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Contact toxicity and proximate effect of fipronil on insect pest and predatory ant community structure in cocoa agro-ecosystem

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ABSTRACT

Although usage persists in some countries, fipronil is banned or restricted in many others. Prior to its ban on cocoa in Ghana, concerns about its effect on non-target insects and secondary outbreak of *Anomis leona* were conflicting. This study, which predates the ban, assessed the toxicity and the short-term effect of fipronil on specific insect community structure in the cocoa agro-ecosystem alongside bifenthrin and a non-insecticide control. Although the insecticides induced a high mortality (90-100%) on the target (mirid: *Sahlbergella singularis*, stink bug: *Bathycoelia thalassina* and coreid bug: *Pseudotheraptus devastans*) and non-target (ants: *Oecophylla longinoda*, *Crematogaster africana*, *Pheidole megacephala* and *Camponotus consobrinus*) insects, the knockdown to fipronil was very low compared to bifenthrin. On the field, fipronil was more detrimental to the ants. Insecticide-treated plots recorded relatively lower post-treatment pest diversity compared to the control, except the last sampling month while ant abundance, richness and diversity were lowest on the fipronil-treated plots at the end of the study period. This study demonstrates that although fipronil was effective against pests and did not result in acute secondary pest outbreak, it was harmful to the ants. This effect could potentially be replicated on these ant species in other cropping systems where the insecticide is used, adversely affecting ecosystem service delivery. Hence, research on its impact on non-target organisms in other cropping systems is needed to regulate and monitor its use.

1. Introduction

Cocoa (*Theobroma cacao*) is a major driver of the Ghanaian economy, generating foreign exchange for the country and providing employment for several households [1]. The cocoa tree is host to numerous arthropods with diverse functional roles that impact productivity [2]. The functional groups include pests, natural enemies and pollinators. The major pests in cocoa cultivation in Ghana include mirids, coreid bugs, stink bugs and mealybugs [3]. These pests are suckers of sap from various parts of the cocoa plant. Their feeding points may also be exploited by opportunistic pathogens to cause further damage. The mealybugs are vectors of the cocoa swollen shoot virus (CSSV), which is currently managed by cutting down diseased cocoa trees. Activities of the different pests account for varying yield losses (about 40% in some instances) [4] and are major limiters of production.

The main insect pest management strategies employed in the cocoa agro-ecosystem in Ghana include cultural and chemical practices with a greater leaning towards chemical use via synthetic insecticide application [5], particularly in the absence of pest-resistant cocoa varieties. However, insecticide usage subjects non-target arthropods including natural enemies to insecticide stress, potentially leading to a reduction in abundance, diversity and behaviour. Generally, synthetic insecticide usage is an occupational hazard [6] and poses environmental and dietary risks [7,8] necessitating environmentally benign and selective compounds for pest control [9–11].

Chemical products for insect pest management in Ghana are dominated by synthetic insecticides [12] with potentially adverse consequences for agro-ecosystems if they are not selective. Sustained synthetic insecticide application may also alter the agro-ecology, thereby affecting insect community structure, diversity and functioning. Due to their effectiveness as bio-indicators, insect diversity in insecticide-altered and other anthropogenically-modified ecosystems can be a measure of environmental health [13–15].

A suite of insecticides is available for use on cocoa in Ghana [3].

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However, non-compliance to insecticide application regimen, as well as risky application and handling practices, are common despite efforts by Ghana Cocoa Board (COCOBOD) and other stakeholders to reduce these aberrant practices which impact insecticide efficacy, residue risk and non-target organism toxicity [6,16,17].

Fipronil is an active ingredient for pest management on diverse crops in Ghana [3,12,18]. However, the foliar application of fipronil on cocoa was disallowed by COCOBOD after 2018, although usage on crops which are outside COCOBOD's mandate and for termite control persists. Before its ban, there were concerns about its efficacy on mirids, a key pest of cocoa, as well as contrasting reports about its acute effect on secondary outbreak of a defoliator and pod feeder, *Anomis leona*, on cocoa [19]. While an increase in *A. leona* larvae was perceived to be triggered by a single fipronil application, others attributed it to a repeat application. *Anomis leona* is an occasional pest [3] that is abundant in December and January in Ghana and March to June in Western Nigeria [2]. It is also present from February to May [2] with a previous outbreak in Ghana reported in February, March and April [20]. Although farmers are aware of the recommended insecticide application regime in cocoa, their knowledge and practice are discordant [21]. Cocoa farmers' insecticide application is mostly influenced by pest presence and damage, however, their knowledge on the identification of the major insect pests and their associated damage is limited [17,22].

Known to be disservice providers in the cocoa ecosystem due to their activity in relation to the vectors of CSSV, ants are also important service providers such as ecosystem engineers and biological control agents of pests [2,13,23,24]. They provide similar ecosystem benefits in other tree cropping systems in the tropics [25–28]. Insecticide usage could cause an ecological imbalance by being lethal to the natural enemies of pests, resulting in pest outbreaks. Hence, ensuring that insecticides are less toxic to non-target insects is of major concern. Coupling this with the contrasting reports of fipronil efficacy and the perception that *A. leona* outbreak was outcomed by its application, it was necessary to ascertain these claims. To address these gaps and provide a better understanding of the proximate effect of the insecticide on insect fauna, this study assessed the effect of fipronil on target (pest) and non-target (specifically predatory ants) insects and its impact on *A. leona* abundance in comparison with a historically-used insecticide, bifenthrin, in the Ghanaian cocoa landscape. Since it is no longer permissible for use on cocoa, the findings of this study will also be of benefit to cropping systems that use fipronil (including termiticidal use that may endanger edaphic ants) and have similar ant community structure due to the ubiquity of the ant species.

2. Materials and methods

2.1. Insects

Field-collected insects from research plots at the Tafo station of the Cocoa Research Institute of Ghana (CRIG) were used for the laboratory studies. This consisted of 3 cocoa insect pests namely mirid (*Sahlbergella singularis*), stink bug (*Bathycoelia thalassina*) and coreid bug (*Pseudotheraptus devastans*) and 4 predatory ants, thus the Weaver ant (*Oecophylla longinoda*), Saint Valentine ant (*Crematogaster africana*), Carpenter ant (*Camponotus consobrinus)* and the Big-headed ant (*Pheidole megacephala*). These are among the most dominant ant species in the cocoa ecosystem [2,3,23].

2.2. Treatments

Two fipronil-based insecticides (200 g/L for foliar application against mirids and other insect pests at 15 mL per 11 L of water [0.0273% a.i.] and 50 g/L for soil drenching against termites at 100 mL per 15 L of water [0.0333% a.i.]) and bifenthrin (27 g/L) with a field application rate of 100 mL per 11 L of water [0.0245% a.i.] were used. Bifenthrin served as the reference (positive control) while water (distilled and tap for laboratory and field work, respectively) served as the control (negative control).

2.3. Laboratory bioassays

Ten adult mirids or worker ants were placed on an insecticide-impregnated filter paper used to line the internal base of a petri dish (Supplementary Material 1a) as described in Ackonor and Adu-Acheampong [29]. The dish was closed and insect knockdown was monitored for 1 h at 1, 5, 10, 20, 30, 40, 50 and 60 min while mortality was observed at 24 h post exposure. Distilled water-impregnated filter paper was used as the control. Each set-up was done concurrently for the insecticides and the control for each insect and 6 replicates (batches of 3 replicates at a time) were done in a completely randomized design.

The bioassay for stink bug and coreid bug was similar to mirid with the only modification being the exposure chamber; an opened-ended lantern glass placed inside a petri dish lined with a filter paper and covered at the top with one half of the petri dish (Supplementary Material 1b). The insects were placed on a cocoa pod inside the lantern and the insecticide or distilled water was directly applied on them using a hand-held sprayer. Knockdown and mortality were assessed as indicated above.

2.4. Experimental area and design

This was conducted on an experimental field (Supplementary Material 2) at Tafo, CRIG, from December 2018 to April 2019. Farmers' recall of the month of insecticide application that resulted in *A. leona* larval outbreak varied widely, hence the study period reflected the months of abundance and outbreak in some parts of Ghana [2,20] and months with high farmer-supplemented insecticide applications [22]. The study site contained mixed hybrid cocoa trees aged 9 years with a 3 × 3 m planting distance. The last insecticide (bifenthrin 30 g/L) application at the experimental site was 2 months, 2 weeks and 3 days prior to the study. The area lies within the moist semi-deciduous forest zone of Ghana and the soil belongs to the ferric lixisol group.

Three treatments (T1: 200 g/L fipronil, T2: 27 g/L bifenthrin and T3: tap water) in a randomized complete block design with four replicates were used. Each treatment plot was 1 acre; hence a total of 12 acres for the treatments, with an inter-plot buffer of 20 m. The recommended insecticide application rate was 15 mL (fipronil) and 100 mL (bifenthrin) with a water delivery rate of 11 L per 0.5 acres. The treatments were applied at monthly intervals for 4 months using a motorized knapsack with the restrictor knob on the 2nd position.

2.5. Insect count and damage assessment

One hundred cocoa trees were randomly sampled in each treatment plot. Insect assessment on each tree was done using the hand–height method of Collingwood [30] by inspecting insect-inhabiting sites including pods, trunk, pod-trunk interface, branches and underside of pods within a height of 2 m above ground level for insects. The trees were also examined visually within the sampling height for insect damage on the pods, trunk and branches [4,19]. Insect count and damage assessment were done before treatment and on 7, 14, 21 and 28 days after treatment. Weather data of the experimental area was obtained from the weather station of the Institute.

2.6. Data analysis

Mortality data was angular transformed and subjected to analysis of variance (ANOVA) to observe if differences in mortality existed. Data on insect count and insect-damaged trees was logarithm- and square root-transformed, respectively before ANOVA. Whenever differences were significant at $p<0.05$, mean separation was done with Tukey HSD test.

Insect counts for each treatment for each month were pooled and

used to compute species abundance, richness and diversity indices such as Shannon-Weiner diversity index (H'), Shannon-Weiner evenness index (Pielou J) and Simpson index D using Genstat ver.9. Shannon-Weiner index was computed using $H' = -\sum_i (n_i/N) \times \log(n_i/N)$ where n_i is the number of individuals in the ith species and N is the total number of individuals, Pielou J was computed by $J' = H'/\log(S)$, where H' is the Shannon index and S is the total number of species. Simpsons index D was calculated by $D = \sum_i \{n_i \times (n_i - 1)\}/(N \times (N - 1))$ and expressed in the output as 1-D.

3. Results

3.1. Knockdown and mortality effect

Within the 60 min period, insect knockdown due to fipronil was very low (0-5%). Bifenthrin however knocked down 100% of the insects (Table 1). The insecticides were acutely toxic to all the insect species tested. The mortality responses to the insecticides were not significantly different for each insect species (Table 1). There was no effect (knockdown or mortality) on insects in the control treatments.

3.2. Field efficacy of insecticides

Abiotic conditions of the experimental area during the study period ranged from a temperature of 22.4 to 34.8 °C, relative humidity of 53.0 to 79.5 %, rainfall level of 0 to 55 mm and total sunshine duration of 177.0 to 217.7 h (Supplementary Material 3). Mirid, stink bug and coreid bug numbers were quickly suppressed by the insecticides (Fig. 1) to very low levels compared to the control. *Anomis leona* abundance was also very low, although there was a sudden increase at 28 days after the 2nd application of bifenthrin. However, this reduced steeply at 7 days after the next application of bifenthrin.

Comparing the different sampling points within the study period, insect pest abundance, especially mirids, was relatively high before treatment. This however reduced after the first insecticide application (Fig. 1). In the control, the abundance of mirids peaked after 1 month.

Mean numbers for mirids, coreid bugs and *A. leona* for the sampling days were significantly different ($p<0.05$). Differences in mirids and *Anomis* numbers for the treatments were also significant ($p<0.05$). Mean mirid and stink bug numbers on fipronil plots were the lowest among the treatments. Coreid bugs were not observed on fipronil and bifenthrin plots.

Ant population reduced drastically after the 1st insecticide application. The population however began to recover afterwards on the bifenthrin-treated plots, although *O. longinoda* population remained below the pre-treatment level (Fig. 2). Population of *O. longinoda* increased at 7 days after the 1st application of the control treatment and although this decreased afterwards, it fluctuated throughout the study period.

Mean ant numbers for the 3 species were significantly different ($p<0.05$) for the sampling days and treatments. Abundance of *P. megacephala* between the fipronil and control plots was not significantly different ($p>0.05$). *Camponotus* sp abundance on the bifenthrin and control plots was also not significantly different. The abundance of *O. longinoda* on bifenthrin plots was not significantly different from the control plots; however, abundance between fipronil and the control was significantly different.

Pest damage was acutely reduced after insecticide application (Fig. 3). While insect damage remained very low or non-existent until the 3rd week after the 3rd application on the fipronil plots, higher insect damage was observed on the bifenthrin-treated plots on the 4th week after the 2nd application and the 1st week after the 3rd application. *Anomis leona* damage on the control plots was generally very low, however, peaks (higher than pre-treatment levels) of mirid and stink bug damage were observed from 7 to 14 days after the 2nd application and 7 days after the 3rd application, respectively.

Mean number of trees with mirid damage was significantly different ($p = 0.000$) for the different sampling days. Stink bug-damaged trees and *Anomis*-damaged trees were also significantly different ($p = 0.000$ for each) for the sampling days. However, the mean number of damaged trees for each pest species was not significantly different ($p>0.05$) among the treatments.

3.3. Species composition, abundance and diversity

Insect number totalling 4117 and comprising individually of 698 pests and 3419 ants were observed (Table 2). These were in the orders Hemiptera, Lepidoptera, Blattodea and Hymenoptera and consisted of mirid (*Sahlbergella singularis*), stink bug (*Bathycoelia thalassina*), mealybug (*Formicococcus njalensis*), coreid bug (*Pseudotheraptus devastans*), psyllid (*Mesohomotoma* sp), ants (*Pheidole megacephala*, *Camponotus* sp, *Oecophylla longinoda*), stem borer (*Eulophonotus* sp.), pod borer (*Characoma* sp), termite (*Macrotermes bellicosus*) and *Anomis leona* (Table 2, Supplementary Material 4).

The rank abundance curves depict the species richness and evenness of insects on the various treatment plots (Fig. 4). Species richness on the fipronil-assigned plots was highest during the pre-treatment assessment compared to the control- and bifenthrin-assigned plots. The richness on the fipronil plots however reduced after the 1st application but the evenness was higher compared to the other treatment plots. Species richness on the fipronil plot was similar to the control plot after the 2nd application but the evenness was higher than the control and bifenthrin plots. After the 3rd treatment, the control had the highest species richness but lower species evenness compared to the fipronil plots. At the end of the 4th application, all the treatments had the same species richness but dissimilar species evenness with the highest evenness on the fipronil plots.

Post first application assessment indicated an increase in insect numbers in the bifenthrin and control plots but a decrease in the fipronil plots (Table 3). Insect pest numbers on the insecticide-treated plots however decreased while ant numbers on all the plots except the fipronil plots increased. Except for insect diversity before treatment, the diversity on fipronil plots was higher than the other plots although the individual numbers were generally lower. Ant diversity was however

Table 1
Knockdown and mortality effect of fipronil and bifenthrin on insect pest and ant species.

Status	Insect species	Fipronil [0.0273% a.i.]		Fipronil [0.0333% a.i.]		Bifenthrin [0.0245% a.i.]	
		%KD	Mortality±SE (%)	%KD	Mortality±SE (%)	%KD [T_{100}]	Mortality±SE (%)
Target (Pests)	*Sahlbergella singularis*	5	100.0 ± 0.0	0	100.0 ± 0.0	100 [20 min]	100.0 ± 0.0
	Pseudotheraptus devastans	0	100.0 ± 0.0	0	90.0 ± 5.8	100 [30 min]	100.0 ± 0.0
	Bathycoelia thalassina	0	100.0 ± 0.0	0	100.0 ± 0.0	100 [60 min]	100.0 ± 0.0
Non-target (Ants)	*Oecophylla longinoda*	0	100.0 ± 0.0	0	100.0 ± 0.0	100 [10 min]	100.0 ± 0.0
	Crematogaster africana	0	100.0 ± 0.0	0	100.0 ± 0.0	100 [5 min]	100.0 ± 0.0
	Camponotus consobrinus	0	100.0 ± 0.0	0	100.0 ± 0.0	100 [10 min]	100.0 ± 0.0
	Pheidole megacephala	0	100.0 ± 0.0	0	100.0 ± 0.0	100 [10 min]	100.0 ± 0.0

%KD: % of insects knocked down after 60 min of exposure; T_{100}: Time to knock down 100% of exposed insects; SE: Standard error; a.i: Active ingredient. Knockdown and mortality in the control for each insect species were nil.

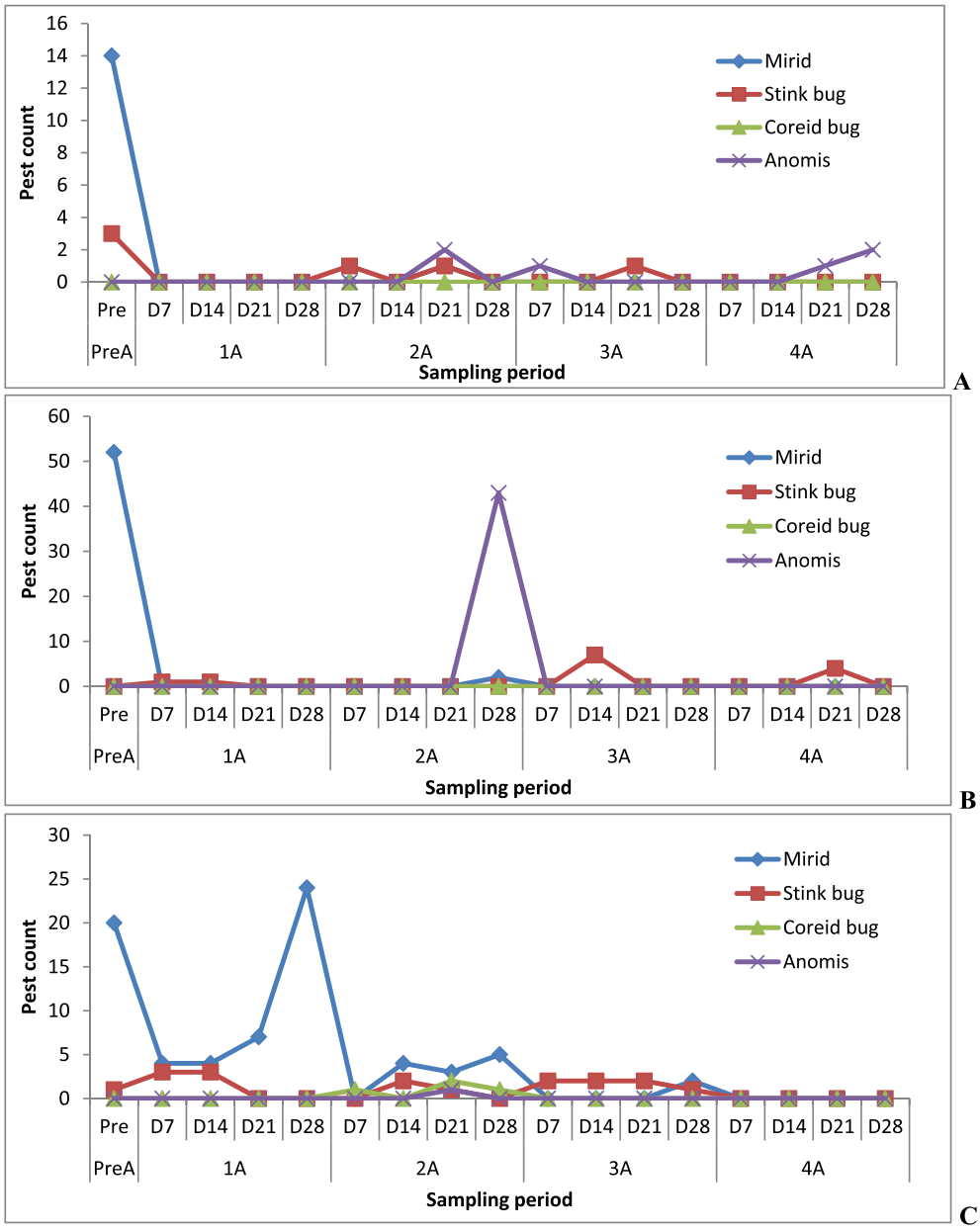

Fig. 1. Insect pest abundance before and after treatment. A: Fipronil; B: Bifenthrin; C: Control; PreA: Pre-treatment; 1A: 1st treatment; 2A: 2nd treatment; 3A: 3rd treatment; 4A: 4th treatment; D7: 7 days after treatment; D14: 14 days after treatment; D21: 21 days after treatment; D28: 28 days after treatment. Anomis: *Anomis leona*.

highest on the control plots at the end of the period while pest diversity was highest on the fipronil plots.

4. Discussion

In this study, fipronil did not induce any significant insect knockdown aligning with the observation of Halos et al. [31] on fleas. The low knockdown effect is advantageous to the insect since knockdown immobilizes it, disrupts its activity and predisposes it to other factors of mortality such as predation and desiccation. However, it implies an uninterrupted contact with fipronil, hence sustaining its uptake (more contact) by the insect. Knockdown effect by bifenthrin was more inimical to ants (total knockdown within 5 to 10 min) compared to the pests (total knockdown within 20 to 60 min). This could be due to an adaptation by the pests to the chemical since they are the target organisms and hence might have evolved resistance against a quick knockdown. The activity of bifenthrin-exposed ants such as foraging, predation and nest building will also be disrupted due to the high knockdown compared to fipronil-exposed ants.

The high mortality induced by the insecticides is similar to the findings on other insects [19,31] including ants [32–35] and portrays the lethality of the insecticides to both target and non-target insects at the recommended doses. Direct insecticidal effects such as mortality, knockdown and reduced fecundity could be amplified or compensated by their indirect effects in the environment. Insecticide application can also cause secondary poisoning of natural enemies and pathogens and diseases in pollinators [36,37]. The cumulative (direct and indirect) effects associated with insecticide use may alter insect community structure leading to pest resurgence and secondary pest outbreaks [37]. In our study, the insecticides reduced pest abundance on the field and except for a brief spike in *A. leona* abundance on bifenthrin-treated plots, its population was also suppressed. Although pest populations on the

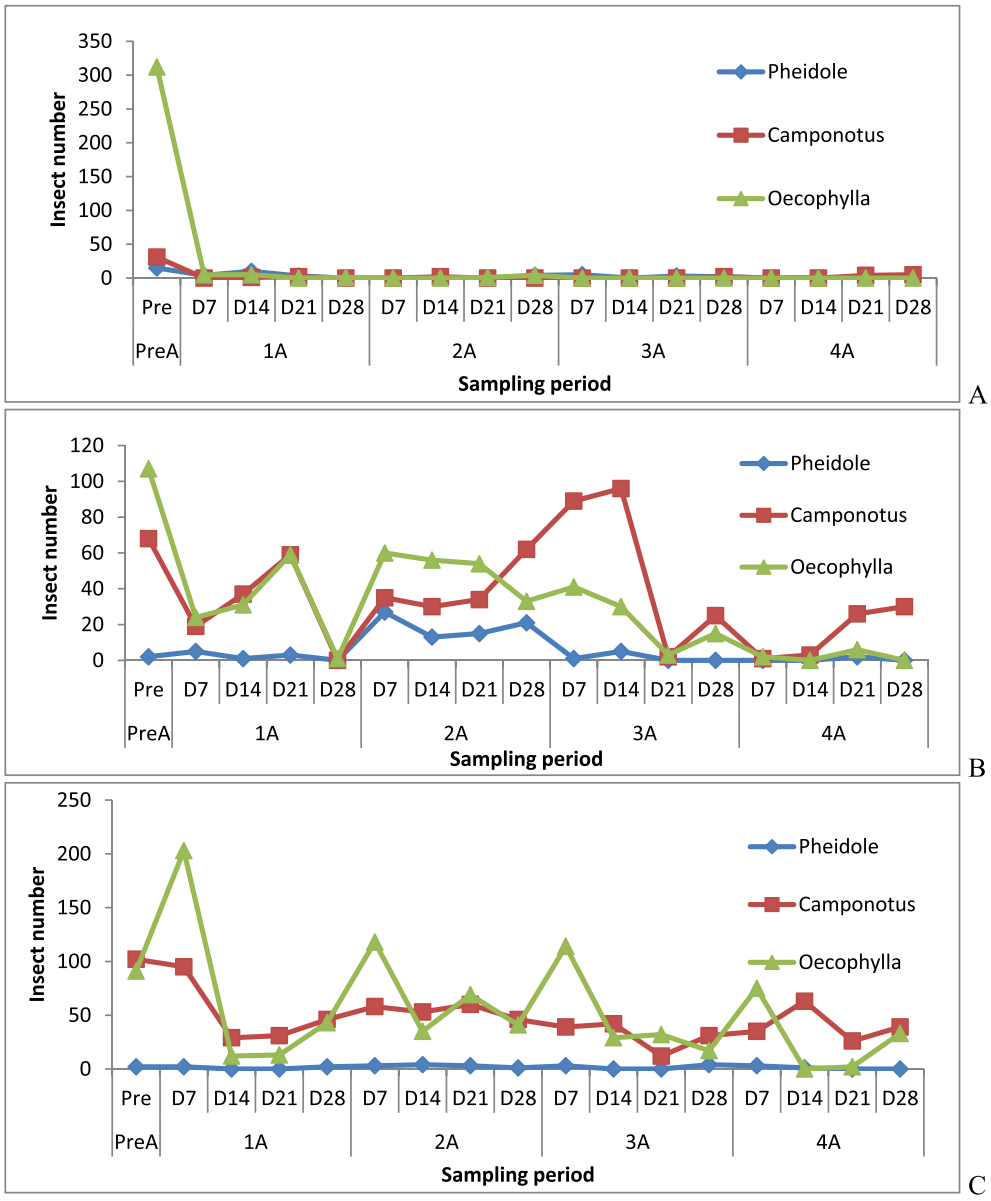

Fig. 2. Ant abundance before and after treatment. A: Fipronil; B: Bifenthrin; C: Water; PreA: Pre-treatment; 1A: 1st treatment; 2A: 2nd treatment; 3A: 3rd treatment; 4A: 4th treatment; D7: 7 days after treatment; D14: 14 days after treatment; D21: 21 days after treatment; D28: 28 days after treatment. Pheidole: *Pheidole megacephala*; Camponotus: *Camponotus* sp; Oecophylla: *Oecophylla longinoda*.

control plots were low, they were higher than the insecticide-treated plots. The residual effect of the insecticide applied prior to the study could have contributed to the low levels on the control plots. Nonetheless, the higher count compared to the insecticide-treated plots indicates the reductive effect of the insecticides on the pests.

Field effect of fipronil on ants mirrored its ex situ toxicity, drastically reducing the ant population after the 1st application and with no sign of recovery after a month. Although *Camponotus* sp and *O. longinoda* were quickly suppressed after the 1st application of bifenthrin, their population rebounded with a fluctuating abundance. This indicates that although bifenthrin acutely suppressed ant populations, the populations recovered and in the case of *Camponotus* sp, reached pre-treatment levels unlike fipronil.

The foraging activity of ants increases their chances of contact with toxicants such as insecticides which could be dispersed in the ant community via horizontal transfer [35]. A study involving fipronil, bifenthrin and other insecticides revealed that fipronil was the only and most effectively transferred insecticide from exposed to non-exposed ants [33]. The translocated insecticide induced secondary mortality and this was attributed to several factors including necrophoresis. Other studies also indicate that secondary and tertiary mortality of ants due to horizontal transfer could be due to direct contact, mutual grooming, trophyllaxis and necrophoresis [35,38]. These factors could have amplified the insecticidal effect of fipronil on the ants in our study, effectively preventing a population recovery on the field. According to Buczkowski et al. [36], fipronil-baited prey (termite) caused an estimated >98% reduction in Argentine ant (*Linepithema humile*) density within 21 days.

Prolonged exposure of non-target ant communities to fipronil resulted in little population recovery [38] as observed in our study. Translocation of fipronil is boosted by its non-repellent nature, consumption by insects and toxicity at the consumed amount which are attributes of an effective toxic bait [32,39] and ensures its effective transfer within the population to adversely affect its recovery [32,40]. Like fipronil, bifenthrin can also adversely affect non-target organisms either as a sole active ingredient or in a binary mixture [41,42].

However, unlike bifenthrin, the effect of fipronil on ant abundance

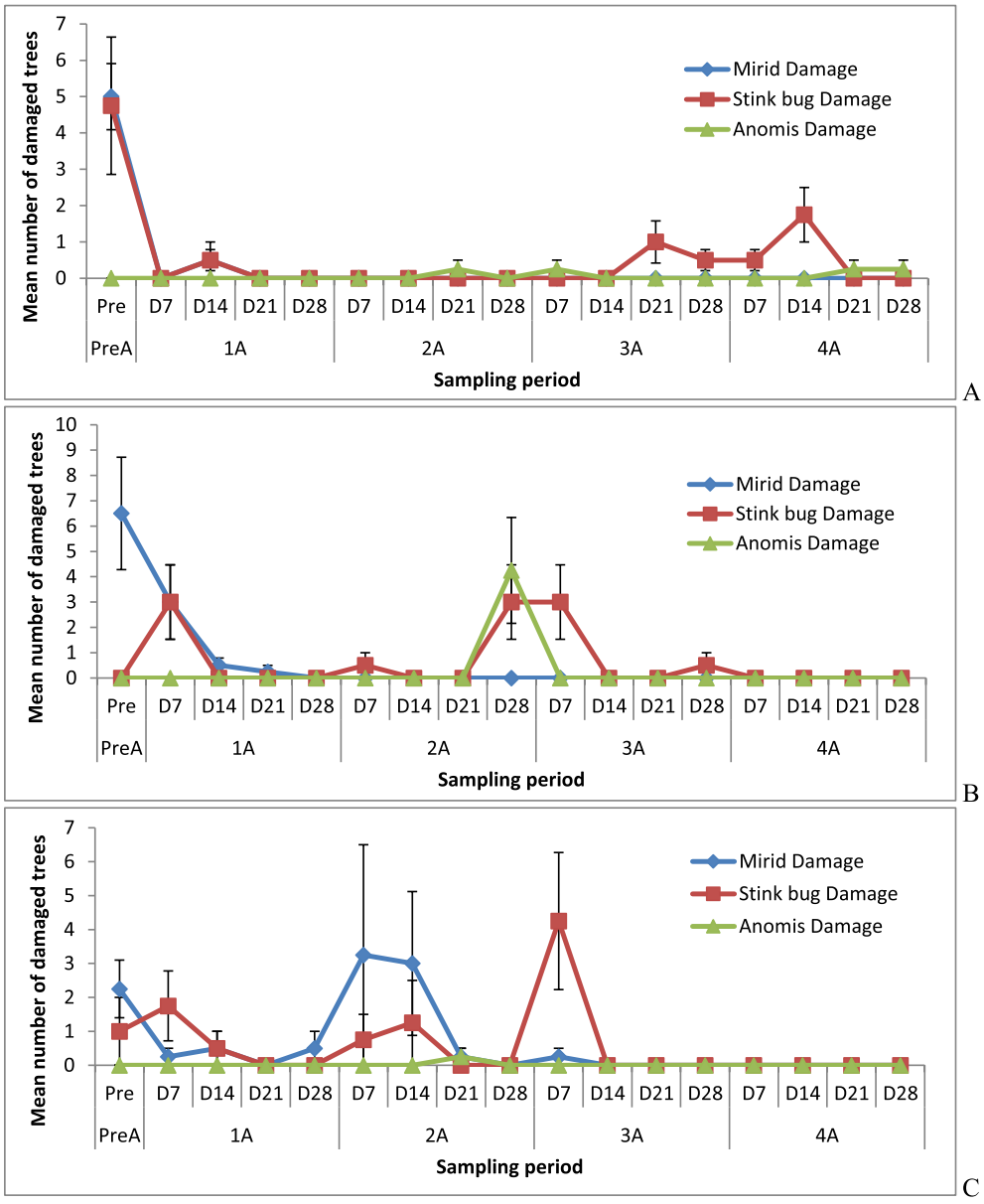

Fig. 3. Number of trees (mean ± SE) damaged by insect pests per 100 trees per acre. A: Fipronil treated plots; B: Bifenthrin-treated plots; C: Control (water)-treated plots; PreA: Pre-treatment; 1A: 1st treatment; 2A: 2nd treatment; 3A: 3rd treatment; 4A: 4th treatment; D7: 7 days after treatment; D14: 14 days after treatment; D21: 21 days after treatment; D28: 28 days after treatment.

Table 2
Insect orders and abundance.

Order	Treatment		
	Control	Bifenthrin	Fipronil
	n (%)	n (%)	n (%)
Blattodea	4 (0.2%)	0 (0%)	5 (0.9%)
Hemiptera	186 (9.4%)	284 (17.8%)	56 (10.3%)
Hymenoptera	1762 (89.1%)	1243 (77.8%)	424 (78.1%)
Lepidoptera	25 (1.3%)	80 (5%)	58 (10.7%)
Total	1977	1597	543

n: Insect count.

was profound, severely reducing *O. longinoda* abundance. This implies an impairment of ant-mediated ecosystem services with continuous use of fipronil, particularly *O. longinoda* which was the most sensitive. *Oecophylla longinoda* is a generalist that preys on several insect species and has been shown to reduce damage due to mirids and coreid bugs in cocoa [23] and cashew [26] and fruit fly in citrus [27] and mango [25]. Repeated or long-term use of fipronil could jeopardise this ecosystem benefit.

In Gupta et al. [43], fipronil and bifenthrin recorded lower damage of the fruit and shoot borer *Earias vittella* on okra compared to indoxacarb and the water control, and the least damage was recorded when fipronil was used. This concurs with our study where both insecticides reduced mirid, stink bug and *Anomis* damage with the least damage on the fipronil plot. Even though damage on the control plots was higher than the other treatment plots, this was still low indicating that adherence to cultural practices on a regular basis can aid in suppressing pest populations. This could also be due to a combination of cultural practices and the prior insecticide application.

While pest diversity 1 month after insecticide application reduced on fipronil plots, this increased on the bifenthrin and control plots, although pest abundance and richness increased on only the control plots and the bifenthrin and control plots, respectively. This corresponds

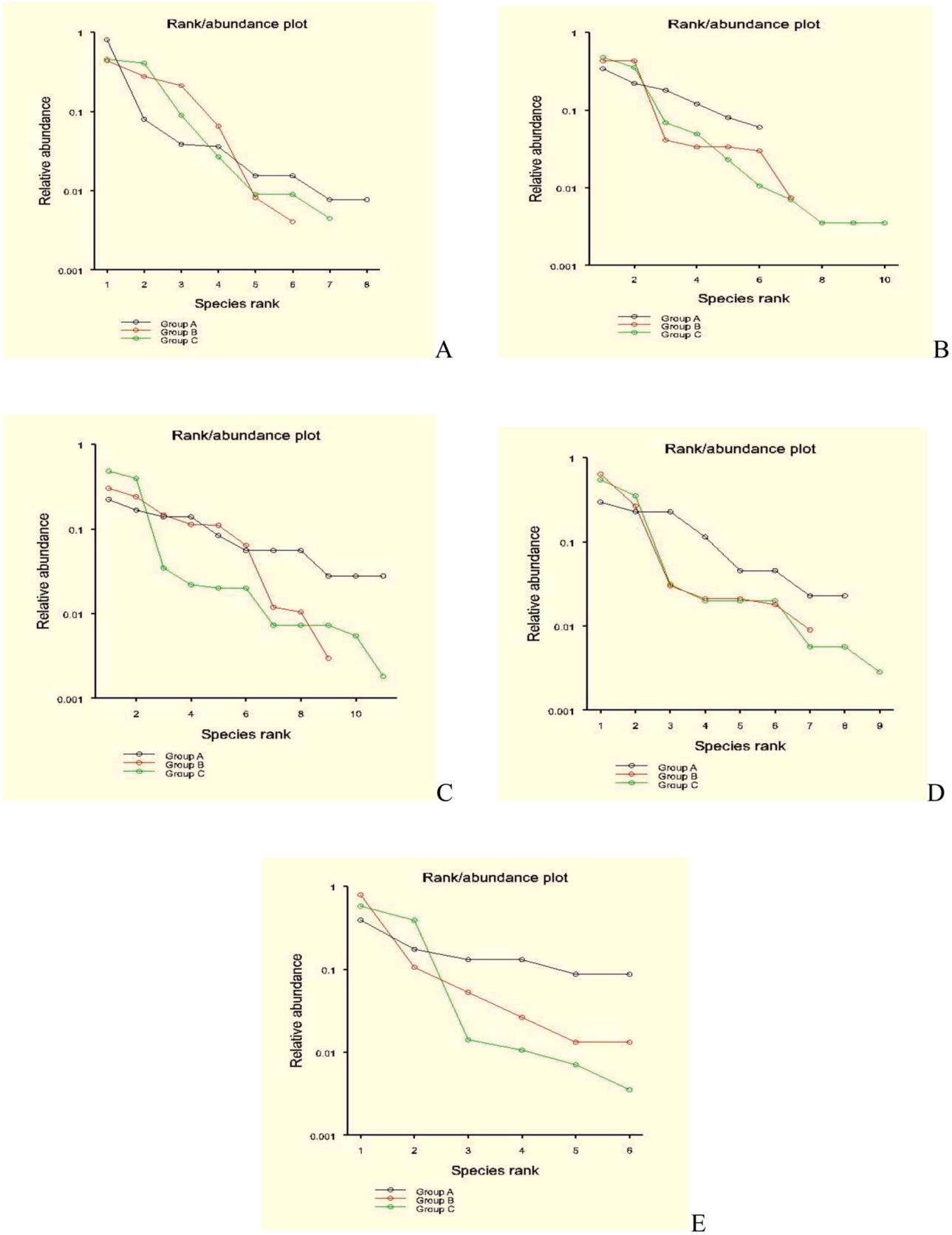

Fig. 4. Species abundance plots A: Pre-treatment B: 1st month post-treatment C: 2nd month post-treatment D: 3rd month post-treatment E: 4th month post-treatment. Group A: Fipronil; Group B: Bifenthrin; Group C: Control.

Table 3
Diversity indices of insect pests and ants in different treatment plots.

Organism	DI	Pre-treatment			Post-treatment											
					Month 1			Month 2			Month 3			Month 4		
		Ctrl	Ref	Reg	Ctrl	Ref	Reg	Ctrl	Ref	Reg	Ctrl	Ref	Reg	Ctrl	Ref	Reg
Pests	H	0.883	0.613	1.433	1.430	1.262	1.046	1.783	1.304	1.784	1.521	1.312	1.409	1.011	0.868	1.358
	J	0.637	0.558	0.891	0.735	0.910	0.952	0.858	0.728	0.858	0.849	0.946	0.787	1.011	0.790	0.980
	D	0.493	0.384	0.744	0.710	0.724	0.667	0.811	0.686	0.826	0.772	0.744	0.730	0.733	0.600	0.803
	S	4	3	5	7	4	3	8	6	8	6	4	6	3	3	4
	N	29	69	32	92	30	19	58	232	24	30	27	32	6	6	12
Ants	H	0.742	0.722	0.465	0.725	0.827	0.923	0.780	1.028	1.028	0.760	0.692	0.4506	0.740	0.482	0.4741
	J	0.675	0.658	0.423	0.660	0.753	0.840	0.710	0.936	0.936	0.692	0.629	0.6500	0.674	0.438	0.6840
	D	0.511	0.490	0.232	0.499	0.538	0.583	0.518	0.625	0.682	0.500	0.440	0.3030	0.498	0.255	0.3273
	S	3	3	3	3	3	3	3	3	3	3	3	2	3	3	2
	N	195	177	358	476	239	31	491	440	12	323	307	12	277	70	11
Insects	H	1.145	1.285	0.828	1.282	1.226	1.634	1.224	1.768	2.169	1.115	1.023	1.734	0.848	0.788	1.627
	J	0.589	0.717	0.398	0.557	0.630	0.912	0.510	0.804	0.904	0.508	0.525	0.834	0.474	0.440	0.908
	D	0.622	0.688	0.351	0.640	0.632	0.795	0.613	0.802	0.890	0.580	0.526	0.810	0.519	0.367	0.802
	S	7	6	8	10	7	6	11	9	11	9	7	8	6	6	6
	N	224	246	390	568	269	50	549	672	36	353	334	44	283	76	23

Ctrl: Control (water)-treated plots; Ref: Bifenthrin-treated plots; Reg: Fipronil treated plots; DI: Diversity Indices; H: Shannon-Weiner diversity index; J: Shannon-Weiner evenness index; D: Simpson 1-D index; S: Species richness; N: Number of individuals.

with the toxic effect of fipronil on the insects which transcends organismal toxicology and behavioural effects to population-level effects. Generally, pest diversity on the control and bifenthrin plots increased compared to their pre-treatment levels and although fipronil had the highest pest diversity at the end of the study period, this was below its pre-treatment level. An increase in post-treatment abundance of pests after the 2nd month could be due to a reduction in interspecies competition as a result of the insecticidal effect on the major insect pests giving rise to the increase in the other insect pests which were however also suppressed after the next insecticide application.

A pre and post-treatment comparison of all the plots indicates that ant diversity was highest on the control plots at the end of the study. Although ant abundance on the control plots was lower than the fipronil plots before treatment, at the end of the study, ant abundance on the control was the highest. Ant abundance on bifenthrin plots was also higher than that of fipronil-treated plots. Variations in pest and ant diversity, evenness, richness and abundance were observed during the study period. In the field, organisms are simultaneously exposed to multiple stressors including multiple pesticide and non-pesticide stressors which impact their development, survival, interaction and abundance. These effects could be disproportionate on different organisms. As stated in van der Sluijs et al. [44], the interactive and cumulative effect of these stressors could have a synergistic effect on non-target organisms including their susceptibility to viral diseases. Aufauvre et al. [45] also observed high mortality in bees exposed (either sequentially or simultaneously) to fipronil and *Nosema ceranae*, as a result of the synergistic lethal effect of both stressors. In our study, insecticide-treated plots in some sampling months had similar or higher pest abundance than the control. Such an observation has been reported by Bommarco et al. [46] who attributed this phenomenon to a combined effect of insecticide resistance in the pest and lower predation and parasitism from natural enemies of the pest that were adversely affected by insecticide applications. Advocating integrated pest management (IPM) as a sustainable approach in cropping systems is laudable, particularly when insecticide usage is judicious, selective (to ensure its compatibility with other management methods) and the last resort since it minimises insecticide use [47].

Species richness of all the treatments was the same at the end of the sampling period, although abundance (highest on control plots) and diversity (highest on fipronil plots) differed. The high diversity on the fipronil plots could be due to the high evenness although abundance was low. This however masks the reduced ant richness when fipronil was used compared to the other treatments. Although insecticide exposure decreases species richness and diversity, short-term insecticide exposure or intermediate disturbance may increase richness and diversity [48–50]. Further understanding of the impact of fipronil on species diversity could be enhanced by widening the temporal and spatial scale of this study in other cropping systems and incorporating diverse insect sampling techniques to provide a holistic view of the diversity. Nonetheless, this study is informative on the harmful effect of fipronil on predatory ants in the cocoa ecosystem, a likely effect in other cropping systems that use this insecticide and in cocoa cropping systems where fipronil usage persists.

5. Conclusion

This study indicates the broad-spectrum toxicity of fipronil and bifenthrin to three major insect pests (*Sahlbergella. singularis*, *Bathycoelia thalassina* and *Pseudotheraptus devastans*) and four predatory ant species (*Oecophylla longinoda*, *Crematogaster africana*, *Camponotus consobrinus* and *Pheidole megacephala*) of cocoa under laboratory conditions. Under short-term field use, the insecticides suppressed pest populations. Fipronil was however very detrimental to the ant populations with no population recovery after single and repeated applications. Insect diversity, richness and abundance on the various treated plots fluctuated during the study. However, at the end of the study, the highest pest and ant diversity occurred on the fipronil-treated and control plots, respectively. Although fipronil usage is no longer permitted on cocoa in Ghana, its utility in other agricultural production systems should be regulated and guided by its impact on non-target organisms such as natural enemies of the target pest(s) or vector(s).

Author contributions

Conceptualization: SWA, GKA, RA-A; Data curation: SWA, PB-D; Formal analysis: SWA; Investigation: SWA, GKA, PB-D; Methodology: SWA, GKA, RA-A, SA-A; Project administration: SWA, GKA; Resources: SWA, GKA, RA-A, SA-A; Supervision: SWA, GKA, RA-A; Validation: SWA, PB-D; Visualization: SWA, GKA, SA-A; Writing - original draft: SWA; Writing - review & editing: SWA, GKA, RA-A, SA-A. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is within manuscript and Supplementary file

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Appendix A. Supplementary data

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References

[1] Institute of Statistical, Social and Economic Research, The State of the Ghanaian Economy in 2020, Institute of Statistical Social and Economic Research, University of Ghana, Ghana, 2021.
[2] P.F. Entwistle, Pests of Cocoa, Longman Group Ltd, London, 1972.
[3] G.K. Awudzi, R. Adu-Acheampong, S.K. Ahadzi, S.W. Avicor, Field Guide for Cocoa Insect Pests Identification, Damage Symptoms and Management, Cocoa Research Institute of Ghana Technical Bulletin No. 28, Cocoa Research Institute of Ghana, New Tafo-Akim, Ghana, 2019.
[4] G.K. Awudzi, M. Asamoah, F. Owusu-Ansah, P. Hadley, P.E. Hatcher, A. J. Daymond, Knowledge and perception of Ghanaian cocoa farmers on mirid control and their willingness to use forecasting systems, Int. J. Trop. Insect Sci. 36 (2016) 22–31, https://doi.org/10.1017/S1742758415000247.
[5] R. Adu-Acheampong, J.E. Sarfo, E.F. Appiah, A. Nkansah, G. Awudzi, E. Obeng, P. Tagbor, R. Sem, Strategy for insect pest control in cocoa, Am. J. Exp. Agric. 6 (2015) 416–423, https://doi.org/10.9734/AJEA/2015/12956.
[6] B.C. Umeh, S.W. Avicor, E. Dankyi, R. Kyerematen, Farmers' knowledge and practices on pollination and insecticide use in cocoa farming in Ghana, Int. J. Agric. Sustain. 20 (2022) 1294–1306, https://doi.org/10.1080/14735903.2022.2106656.
[7] E. Dankyi, C. Gordon, D. Carboo, I.S. Fomsgaard, Quantification of neonicotinoid insecticide residues in soils from cocoa plantations using a QuEChERS extraction procedure and LC-MS/MS, Sci. Total Environ. 499 (2014) 276–283, https://doi.org/10.1016/j.scitotenv.2014.08.051.
[8] E. Dankyi, D. Carboo, C. Gordon, I.S. Fomsgaard, Application of the QuEChERS procedure and LC–MS/MS for the assessment of neonicotinoid insecticide residues in cocoa beans and shells, J. Food Compos. Anal. 44 (2015) 149–157, https://doi.org/10.1016/j.jfca.2015.09.002.
[9] P.O. Asekunowo, R.A. Haque, M.R. Razali, S.W. Avicor, M.F.F. Wajidi, Synthesis and characterization of nitrile functionalized silver(I)-N-heterocyclic carbene complexes: DNA binding, cleavage studies, antibacterial properties and mosquitocidal activity against the dengue vector, *Aedes albopictus*, Eur. J. Med. Chem. 150 (2018) 601–615, https://doi.org/10.1016/j.ejmech.2018.03.029.
[10] S.W. Avicor, M.F.F. Wajidi, E.S. Achoribo, M.T. Ong, S.N. Hamzah, Tiger nut (*Cyperus esculentus*) as a potential phytoinsecticide: larvicidal activity of crude extracts on *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae), Trop. Biomed. 38 (2021) 186–191, https://doi.org/10.47665/tb.38.2.056.
[11] Y. Bukari, S.W. Avicor, G. Awudzi, M.K. Ainooson, E.K. Asare, I. Amoako-Attah, In vitro activity of soybean extract on cocoa disease pathogens (Erythricium salmonicolor and Marasmiellus scandens) and termite pest (Microtermes subhyalinus), Cogent Food Agric. 8 (2022), 2147474, https://doi.org/10.1080/23311932.2022.2147474.
[12] Environmental Protection Agency-Ghana, Register of Pesticides, Chemicals Control and Management Centre, Environmental Protection Agency, Ghana, 2021.
[13] L. Diamé, J.-Y. Rey, J.-F. Vayssières, I. Grechi, A. Chailleux, K. Diarra, Ants: major functional elements in fruit agro-ecosystems and biological control agents, Sustainability 10 (2018) 23, https://doi.org/10.3390/su10010023.
[14] R. Kyerematen, S. Adu-Acheampong, D. Acquah-Lamptey, R.S. Anderson, Using Orthoptera and Hymenoptera indicator groups as evidence of degradation in a mining concession (Tarkwa gold mine) in Ghana, Int. J. Trop. Insect Sci. 40 (2020) 221–224, https://doi.org/10.1007/s42690-019-00053-2.
[15] G. Akesse-Ransford, E.O. Owusu, R. Kyerematen, S. Adu-Acheampong, Arthropod diversity of cocoa farms under two management systems in the Eastern and Central regions of Ghana, Agrofor. Syst. 95 (2021) 791–803, https://doi.org/10.1007/s10457-020-00568-5.
[16] E.K. Denkyirah, E.D. Okoffo, D.T. Adu, A.A. Aziz, A. Ofori, E.K. Denkyirah, Modeling Ghanaian cocoa farmers' decision to use pesticide and frequency of application: the case of Brong-Ahafo Region, SpringerPlus 5 (2016) 1113, https://doi.org/10.1186/s40064-016-2779-z.
[17] G.K. Awudzi, R. Adu-Acheampong, S.W. Avicor, Y. Bukari, M.A. Yeboah, E.K. O. Boateng, S.K. Ahadzi, Farmers' knowledge and perception of cocoa insect pests and damage and the implications for pest management on cocoa in Ghana, J. Plant Protect. Res. 61 (2021) 145–155, https://doi.org/10.24425/jppr.2021.137022.
[18] S.W. Avicor, E.O. Owusu, V.Y. Eziah, Farmers' perception on insect pest control and insecticide usage pattern in selected areas of Ghana, New York Sci. J. 4 (2011) 23–29.
[19] S.W. Avicor, R. Adu-Acheampong, G.K. Awudzi, Outbreak and insecticide susceptibility of pod feeding-larvae on cocoa in Ghana, Pertanika J. Trop. Agric. Sci. 45 (2022) 55–73, https://doi.org/10.47836/pjtas.45.1.04.
[20] J.E. Sarfo, I.Y. Opoku, Report on an unusual attack on cocoa by a defoliator, *Anomis leona* Schaus (Coleoptera: Noctuidae), in Ghana, in: A.Y. Akrofi, F. Baah (Eds.), Proceedings of INCOPED 5th International Seminar on Cocoa Pests and Diseases, 15th-17th October 2006, INCOPED Secretariat, Cocoa Research Institute of Ghana, 2007, pp. 114–117, in: https://www.incocoa.org/data/incoped_workshop_5_proceedings_2006.pdf.
[21] K.O. Boateng, E. Dankyi, I.K. Amponsah, G.K. Awudzi, E. Amponsah, G. Darko, Knowledge, perception, and pesticide application practices among smallholder cocoa farmers in four Ghanaian cocoa-growing regions, Toxicol Rep 10 (2023) 46–55, https://doi.org/10.1016/j.toxrep.2022.12.008.
[22] F. Amon-Armah, F. Baah, F. Owusu-Ansah, R. Adu-Acheampong, G.K. Awudzi, Farmers' knowledge of major insect pests and their occurrence in cocoa plantations in Ghana, Int. J. Pest Manag. 69 (2020) 1–13, https://doi.org/10.1080/09670874.2020.1842551.
[23] P.M. van Wijngaarden, M. van Kessel, A. van Huis, *Oecophylla longinoda* (Hymenoptera: Formicidae) as a biological control agent for cocoa capsids (Hemiptera: Miridae), Proc. Neth. Entomol. Soc. Meet. 18 (2007) 21–30, in: https://secties.nev.nl/pages/publicates/proceedings/nummers/18/21-30.pdf. (Accessed 2 May 2022).
[24] D.H.B. Bisseleua, D. Begoude, H. Tonnang, S. Vidal, Ant-mediated ecosystem services and disservices on market yield in cocoa, Agric. Ecosyst. Environ. 247 (2017) 409–417, https://doi.org/10.1016/j.agee.2017.07.004.
[25] P. Van Mele, J.F. Vayssières, E. Van Tellingen, J. Vrolijks, Effect of an African weaver ant, *Oecophylla longinoda*, in controlling mango fruit flies (Diptera: Tephritidae) in Benin, J. Econ. Entomol. 100 (2007) 695–701, https://doi.org/10.1603/0022-0493(2007)100[695:eoaawa]2.0.co;2.
[26] E.A. Dwomoh, J.V.K. Afun, J.B. Ackonor, V.N. Agene, Investigations on *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) as biocontrol agents in the protection of cashew plantations, Pest Manag. Sci. 65 (2009) 41–46, https://doi.org/10.1002/ps.1642.
[27] I.N. Ativor, K. Afreh-Nuamah, M.K. Billah, D. Obeng-Ofori, Weaver ant, *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) activity reduces fruitfly damage in citrus orchards, J. Agric. Sci. Technol. 2 (2012) 449–458.
[28] J.H. Thurman, T.D. Northfield, W.E. Snyder, Weaver ants provide ecosystem services to tropical tree crops, Front. Ecol. Evol. 7 (2019) 120, https://doi.org/10.3389/fevo.2019.00120.
[29] J.B. Ackonor, R. Adu-Acheampong, Standard protocol for screening conventional insecticides at the Cocoa Research Institute of Ghana for mirid control, J. Ghana Sci. Assoc. 9 (2007) 117–121, https://doi.org/10.4314/jgsa.v9i2.18020.
[30] C.A. Collingwood, A comparison of assessment methods in cocoa mirid count trials, in: Proceedings of 3rd International Cocoa Research Conference, Cocoa Research Institute of Ghana, 1971, p. 161.
[31] L. Halos, J.J. Fourie, B. Fankhauser, F. Beugnet, Knock-down and speed of kill of a combination of fipronil and permethrin for the prevention of *Ctenocephalides felis* flea infestation in dogs, Parasites Vectors 9 (2016) 57, https://doi.org/10.1186/s13071-016-1345-4.
[32] G. Buczkowski, M.E. Scharf, C.R. Ratliff, G.W. Bennett, Efficacy of stimulated barrier treatments against laboratory colonies of pharaoh ants, J. Econ. Entomol. 98 (2005) 485–492, https://doi.org/10.1093/jee/98.2.485.
[33] D.H. Choe, M.K. Rust, Horizontal transfer of insecticides in laboratory colonies of the Argentine ant (Hymenoptera: Formicidae), J. Econ. Entomol. 101 (2008) 1397–1405, https://doi.org/10.1603/0022-0493(2008)101[1397:htoiil]2.0.co;2.
[34] W. Jiang, A. Soeprono, M.K. Rust, J. Gan, Ant control efficacy of pyrethroids and fipronil on outdoor concrete surfaces, Pest Manag. Sci. 70 (2014) 271–277, https://doi.org/10.1002/ps.3555.
[35] G. Buczkowski, Trap-treat-release: horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*, Pest Manag. Sci. 75 (2019) 2195–2201, https://doi.org/10.1002/ps.5345.
[36] G. Buczkowski, N.P. Mothapo, T.C. Wossler, Let them eat termites-prey baiting provides effective control of Argentine ants, Linepithema humile, in a biodiversity hotspot, J. Appl. Entomol. 142 (2018) 504–512, https://doi.org/10.1111/jen.12501.
[37] F. Sánchez-Bayo, Indirect effect of pesticides on insects and other arthropods, Toxics 9 (2021) 177, https://doi.org/10.3390/toxics9080177.
[38] Y. Sakamoto, T.I. Hayashi, M.N. Inoue, H. Ohnishi, T. Kishimoto, K. Goka, Effects of fipronil on non-target ants and other invertebrates in a program for eradication of the Argentine ant, Linepitherma humile, Sociobiology 66 (2019) 227–239, https://doi.org/10.13102/sociobiology.v66i2.3772.

[39] X.P. Hu, Evaluation of efficacy and nonrepellency of indoxacarb and fipronil-treated soil at various concentrations and thicknesses against two subterranean termites (Isoptera: Rhinotermitidae), J. Econ. Entomol. 98 (2005) 509–517, https://doi.org/10.1093/jee/98.2.509.

[40] L.M. Hooper-Bui, M.K. Rust, Oral toxicity of abamectin, boric acid, fipronil and hydramethylnon to groups of workers and queens of the Argentine ant, *Linepithema humile* (Mayr), J. Econ. Entomol. 93 (2000) 858–864, https://doi.org/10.1603/0022-0493-93.3.858.

[41] Z.-Y. Zhang, D.-L. Wang, Z.-J. Chi, X.-J. Liu, X.-Y. Hong, Acute toxicity of organophosphorus and pyrethroid insecticides to *Bombyx mori*, J. Econ. Entomol. 101 (2008) 360–364, https://doi.org/10.1093/jee/101.2.360.

[42] R. Adu-Acheampong, J.B. Ackonor, The effect of imidacloprid and mixed pirimiphos-methyl and bifenthrin on non-target arthropods of cocoa, Trop. Sci. 45 (2006) 153–154.

[43] S. Gupta, R.K. Sharma, R.K. Gupta, S.R. Sinha, R. Singh, V.T. Gajbhiye, Persistence of new insecticides and their efficacy against insect pests of okra, Bull. Environ. Contam. Toxicol. 82 (2009) 243–247, https://doi.org/10.1007/s00128-008-9581-8.

[44] J.P. van der Sluijs, V. Amaral-Rogers, L.P. Belzunces, M.F.I.J.B. van Lexmond, J.-M. Bonmatin, M. Chagnon, C.A. Downs, L. Furlan, D.W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E.A.D. Mitchell, C.A. Morrissey, D.A. Noome, L. Pisa, J. Settele, N. Simon-Delso, J.D. Stark, A. Tapparo, H. Van Dyck, J. van Praagh, P. R. Whitehorn, M. Wiemers, Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning, Environ. Sci. Pollut. Res. 22 (2015) 148–154, https://doi.org/10.1007/s11356-014-3229-5.

[45] J. Aufauvre, D.G. Biron, C. Vidau, R. Fontbonne, M. Roudel, M. Diogon, B. Viguès, L.P. Belzunces, F. Delbac, N. Blot, Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee, Sci. Rep. 2 (2012) 326, https://doi.org/10.1038/srep00326.

[46] R. Bommarco, F. Miranda, H. Bylund, C. Björkman, Insecticides suppress natural enemies and increase pest damage in cabbage, J. Econ. Entomol. 104 (2011) 782–791, https://doi.org/10.1603/EC10444.

[47] J.R. Pecenka, L.L. Ingwell, R.E. Foster, C.H. Krupke, I. Kaplan, IPM reduces insecticide applications by 95% while maintaining or enhancing crop yields through wild pollinator conservation, Proc. Natl. Acad. Sci. U.S.A. 118 (2021), e2108429118, https://doi.org/10.1073/pnas.210842911.

[48] J.H. Connel, Diversity in tropical rain forests and coral reefs, Science 199 (1978) 1302–1310, https://doi.org/10.1126/science.199.4335.1302.

[49] B.A. Menge, J.P. Sutherland, Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment, Am. Nat. 130 (1987) 730–757.

[50] T. Hanazato, Response of a zooplankton community to insecticide application in experimental ponds: a review and the implications of the effects of chemicals on the structure and functioning of freshwater communities, Environ. Pollut. 101 (1998) 361–373, https://doi.org/10.1016/S0269-7491(98)00053-0.