Using Trap crops and Entomopathogenic Nematodes to Manage Caterpillar Pests on Head Cabbage

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Introduction

Fig. 1. A) Mustard greens as a trap crop for imported cabbageworm (ICW) and diamondback moth when intercropped with head cabbage, B) ICW cadaver colonized by entomopathogenic nematodes (EPN), and C) infective juveniles of EPN swarming out from insect cadavers.

Head cabbage (*Brassica oleracea* L. var. capitata) is a top-volume producing crop in Hawaii with an estimated production of $3.93 million farm gate value (NAAS 2019). While the tropical climate of Hawaii allows year-round cabbage production, it also allows year-round insect pest pressure from diamondback moth (*Plutella xylostella*), imported cabbageworm (*Pieris rapae*), cabbage webworm (*Hellula undalis*), and cabbage looper (*Trichoplusia ni*). Among these insect pests, diamondback moth (DBM) is most challenging due to its propensity to develop insecticide resistance. The other insects listed are of a lesser challenge because most labelled insecticides control them effectively. However, during cool and dry seasons in Hawaii, imported cabbageworm (ICW) larval populations can be very high and inflict significant yield loss. Severe feeding of DBM and ICW larvae on young cabbages can cause cabbage seedlings to abort or stunt cabbage heads. Farmers in Hawaii have experienced 20-40% and sometimes up to 100% of yield loss despite intensive attempts to manage the pest (Shimabuku et al., 1995). Several insecticides are available and have been used, however, continuous use of insecticide with the same mode of action provides consistent insecticide selection pressure, yielding DBM populations that are resistant to organophosphate, carbamate, pyrethroid, organochlorines and other insecticides (Tabashnik et al., 1987). CTAHR researchers and extension agents have a long track record of studying and developing insecticide resistance management (IRM) programs against DBM (Tabashnik et al., 1987; Mau and Gusukuma-Minuto, 2001; Zhao et al., 2006). With stringent practice of IRM, the battle against *Bt* (*Bacillus thuringiensis*) and spinosad-resistant DBM was successful, and ICW can also be managed successfully. However, the IRM program recommends a complete 6-month insecticide rotation with different mechanism of action groups rotated every month (Chou et al., 2018; Mau and Gusukuma-Minuto, 2001). While conventional growers have a handful of effective synthetic-based pesticides to rotate in the 6-month rotational program, organic growers only have a couple of pesticides that are effective such as *Bt* and Spinosad. Thus, it is imperative to identify additional microbial biocontrol agents that can add to the organic insecticide rotation program and cultural practices that can assist organic farmers in managing DBM and ICW effectively. Please visit “Non-chemical approaches to manage diamondback moth” YouTube video to see a list of approaches.

Trap Crop: The push-pull integrated pest management strategy involves the behavioral manipulation of insect pests and their natural enemies by the use of behavior modifying stimuli. One aspect of the push-pull tactic is to control agricultural pests by using repellent "push" plants and trap "pull" plants. Pull crops or trap crops can attract, divert, intercept, and/or retain targeted insect pests in order to reduce damage to the cash crop (Shelton and Badenes-Pérez, 2006). Cruciferous crops are variable in attractiveness to DBM and ICW (Piñero & Manandhar, 2015). Waxy collard greens (*Brassica oleracea* var. viridis), brown mustard (*Brassica juncea*), yellow rocket (also called bittercress, *Barbarea vulgaris*), are some of the most preferred trap crops for DBM due to their high glucosinolate content (Sherbrooke et al., 2020). Thus, our first cultural strategy to approach these pests is to identify trap crops that attract DBM and ICW in preference to cabbage.

Entomopathogenic nematodes: Entomopathogenic nematodes (EPNs) are microscopic roundworms that are obligate parasites of insect hosts and cause host mortality within 24-48 hours upon infection (Kaya and Gaugler, 1993). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological control agents for pest management (Grewal et al. 2005). Both *Heterorhabditis* and *Steinernema* have mutualistic relationships with the bacteria *Photorhabdus* and *Xenorhabdus*, respectively. EPNs enter into the insect via body openings such as spiracles, the mouth, anus, or in some species through intersegmental membranes of the cuticle, and infect the hemolymph (Bedding and Molyneux 1982). Once inside the host, the nematodes release symbiotic bacteria that colonize and kill the host within 24-48 hours (Godjo et al., 2017). This project examined if integrating the use of trap crop and application of EPNs would have synergistic effect against DBM and ICW on head cabbage.

Field Trials Summary

Host Preference Test: A field trial was conducted at Waianae, Oahu to compare damage and abundance of DBM and ICW on curly kale (*Brassica oleracea* variety acephala), ‘Mei Qing’ pak choi (*Brassica rapa* subsp. chinensis), ‘Joi Choi’ pak choi, ‘Hirayama’ kai choi (*Brassica juncea*), and ‘KK’ head cabbage (*Brassica oleraceae* Capitata Group). Seedlings of pak choi, kai choi, and head cabbage were transplanted 5 weeks after germination on December 19, 2019 at 30-cm spacing between plants in a 1-month old curly kale field that had a natural infestation of DBM and ICW (Fig. 2A). Plants were examined for percent of leaves with DBM or ICW damage (Fig. 2B), and abundance of eggs, larvae, pupae and adult stages per plant from 5 plants per plot, with 4 replicated plots of each variety tested over two sampling times (January 16 and January 23, 2020). Data from the two dates were combined and subjected to one-way analysis of variance using SAS 9.4 (SAS Inc., Cary, NC).

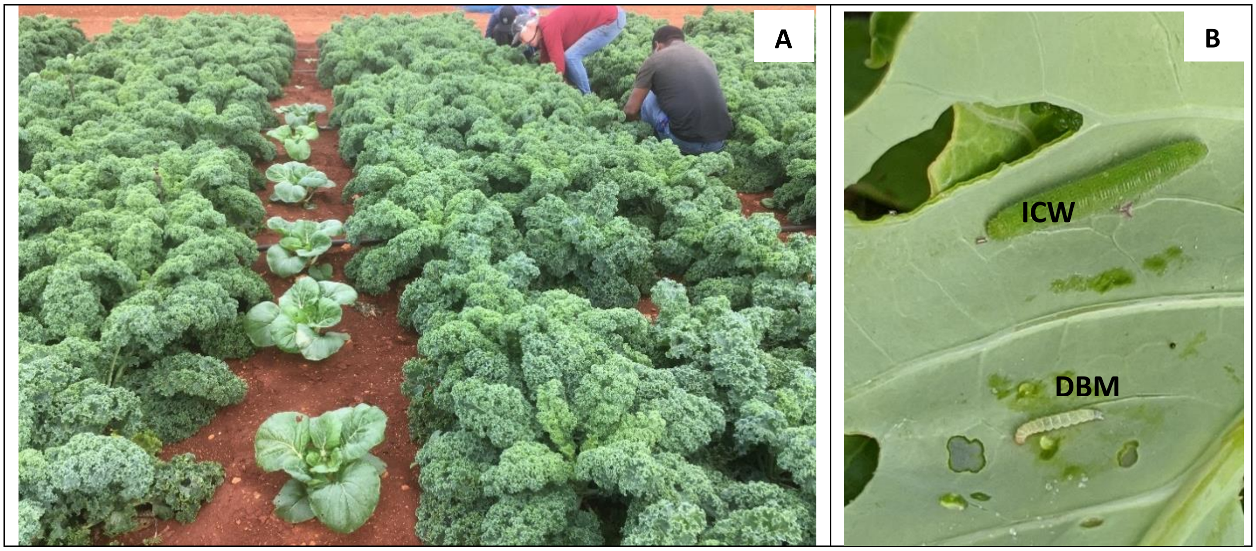


Fig. 2. A) Alternate hosts for imported cabbageworm (ICW) and diamondback moth (DBM) planted into a kale field in Waianae. B) Damage caused by DBM and ICW were monitored.

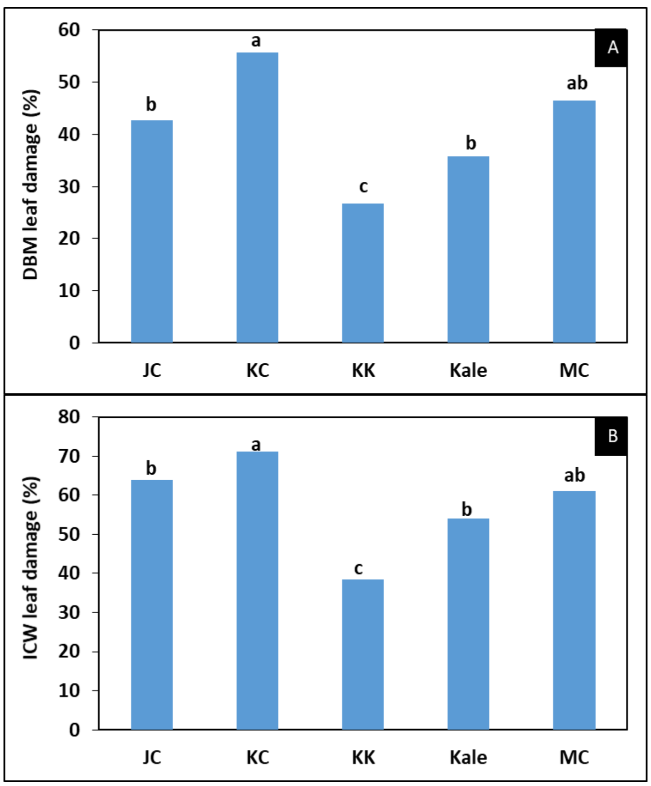


Fig. 3. Percent of leaves with diamondback moth (DBM) and imported cabbageworm (ICW) damage on 'Joy Choi' (JC), kai choi (KC), KK (cabbage), kale and 'Mei Qing' pak choi (MC) (n = 40). Means followed by the same letter(s) are not different based on Waller-Duncan (k-ratio) t-test.

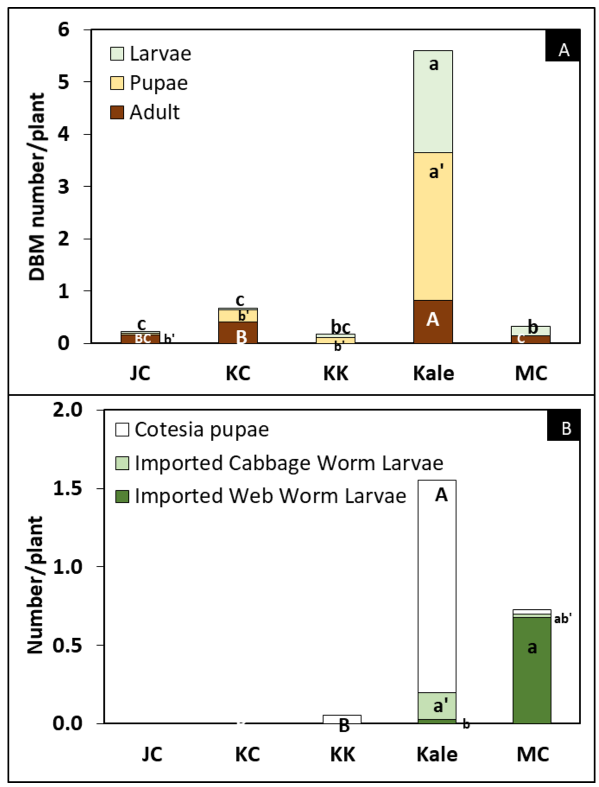


Fig. 4. Number of A) diamondback moth (DBM) and B) imported cabbageworm (ICW) on 'Joy Choi' (JC), kai choi (KC), KK (cabbage), kale and 'Mei Qing' pak choi (MC) (n = 40). Means of each insect stage or type followed by the same letter(s) are not different based on Waller-Duncan (k-ratio) t-test.

Results – Among the five plant species tested, kai choi was most prone to DBM and ICW damage with significantly higher percentage of leaves damaged by both insects compared to that on 'KK' head cabbage, and kale (Fig. 3). Although DBM larvae and adults, as well as ICW larvae and webworm larvae preferred to roost on kale than other plants tested in this experiment (Fig. 4), DBM and ICW preferred to feed on kai choi (Fig. 3). Based on these results, we selected mustard greens (kai choi) as a trap crop in subsequent studies.

Fig. 5. Kai choi as a border trap crop for head cabbage production at Waialua at 4 (left) and 7 (right) weeks after transplanting.

Mustard Greens Trap Crop and Steinernema feltiae EPN foliar spray: A second field trial was conducted at Waialua in an organic farm plot naturally infested with DBM and ICW on 'KY' head cabbage to examine the pest suppressive effect of inter-planting mustard greens ('Hirayama' kai choi) with 'KY' and foliar application of *Steinernema feltiae* MG14 (previously isolated from soil in Kahului, Maui). Six week-old KY seedlings were transplanted into the field on March 19, 2020 at 30-cm spacing between plants and rows. The experiment was laid in 2 × 2 (trap crop × EPN) factorial design with 4 replicated plots. Each plot was composed of 5 cabbage plants. Half of the field was inter-planted with kai choi in between head cabbage plants on the border as a trap crop, whereas the other half was not. On April 23, each of the main plot was split into half to be sprayed or not sprayed with 1000 infective juveniles (IJs) of *S. feltiae* per plant delivered through 8 ml of water suspension containing Barricade®(Barricade International, Inc., Hobe Sound, FL) as an anti-desiccant and para-aminobenzoic acid (PABA) (Sigma Chemical, Steinheim, Germany) as a UV protectant (Fig. 6). Plants were examined for percent of leaves with DBM or ICW damage at weekly intervals from transplanting to harvesting (May 15, 2020). Abundance of eggs, larvae, pupae and adult stages per plant from 4 plants per plot were also counted. Data from all dates were subjected to 2 × 2 analysis of variance using SAS 9.4 (SAS Inc., Cary, NC).

Results – No interaction among sampling dates, trap crop and EPN effects occurred, thus data from all dates were combined and only the main treatment effects were shown. Planting kai choi as a trap crop suppressed abundance of ICW (eggs and larval counts per plant) and DBM (larval and pupae) significantly ($P \leq 0.05$) (Fig. 7 A, B). Trap crop also reduced ($P \leq 0.05$) leaf damage of ICW and DBM by 33 % and 45%, respectively on the head cabbage (Fig. 7C, D). On the other hand, EPN suppressed abundance of ICW (combination of all stages) ($P \leq 0.05$) by 43.4% as compared to the no EPN treatment (Fig. 8). Interestingly, both ICW and DBM preferred to roost or lay eggs on head cabbage more than on kai choi, but they preferred to feed on kai choi compared to head cabbage ($P \leq 0.05$) (Fig. 9). There was no interaction between trap crop and EPN treatment effect for all parameters measured except for the DBM larvae abundance.

Fig. 6. Entomopathogenic nematodes foliar spray using handheld sprayer.

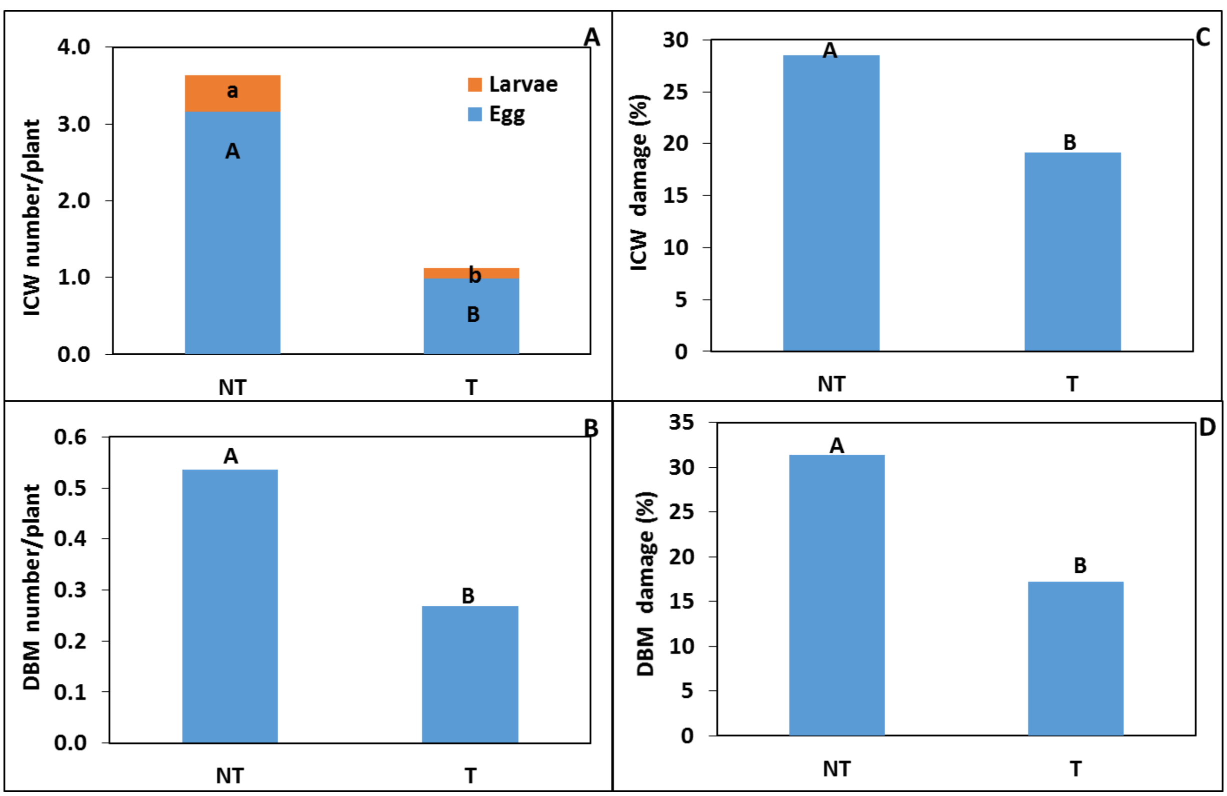


Fig. 7. Effect of kai choi as a trap crop on A) abundance of imported cabbageworm (ICW), B) diamondback moth (DBM), and percent of leaves damaged by C) ICW and D) DBM in the Waialua Trial. T = Trap crop, NT = No trap crop. Columns (n=224) followed by the same letters in a graph are not different based on analysis of variance.

Overall, trap cropping by kai choi was more effective in suppressing DBM than ICW. However, ICW can further be suppressed by EPN foliar spray. More research is needed to improve efficacy of EPN as a biocontrol agent against DBM on cabbage as effective EPN control of DBM had been reported on watercress (*Nasturtium officinale*) (Baur et al. 1998); and on head cabbage when different species of EPN (*Heterorhabditis bacteriophora*) was applied at a much higher concentration (1200 IJs/cm^2) in combination with *Metarhizium anisopliae* (Sáenz-Aponte et al., 2020).

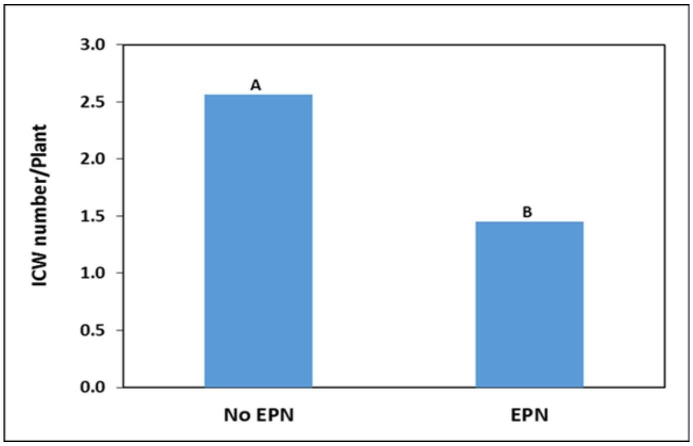


Fig. 8. Effect of entomopathogenic nematodes (EPN) as foliar spray on A) abundance of imported cabbageworm (ICW) in the Waialua Trial. Columns (n=224) followed by the same letters in a graph are not different based on analysis of variance.

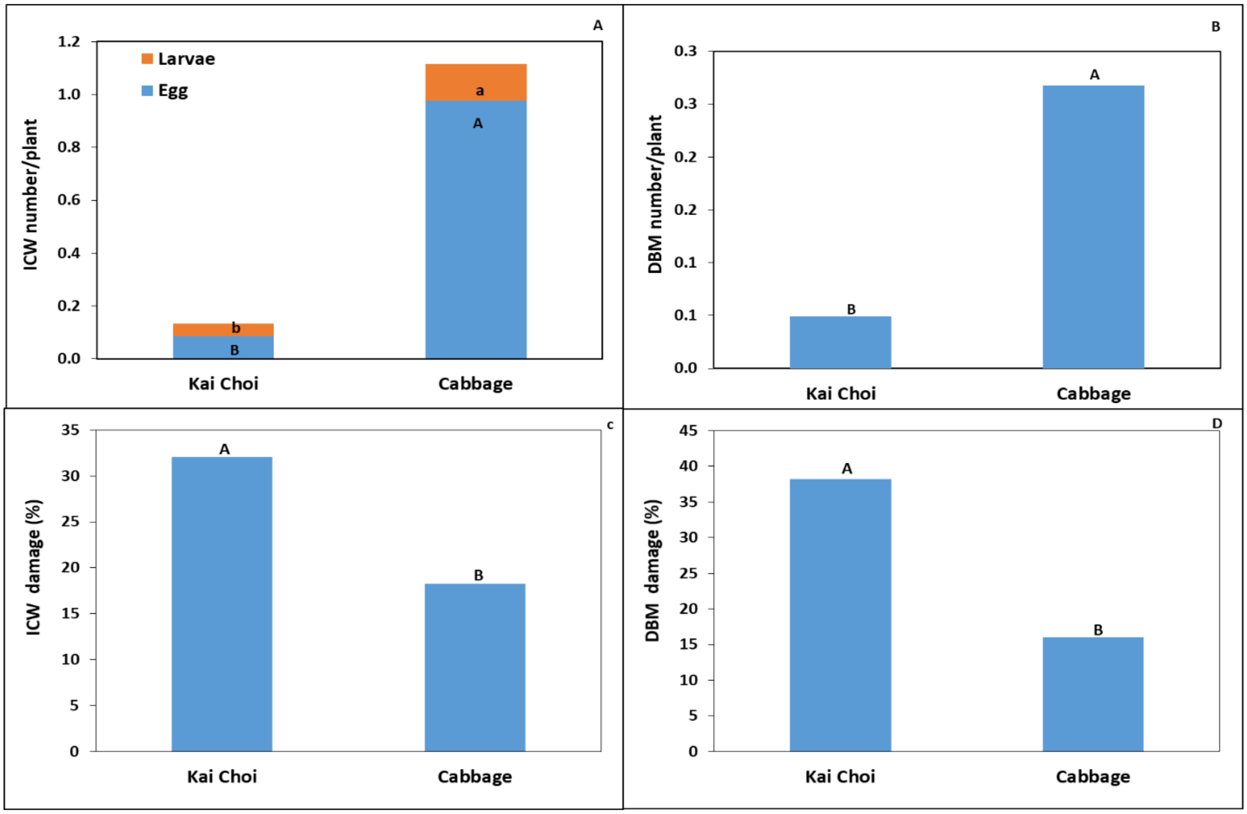
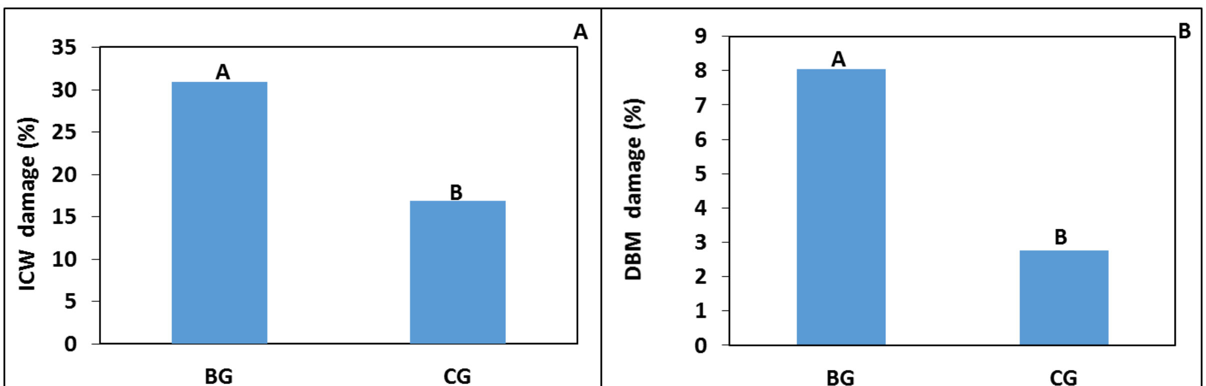


Fig. 9. Abundance of A) imported cabbage worm (ICW) larvae and eggs, and B) diamondback moth (DBM) (all stages), and percentage of C) ICW and D) DBM leaf damage on kai choi and head cabbage in the Waialua Trial. Columns (n=224) followed by the same letter in a graph are not different based on analysis of variance.

Collard Greens Trap Crop: Another field trial was conducted at Magoon Teaching Facility to examine if collard greens can be another effective trap crop against ICW and DBM. Six-week old 'KK' head cabbage seedlings were transplanted into the field on February 10, 2020. Two treatments examined were: cabbage with collard greens as field borders, vs without collard greens as the control. Each treatment was composed of 18 cabbage plants planted at 30-cm between plants and 90-cm between rows. Each treatment had three replications, each with 3 plants being monitored for percent leaves with DBM damage or ICW damage, and abundance of eggs, larvae, pupae and adult stages on cabbage at weekly intervals for 7 weeks. Same number of collard green plants were also monitored. Repeated measure analysis over the 7 weeks were subjected to one way analysis of variance using SAS 9.4 (SAS Inc., Cary, NC).



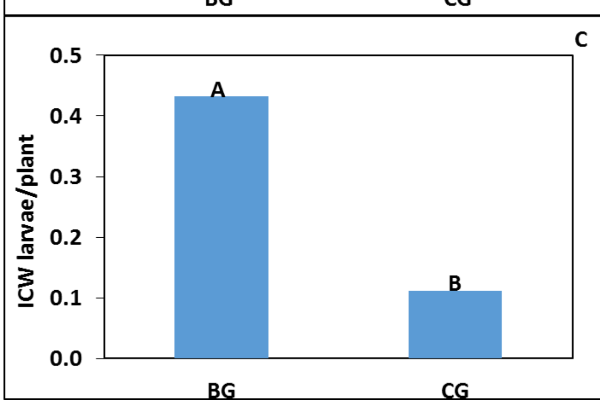


Fig. 10. Effect of collard greens trap crop on percent leaves damaged by A) ICW and B) DBM, and abundance of ICW larvae on cabbage plants. CG = Collard greens surrounding cabbage plant as trap crop, BG = bare ground control plot without collard green. Means are average from repeated measure over 7 weeks (n=45). Columns followed by the same letters in a graph are not different ($P > 0.05$) based on one-way analysis of variance.

Results – No interaction among sampling dates and trap crop treatments occurred, thus data from all dates were combined. Planting collard green as a trap crop surrounding the head cabbage reduced ($P \leq 0.05$) ICW and DBM damage by 45 % and 65 %, respectively on the head cabbage (Fig.10 A, B) and suppressed the abundance of ICW larvae ($P \leq 0.05$) (Fig. 10 C) compared to the cabbage planted without collard greens borders. Although no significant difference in leaf damage or insect counts were observed between collard green and head cabbage planted side by side ($P > 0.05$), both ICW and DBM tend to feed more on collard greens than head cabbage. Thus, collard greens is also a good trap crop to reduce feeding damage of ICW and DBM on cabbage.

Conclusion

Results from these field trials demonstrated the potential of using mustard green ('Hirayama' kai choi) and collard greens as trap crops inter-planted with or bordered around head cabbage followed by foliar spray of the EPN, *S. feltiae* MG 14 at 1,000 IJs per plant around 1 month after transplanting. More research is needed on the planting distance between head cabbage and the trap crop. Although trap cropping or EPN did not totally suppress DBM and ICW damage, these organic pest management tools can improve organic insecticide rotation program to reduce the insecticide selection pressure and pest damage on head cabbage.

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