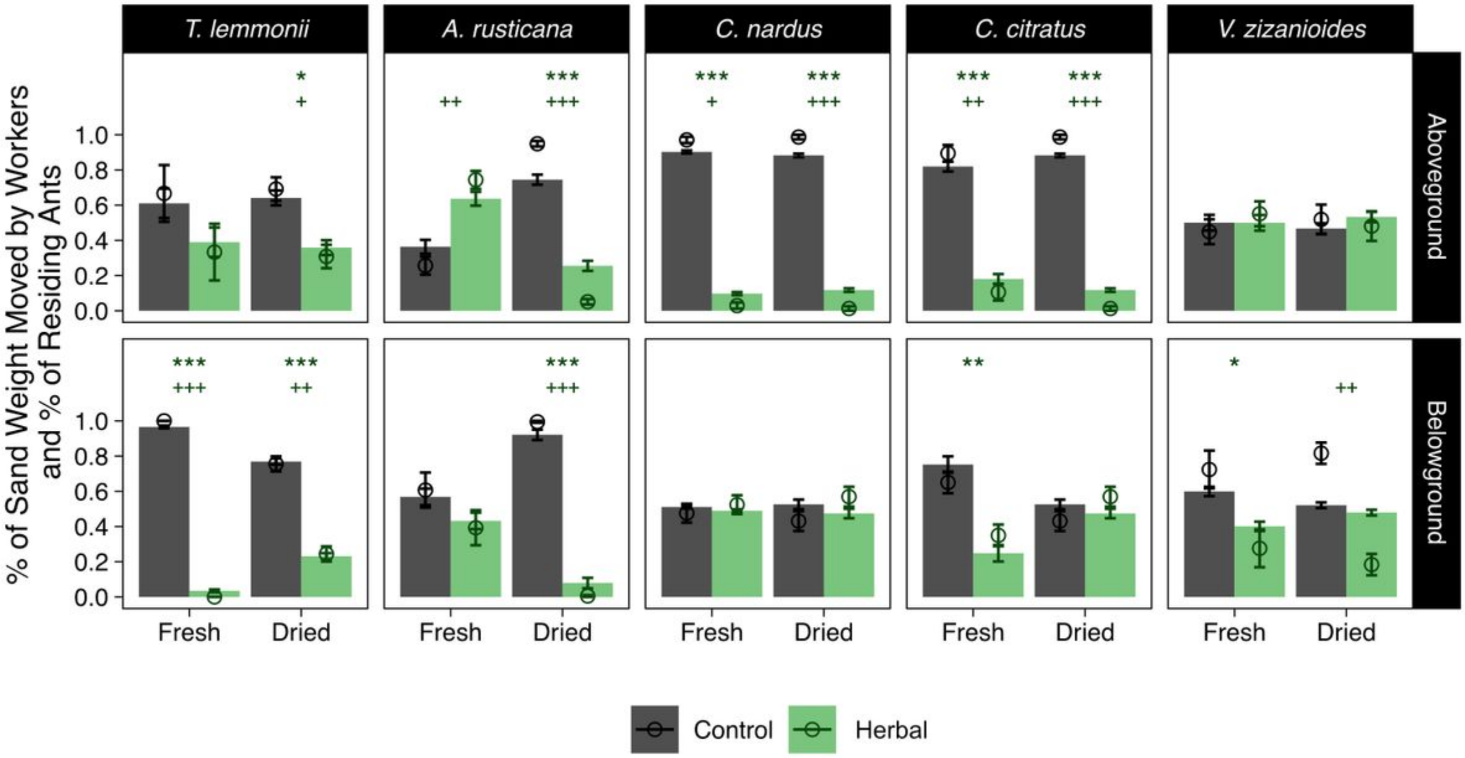

Figure 5

Repellence of tissue-based plant crude extracts in terms of sand weight moved by workers (columns) and residing ants (circles) tested with the two-choice digging bioassay * Significance for sand weight + Significance for the number of residing ants Significant code: *, P < 0.05; **, P < 0.01; *** P < 0.001

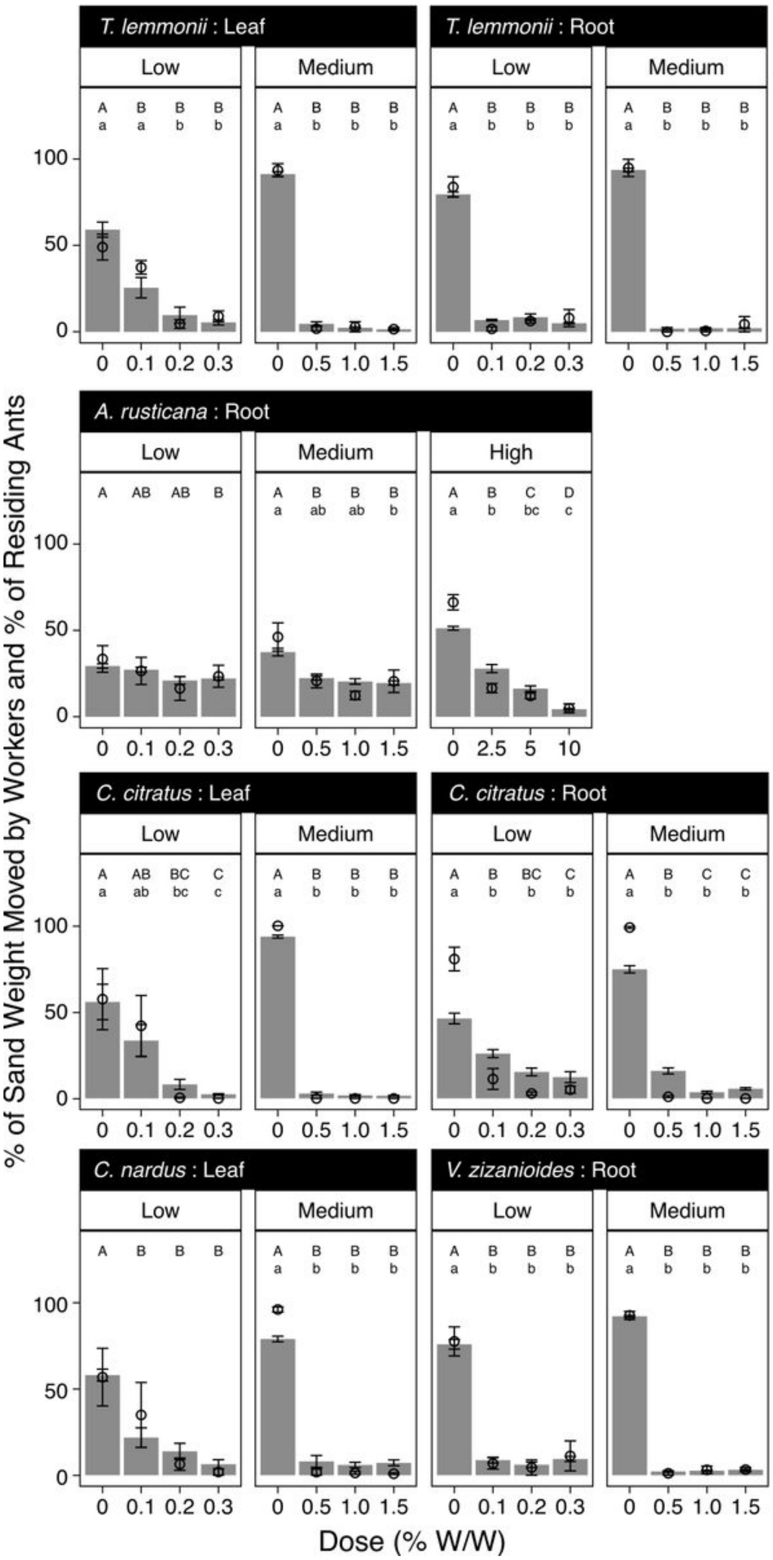

Figure 6

Sand weight moved by workers (columns) and residing ants (circles) of the multiple-choice digging bioassay. Bars represent standard errors. Columns or circles that shared the same letter are not significantly different from each other (P > 0.05, Tukey’s HSD tests). Uppercase letters indicate grouping for sand weight and lowercase ones for residing ants. The multiple comparisons were performed when the null hypothesis of ANOVA was rejected at a 95% confidence level (see Table S5)

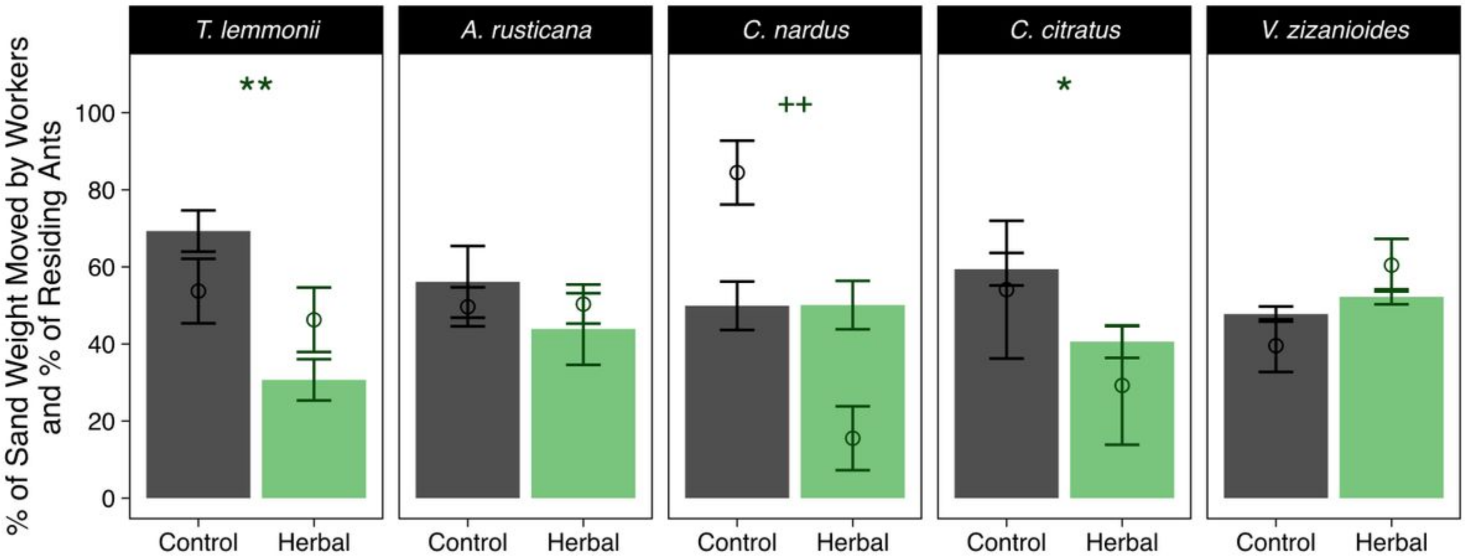

Figure 7

Repellence of the soil collected from the experimental plots regarding sand weight moved by workers (columns) and residing ants (circles). Bars represent standard errors * Significance for sand weight + Significance for the number of residing ants Significant code: *, P < 0.05; **, P < 0.01; *** P < 0.001

Supplementary Files

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- 4.Suppl.pdf