Effects of three chitin synthesis inhibitors on egg masses, nymphs and adults of Halyomorpha halys (Hemiptera: Pentatomidae)

Masetti Antonio (Orcid ID: 0000-0001-6061-2752)
Depalo Laura (Orcid ID: 0000-0002-0664-3854)

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Running title: **Effects of chitin synthesis inhibitors on the brown marmorated stink bug**

Antonio Masetti[1]*, Anna Rathé[2], Nicola Robertson[3], Diane Anderson[4],

Jim Walker[5], Edison Pasqualini[1] and Laura Depalo[1]

[1] Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma mater studiorum* - Università di Bologna, v.le G. Fanin, 42 – 40127 Bologna, Italy

[2] Horticulture New Zealand — Ahumāra Kai Aotearoa, Wellington, New Zealand

[3] New Zealand Apples and Pears Inc., Hawkes Bay, New Zealand

[4] Ministry for Primary Industries, Christchurch, New Zealand

[5] The New Zealand Institute for Plant & Food Research Limited, Hawkes Bay, New Zealand

* Corresponding author, e-mail: antonio.masetti@unibo.it

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Abstract

BACKGROUND: *Halyomorpha halys,* (brown marmorated stink bug, BMSB), is a high-concern invasive species causing severe damage to orchards in many countries outside its native Asian range. Management methods other than frequent sprays of broad-spectrum insecticides are needed to restore integrated pest management (IPM) practices in orchards. Chitin synthesis inhibitors are usually regarded as desirable options in IPM programs because of lower toxicity to beneficial insects and non-target organisms compared to neurotoxic insecticides. In this study, the activity of three chitin synthesis inhibitors (namely buprofezin, novaluron and triflumuron) was investigated on BMSB egg masses, 3rd instars and adults by means of laboratory bioassays.

RESULTS: Novaluron and to a lesser extent triflumuron were detrimental to BMSB nymphs exposed to residues on potted peach plants. Novaluron caused high mortality among early instars emerged from sprayed egg masses. No significant differences were found between buprofezin and water control on eggs or 3rd instars. When sprayed on BMSB adults, none of the chitin synthesis inhibitors affected survival, fecundity or egg hatching.

CONCLUSION: Given the activity on nymphs, but the lack of effects on adults, novaluron and triflumuron might be considered for field applications only as a tool in a wider management strategy along with other methods aimed at preventing the invasion of crops by BMSB adults.

Keywords: Brown marmorated stink bug; Invasive species; Buprofezin; Novaluron; Triflumuron; Integrated pest management.

Introduction

Brown marmorated stink bug (BMSB) - *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) - is native to Asia, but is an invasive pest in the USA and southern Europe where it has become very common in orchards. BMSB is an economically important insect pest in most countries outside its native range (1, 2). Current management strategies rely primarily on neurotoxic broad-spectrum insecticides that are effective on BMSBs only shortly after application (3, 4). This has forced farmers to increase the frequency of insecticide sprays to a 6–7 day intensive schedule (5), causing the disruption of many of the integrated pest management (IPM) practices developed over the last 30 years (6) and the resurgence of secondary pests (2). The identification of more selective insecticides targeting BMSB would significantly reduce the environmental issues associated with its control and could contribute to the restoration of IPM principles.

Inhibitors of chitin biosynthesis (CSIs) are classified into groups 15 and 16 by the Insecticide Resistance Action Committee (IRAC, https://www.irac-online.org/modes-of-action). Group 15 (inhibitors of chitin biosynthesis affecting CHS1) include benzoylphenyl ureas (BPUs) such as diflubenzuron, triflumuron and novaluron, directly interact with the enzyme chitin synthase 1 thus inhibiting chitin polymerization (7). BPUs, have been used as pesticides since the 1970s (8, 9). Buprofezin is the only active ingredient in group 16 (inhibitors of chitin biosynthesis, type 1). The mode of action (MoA) of buprofezin is still incompletely defined; it interferes with chitin biosynthesis, but with a chemistry different from BPUs. In addition, buprofezin may act as a prostaglandin inhibitor leading to the suppression of ecdysis (10). Buprofezin was introduced at the beginning of the 1990s for the control of whiteflies and other homopteran nymphs.

The inhibition of chitin biosynthesis caused by CSIs compromises the integrity of endocuticle and hinders insect molts. Mortality usually occurs at the juvenile stages because of a failed molt. Detrimental effects on adult fecundity and egg viability are reported as well, although with a wide

range of variation among insect groups and active ingredients (11). CSIs show minimal toxicity to mammals and are considered less harmful to beneficial insects and non-target organisms than most insecticides acting on nerves and muscles (12). Therefore, BPUs and buprofezin may be considered as desirable options in IPM programs (10) and are usually included among the so called "reduced-risk insecticides".

The activity of CSIs on BMSB has not been investigated as extensively as was done for neurotoxic insecticides (3). However, Kamminga et al. (3), reported high mortality percentages among nymphs fed with green bean dipped in diflubenzuron and novaluron solutions. More recently, Masetti et al. (13) demonstrated a significant insecticidal activity on BMSB juveniles exerted by residues of the BPU triflumuron, which had been aged on peach plants up to 21 days in glasshouse conditions before exposure of bugs. The same article also reported a significant reduction of BMSB damage on pear orchards treated with triflumuron.

The aim of this study was to test the overall potential of some CSIs as insecticides targeting BMSB. Three active ingredients – novaluron, triflumuron and buprofezin – were tested on egg masses, nymphs and adults of BMSB in laboratory assays. The lack of lethal effects on adult insects and the overall slow speed of kill on juveniles are a well-known drawback of CSIs. Therefore, besides bioassays on nymphs, CSIs were also tested on BMSB adults to check their effects on survival, fecundity and hatch rate. Egg masses were also sprayed to evaluate the capacity of these active ingredients to impair either embryonic development or survival of 1st and 2nd instars. Although usually overlooked, activity on eggs and sub-lethal effects on adults could indeed add up to cause considerable secondary impacts on pest populations (14-16).

Materials and methods

Insects

BMSB egg masses and nymphs were obtained from a laboratory colony established in 2015 at the Department of Agricultural and Food Sciences (University of Bologna). Bugs were reared in a walk-in climatic chamber at 25±2 °C, 50–70% RH, with a 14:10 (L:D) photoperiod. Adults and nymphs were fed twice per week with green beans, carrots and soybean seeds; fresh fruits (pears, apples or kiwi fruits) were provided weekly to adults only. Egg masses were removed daily from adult cages and held in Petri dishes (diameter 10 cm) to wait for hatching. Second instars were moved to 1.5 L-plastic jars and fed as previously described.

Newly laid egg masses (< 24 h) and early and as much as possible coetaneous 3rd instars were used for the experiments. BMSB adults tested in the bioassays were collected in the field from the end of August throughout October 2021 by means of Rescue® hanging pyramid traps (Sterling International Inc., Spokane, WA, USA) baited with standard BMSB Monitoring Lure® (Trécé Inc., Adair, OK, USA), which were checked daily. Collection from overwintering colonies in buildings and sweep netting in soybean fields was also carried out. All bugs belonged to the 2nd generation and were moving into overwintering. Therefore, before being used for the experiments all insects were kept in a walk-in climatic chamber set at the conditions reported above for at least one month to prevent the induction of or to break reproductive diapause. During this period bugs were fed with green beans, carrots, soybean seeds and fresh fruit (pears, apples or kiwifruits).

Insecticides

Insecticide treatments

Two groups of CSIs were used in the bioassays. Alsystin 48 SC (triflumuron 39.34% = 480 g L^{-1}, Bayer Crop Sciences, Milan, Italy) and Rimon 10 EC (novaluron 100 g L^{-1}, Adama Makhteshim, Be’er

Sheva, Israel) are both BPUs included in IRAC group 15. Applaud plus (buprofezin 25% = 250 g kg^{-1}, Sipcam Italia, Milan, Italy), which is the only insecticide in IRAC group 16, was also tested (Table 1). There is a range of doses for each active ingredient on the label of commercial products registered in different countries. Therefore, rather than settling on any particular label dose, two concentrations were tested for each active ingredient in order to encompass what is allowed on most of the labels.

Bioassays on BMSB egg masses

Egg masses, which had been laid either on peach leaves or paper towels, were excised along with ≈3 cm^2 of the substrate. Each egg mass was individually sprayed until run-off with an insecticide solution. Controls were sprayed in the same way with deionized water. After drying, each egg mass was placed individually in a plastic Petri dish (diameter 10 cm) with a piece of wet cotton wool that was used to keep appropriate humidity. A green bean was provided as food for nymphs. Petri dishes were maintained at the same conditions reported for BMSB rearing. Mortality was checked twice a week; green bean and cotton wool were changed as needed. Any deformed or collapsed egg found at the first check was considered not viable because of unsuccessful fertilization or abnormalities during deposition. These eggs were not considered when scoring mortality due to insecticide application on egg masses.

Eight replicates per treatment (56 experimental units, each consisting of a single egg mass) were set up in total. First instars were held in the Petri dishes until they died or molted. Second instars were removed from Petri dishes and held in ventilated 200-ml plastic jars to check the development to 3rd instar, and provided the same food as previously reported. Nymphs that successfully molted to the 3rd instar were considered to have survived the treatment.

Bioassays on BMSB 3rd instars

Potted peach plants (GF-677 rootstock, approximately 30 cm high) were sprayed outdoors using a hand sprayer to the point of runoff and then residues were allowed to dry before trials. Control plants were sprayed in the same way with tap water. Approximately 10 ml of insecticide solution was applied to each plant.

After treatment droplets dried, experimental units were set up by placing potted plants individually in plexiglas cylinders (diameter 8 cm, height 30 cm). These had a fine net lid and a hole (diameter 8 cm) near the base that was also sealed with a fine net to allow air circulation and avoid mold growth. The soil surface on the pot was covered with nonwoven fabric, which was tightly wrapped to the plant stem.

Ten early 3rd instar BMSBs were placed in each cylinder. Given that nymphs must feed on fruit or vegetables to complete development, carrots and green beans were provided ad libitum and changed twice per week. The green beans were hung on the plants to force the nymphs to climb onto the canopy in order to feed. Six replicates per treatment (42 experimental units) were set up in total.

Experimental units were held at the same conditions reported for BMSB rearing, and mortality was checked after 7, 14 and 21 days of continuous exposure of nymphs to plant residues. The number of dead nymphs and instar stage, i.e. the number of molts they successfully completed, was recorded. Moribund nymphs (i.e. unable to upright themselves when flipped on their back) found at the last exposure interval were reared individually in ventilated 200-ml plastic jars at the same conditions until they died or reached the adult stage (recorded as a survived).

Bioassays on BMSB adults

Only the highest concentrations of the chitin synthesis inhibitors were tested on BMSB adults

(Table 1). Groups of 8 bugs (4 females and 4 males) were randomly assembled in a glass cylinder (diameter 5 cm, height 15 cm) and sprayed with a hand sprayer. Control groups were treated in the same way with tap water. Approximately 3 ml of insecticide solution was used to spray each group.

BMSB adults were left to dry for 15 minutes and then placed in insect mesh cages (30 x 30 x 30 cm). Two groups of bugs which had been treated with the same insecticide were housed together in each cage, totaling 8 females and 8 males. Bugs were provided ad libitum with water pads, carrots, green beans and apples which were renewed twice per week. Three replicates were set up for each treatment on September 28th 2021 and two additional replicates on 12th January 2022. Overall 20 experimental units were carried out and 320 BMSB adults were used.

Cages were held for 50 days after treatment at the same conditions reported for BMSB rearing. Twice a week each cage was inspected to check BMSB mortality and to collect egg masses. The number of eggs in each mass were counted to score fecundity. Each egg mass was placed individually in a plastic Petri dish (diameter 10 cm) along with a green bean as food and a piece of wet cotton wool that was included to keep appropriate relative humidity. Egg masses were maintained for 2 weeks at the same conditions reported for BMSB rearing and egg hatch was recorded as a measure of fertility.

Statistical analysis

Data from the bioassays on BMSB egg masses were analyzed by a generalized linear mixed model (GLMM) with first-order autoregressive covariance structure, binomial error distribution, probit link function and Kenward–Roger method for estimating degrees of freedom. The number of unhatched eggs or dead nymphs out of the total number of viable eggs in the egg mass was considered as a dependent variable. The insecticide treatments were used as levels of the

between-subject factor, the developmental stages (egg, 1st instar, 2nd instar) were included as repeated measures and the replicate was considered as a random factor. The treatment × developmental stage interaction was tested as well.

A GLMM with the same features already described for egg masses was run to analyze the mortality of 3rd instars. The number of dead insects out of the total nymphs tested was modeled as the dependent variable. The insecticide treatments were used as levels of the between-subject factor, the exposure intervals were included as repeated measures and the replicate as random factor. The treatment × exposure interval interaction was tested as well. For both egg masses and nymphs, a significant effect of the interaction was detected. Therefore, treatments were compared within each level of the repeated measure with Bonferroni sequential adjustment for multiple comparisons.

Longevity of treated adults was analyzed with Kaplan-Meier estimators, considering dead insects as “events” and insects still alive at the last check 50 days after treatments as “censored data”. A male bug that escaped because of bad handling from a triflumuron cage was also censored. The log-rank (Mantel-Cox) test followed by Holm-Sidak multiple comparison procedure were used to detect significant differences ($p < 0.05$) among the survival curves of different treatments. These tests were run twice: once on the whole data set irrespective of sex and then separately for females and males.

Fecundity was scored as the total number of eggs per cage (i.e. laid by the 8 females over the duration of the experiment). Raw data were $\log_{10}$ transformed to meet the assumption of normality and homoscedasticity and were analyzed by one-way ANOVA including the treatments as factor levels.

A generalized linear model (GLM) with binomial error distribution and probit link function was used to analyze fertility. The proportion of hatched eggs out of the total number of eggs laid per

cage was considered as dependent variable and treatments were used as levels of the fixed factor. All the analyses and graphical representations were carried out with IBM SPSS Statistics (ver. 26).

Results

Bioassays on BMSB egg masses

The significant treatment × stage interaction detected by the GLMM ($F_{(12, 93.3)} = 2.7$; $P=0.003$) revealed that the effect of treatments was dependent on the BMSB developmental stage. Therefore, treatments were tested separately for eggs, 1st instars and 2nd instars.

None of the insecticides affected the hatch of BMSB eggs and no significant differences could be detected in comparison with the water control. Only 1st instars that emerged from eggs sprayed with novaluron at 200 mg L^{-1} showed a statistically significant mortality increase in comparison with the control.

A number of differences among treatments were detected on the 2nd instars. The highest mortality occurred among nymphs emerged from eggs sprayed with the full concentration of novaluron. The half concentration of novaluron (100 mg active ingredient L^{-1}) was the second most effective active treatment, even if the differences between concentrations of triflumuron and the highest concentration of buprofezin were not supported by multiple comparison test. With the exception of novaluron at both concentrations, no other treatments caused significantly higher mortality than the control (Fig.1).

Bioassays on BMSB 3rd instars

Given that GLMM detected a significant effect of the interaction treatment × exposure interval ($F_{(12, 71.3)} = 5.1$; $P<0.001$), treatments were compared to each other within each exposure interval. At 7-day exposure, none of the insecticide caused significantly different mortality in comparison

with the water control. After 14-day exposure, the mortality in nymphs exposed to residues of novaluron at both concentrations was significantly higher than all other treatments. More than 85% of BMSB nymphs exposed to novaluron residues had died by the 14th day. Several differences among treatments emerged after 21-day exposure (Fig. 2). The highest mortalities were scored among nymphs exposed to novaluron either at full (95.2 ± 3.4%) or half concentration (88.3 ± 6.0%). The differences between novaluron at 100 mg L^{-1} and triflumuron at 120 mg L^{-1} (66.6 ± 12.2%) were not supported by the multiple comparison test. No significant differences were detected between the two concentrations of triflumuron.

Bioassays on BMSB adults

Overall, 182 out of 320 bugs (56.9%) were still alive 50 days after spraying. Log-rank test detected some significant differences among treatments for the survival curves of bugs irrespective of sex (Fig. 3) and for females (Fig. 4), whereas no difference was detected by survival analysis on males (Fig. 5). None of the insecticides had a significant effect on survival in comparison with the control. However, female bugs sprayed with buprofezin suffered a slight but statistically significant decrease in survival in comparison with novaluron and triflumuron. The same trend was also found in the survival curve when both sexes were pooled.

One-way ANOVA did not detect any significant differences among treatments in the total number of eggs per cage ($F_{(3, 16)} = 0.9$; $P = 0.44$). The total number of eggs laid in the 50-day period ranged from 206.4 ± 42.7 for buprofezin to 336.2 ± 45.6 for triflumuron. As shown by the size of the standard errors, a wide variation was detected among experimental units (i.e cages). Total egg number for individual cages ranged between a minimum of 23 eggs laid in 50 days by bugs in a control cage to 744 eggs recovered in a cage where bugs treated with novaluron were housed.

A small range of variation among all treatments was detected in the percentage of eggs that

successfully hatched and no significant differences could be scored (Wald $\chi^2_{(3)} = 0.6$; $P = 0.90$). BMSB nymphs emerged from approximately 80% of eggs (ranging from 74.0 ± 4.1% for novaluron to 82.6 ± 3.6% for triflumuron).

Discussion

The insecticidal activity exerted on BMSB showed a wide range of variation among the three CSIs tested in this study; with the life stage of the bugs being a key factor determining the effect of the treatments. While none of the insecticides negatively affected egg hatching or reduced survival, fecundity or fertility of adult bugs, residues of triflumuron and especially novaluron were harmful to nymphs. The lack of activity of CSI sprays on egg masses and adults is in line with the findings of Kamminga et al. (3) and may be due to the low permeability of BMSB chorion and tegument to these active ingredients.

Intake of BPUs through tarsal contact with residues was likely the major means of insecticide poisoning for 3rd instars exposed to treated plants. Although ingestion has been usually considered as the major means of intake for CSIs, contact activity has also been reported (8, 12, 17). As untreated vegetables were provided as food to nymphs in all the experiments, intake via contact was likely prevalent in our study. Theoretically, nymphs on treated peach plants could have also ingested insecticides by probing or piercing plants. However, bugs were never observed feeding on the potted plants. Moreover, the capacity of CSIs to enter in plant tissues is debated and is likely different for the different active ingredients. For example, while Dhadialla et al. (10) reported no systemic or translaminar activity for novaluron, Ishaaya et al. (18) inferred its capacity to enter the leaf blade because of insecticidal activity on leafminer larvae.

While ingestion of CSIs, if any, was likely negligible for bugs on potted peach plants, nymphs that emerged from sprayed egg masses could feasibly have absorbed insecticides through ingestion as

newly hatched 1st instars feed on egg shells (19). However, once nymphs started crawling, intake of CSIs by tarsal contact with residues on eggs or on substrates upon which egg masses had been laid cannot be ruled out.

Considering that nymphs exposed to plant residues were followed for 21 days, an overall low mortality was detected in the control groups (20.0 ± 5.8% at 21-day exposure). On the other hand, 28% mortality was found at the 2nd instar stage among the bugs emerged from egg masses assigned to the control groups. The 2nd instar is likely rather critical in BMSB development since this is the life stage that starts feeding on plant tissues, and considerable mortality is quite common in routinely reared colonies. Our findings are in line with Medal et al. (20) who reported juvenile mortality from 23% to 50% in BMSB rearing, and Fisher et al. (21) who recorded overall low survival of nymphs when testing the influence of temperature and humidity on BMSB.

Novaluron was the most effective active ingredient, either when sprayed directly on egg masses or on potted peach plants, and caused the highest mortality among nymphs at both full and half concentration. Overall, these results aligned with the findings of laboratory assays by Kamminga et al. (3) who fed BMSB nymphs for 7 days with fresh beans dipped in solutions of novaluron. Cutler et al. (22) reported a very similar pattern of mortality among nymphs of the predatory bug *Podisius maculiventris* (Say) (Hemiptera: Pentatomidae) exposed to potato foliage treated with novaluron, thus reinforcing the evidence of toxicity to Pentatomidae juveniles.

With the exception of novaluron at both concentrations, no other treatments caused significantly higher mortality than the water control in the egg mass bioassays. On the other hand, nymphs exposed to residues of triflumuron on plants suffered an overall higher mortality than control groups at the longest exposure period. The mortality among nymphs exposed for 21 days to residues of triflumuron in this experiment is in line with that recorded in our previous study for fresh residues at the same exposure interval (13). A lack of statistical support for the insecticidal

activity of triflumuron in comparison with the controls after 14 days of exposure to residues was found in this study, which differs from previous findings. However, this difference seems likely due to the inclusion in the statistical analysis of all the other insecticides, and overall both bioassays showed a certain insecticidal activity exerted by triflumuron on BMSB nymphs.

Neither the sprays nor exposure to residues of buprofezin had detrimental effects on BMSB at any life stage. Although widely used on hemipterans in the Cicadomorpha and Sternorrhyncha suborders, negligible activity of buprofezin has been reported on Heteroptera. Mostly predatory bugs (pirate bugs) have been tested and no harmful effects were observed on nymphal development of *Orius tristicolor* (White) (23) and *Anthocoris nemoralis* (Hemiptera: Anthocoridae) (24). As far as we know this is the first study to test the effects of buprofezin on Pentatomidae.

Overall, none of the CSIs caused any sub-lethal effects to BMSB when the insecticides were sprayed on adults. The slight significant differences in survival of adult females did not involve comparisons with the control and seem negligible from the standpoint of BMSB management.

Our results on novaluron and triflumuron support the studies reporting no negative impacts of BPUs to adult insects, unless the active ingredients are ingested (25). Effects on fecundity and hatch rate have been described on adults of *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae), which were fed with a diet incorporating novaluron at 600 ppm. However, effects on insects exposed to foliar spray residues were significant only for 1-day old adults and not for older bugs (14). Moreover, continuous exposure of adult Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), to novaluron-treated foliage did not affect either their longevity or fecundity. Egg viability, which was severely decreased by the insecticide, was restored after adult beetles spent a few days on untreated leaves (26). Kamminga et al. (3) investigated the activity of diflubenzuron and novaluron on BMSB adults by feeding them with insecticide-treated green beans and did not find any effects either on bug survival or egg hatch

rate. As far as we are aware, there are no studies on sub-lethal effects caused by triflumuron to adult insects.

A reduction of oviposition rate and egg viability has been reported for buprofezin in adult homopterans by Ali et al. (27), who exposed 3rd instars of *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae) to sub-lethal concentrations. On the other hand, when adults were treated with buprofezin, no significant effects were reported on *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (28) and *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (29). Moreover, exposure of adult *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae) to buprofezin residues had no significant effect on the biological parameters of the parasitoid (30).

Using BPUs as a standalone BMSB tool in orchards is unlikely to provide effective management, due to the lack effect on adults and potential issues with the speed of insecticidal action. Therefore, field applications of novaluron, triflumuron and possibly other BPUs would likely need to be considered in combination with other IPM methods. Notwithstanding, the long-lasting activity of BPUs could be exploited to reduce damage by BMSB nymphs without the need for frequent treatments, which is a major problem of neurotoxic active ingredients (4). Indeed, the residual activity of most neurotoxic insecticides tested on BMSB lasts for less than three days (31), whereas the persistence of insecticidal activity of triflumuron residues aged on plants up to 21 days has been demonstrated on BMSB nymphs by laboratory bioassays (13). Moreover, the environmental persistence of CSIs has been well documented by analyses of residue concentrations (32, 33).

Systems such as sprays at the orchard perimeter (34) and attract-and-kill (35), which are aimed at suppressing the invading bugs before they enter orchards, would particularly benefit from long-lasting BPU treatments aimed at decreasing nymphal populations and hampering the reproduction cycle that can occur within some orchards. Finally, the inclusion of BPUs in BMSB control strategies

could help to restore, at least in part, the rotation of insecticide chemistries, which is key pillar of IPM aimed at delaying the selection of resistant pest strains (9, 36).

While BPUs are usually considered more compatible with IPM in comparison with most broad-spectrum neurotoxic active ingredients (10, 25), they impact on biological control agents. Detrimental effects have been reported on nymphs of predatory bugs (22, 37) and on ladybeetle larvae (38). Furthermore, some sub-lethal effects were found on adult parasitoids either after direct contact with residues (39) or when parasitized eggs of the hosts were treated (40). Therefore, the possible detrimental effects of BPUs on natural enemies of BMSB seem worthy of further investigation to check the compatibility of these insecticides with biological control agents, especially egg parasitoids, using both augmentative (41) and classical (42) approaches.

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Figure Caption

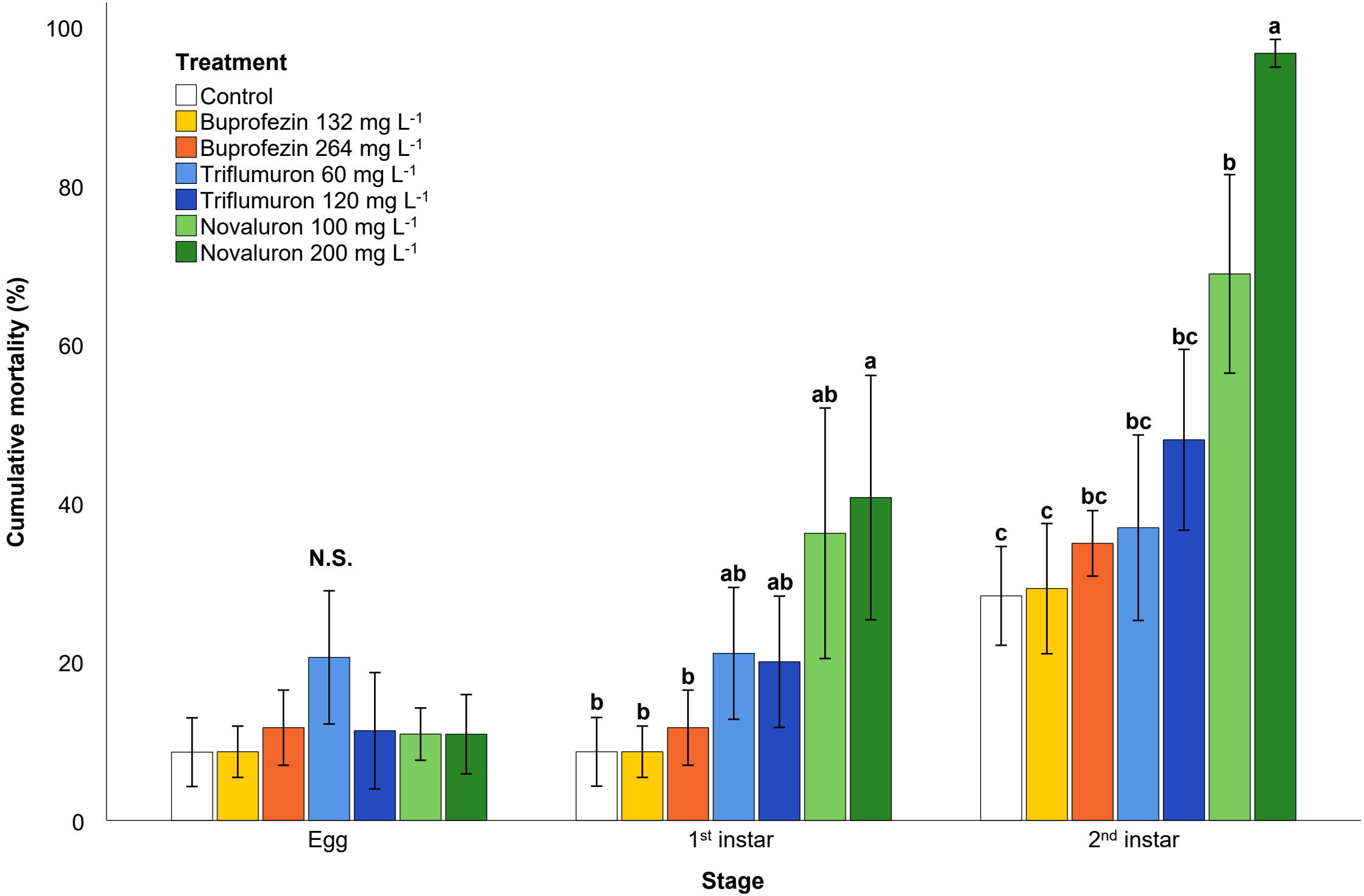
Figure 1. Mortality by developmental stage of *Halyomorpha halys* when egg masses were sprayed with CSI insecticides. Vertical lines indicate standard errors of the means. Different letters indicate significant differences among treatments, which were compared within each developmental stage because of significant interaction of treatment × stage (Bonferroni sequential adjustment, $P<0.05$).

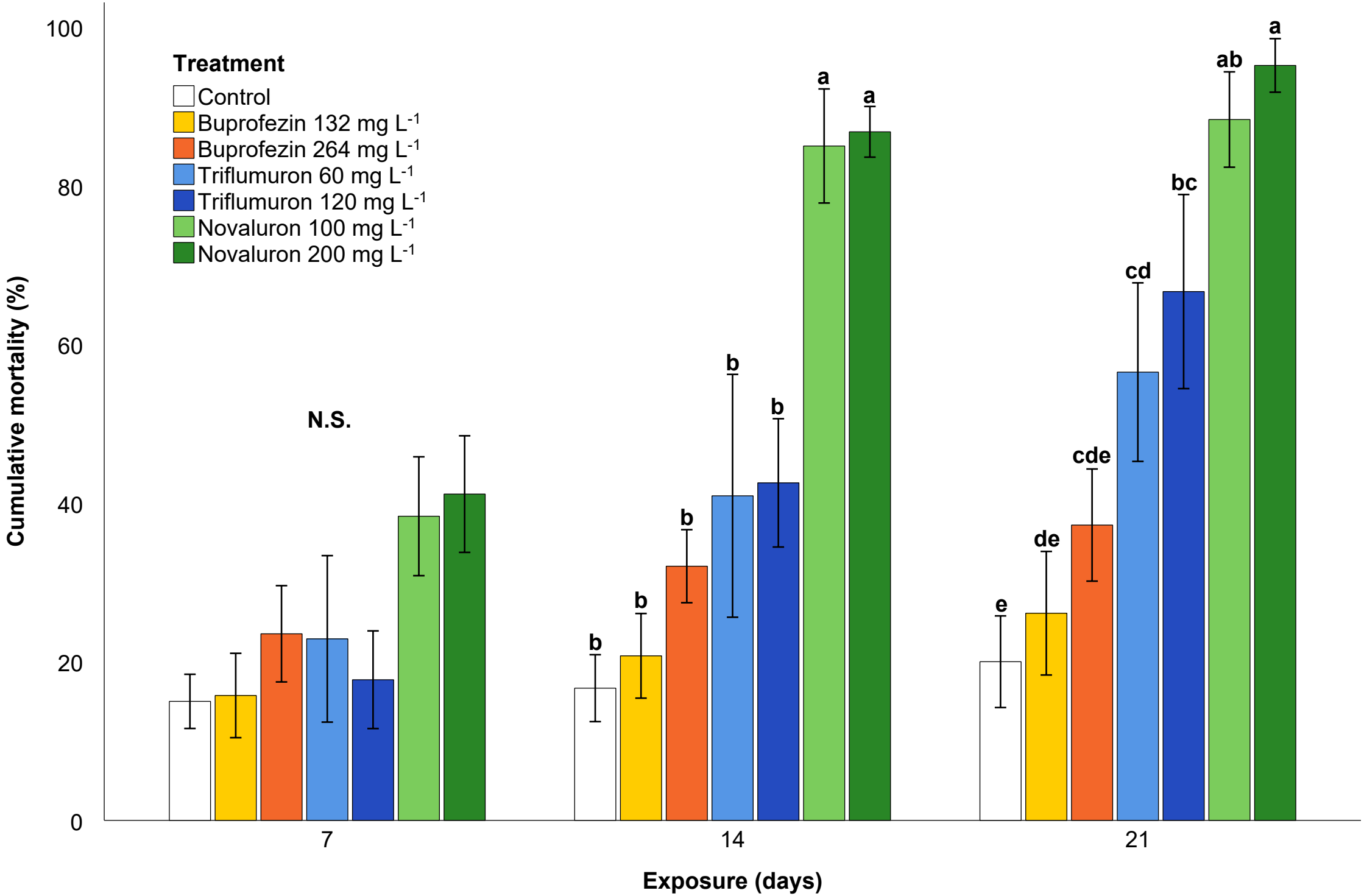
Figure 2. Mortality of *Halyomorpha halys* nymphs exposed for 7, 14 and 21 days to CSI insecticide residues on potted peach plants. Vertical lines indicate standard errors of the means. Different letters indicate significant differences among treatments, which were compared within each exposure interval because of significant interaction of treatment × exposure interval (Bonferroni sequential adjustment, $P<0.05$).

Figure 3. Cumulative survival of *Halyomorpha halys* adults (both sexes pooled, N= 80 per treatment) sprayed with CSI insecticides. Different letters indicate significant differences among survival curves as detected by log-rank (Mantel-Cox) test followed by Holm-Sidak multiple comparison procedure ($P<0.05$).

Figure 4. Cumulative survival of *Halyomorpha halys* females (N= 40 per treatment) sprayed with the CSI insecticides. Different letters indicate significant differences among survival curves as detected by log-rank (Mantel-Cox) test followed by Holm-Sidak multiple comparison procedure ($P<0.05$).

Figure 5. Cumulative survival of *Halyomorpha halys* males (N= 40 per treatment) sprayed with CSI insecticides. Log-rank (Mantel-Cox) test did not detect significant difference among survival curves (P <0.05).


Treatment
Control
Buprofezin 132 mg L-1
Buprofezin 264 mg L-1
Triflumuron 60 mg L-1
Triflumuron 120 mg L-1
Novaluron 100 mg L-1
Novaluron 200 mg L-1
Cumulative mortality (%)
100
80
60
40
20
0
N.S.
b
b
b
ab
ab
ab
a
c
c
bc
bc
bc
b
a
Egg
1st instar
2nd instar
Stage


Treatment
Control
Buprofezin 132 mg L-1
Buprofezin 264 mg L-1
Triflumuron 60 mg L-1
Triflumuron 120 mg L-1
Novaluron 100 mg L-1
Novaluron 200 mg L-1
Cumulative mortality (%)
100
80
60
40
20
0
N.S.
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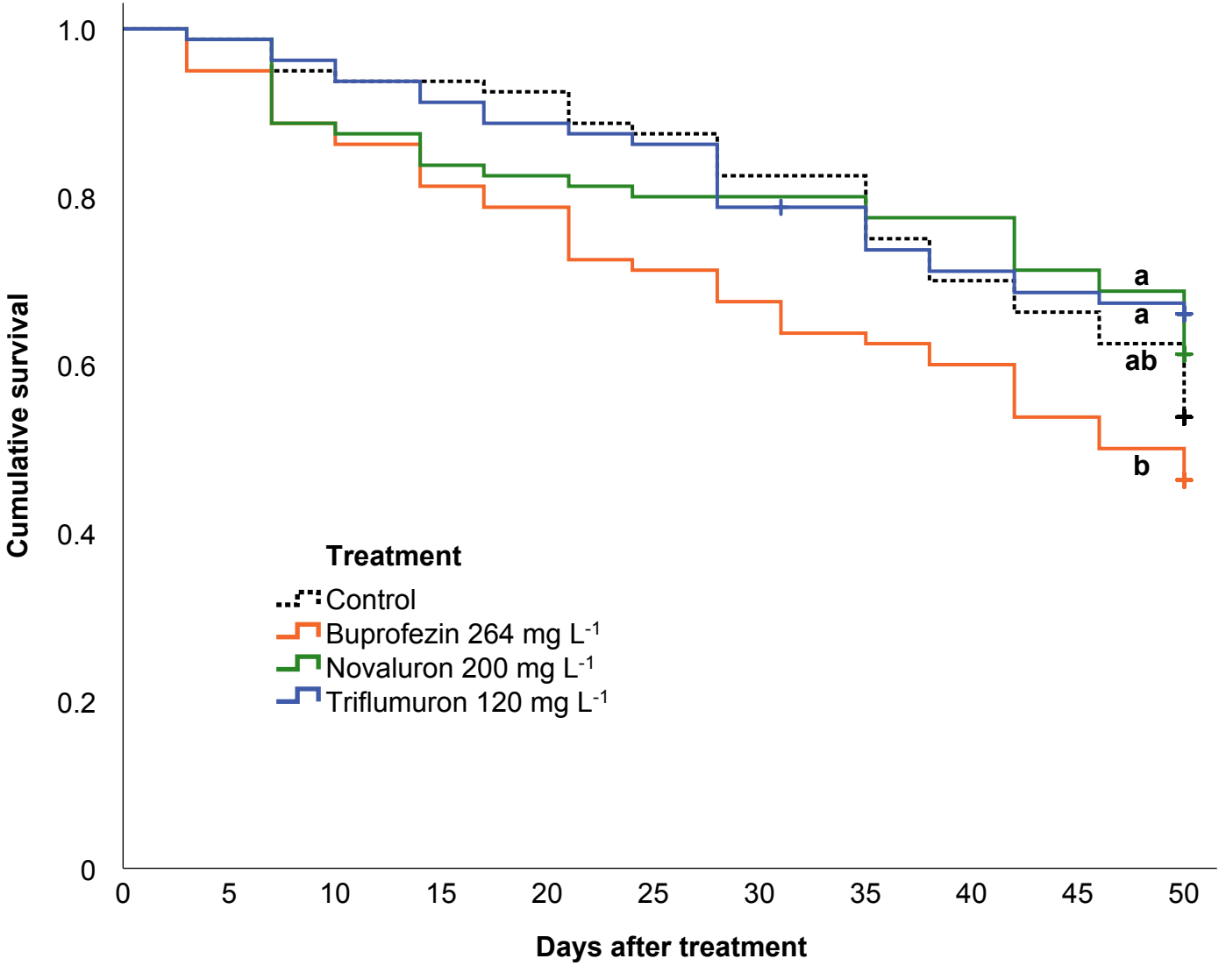
Exposure (days)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Cumulative survival
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Days after treatment
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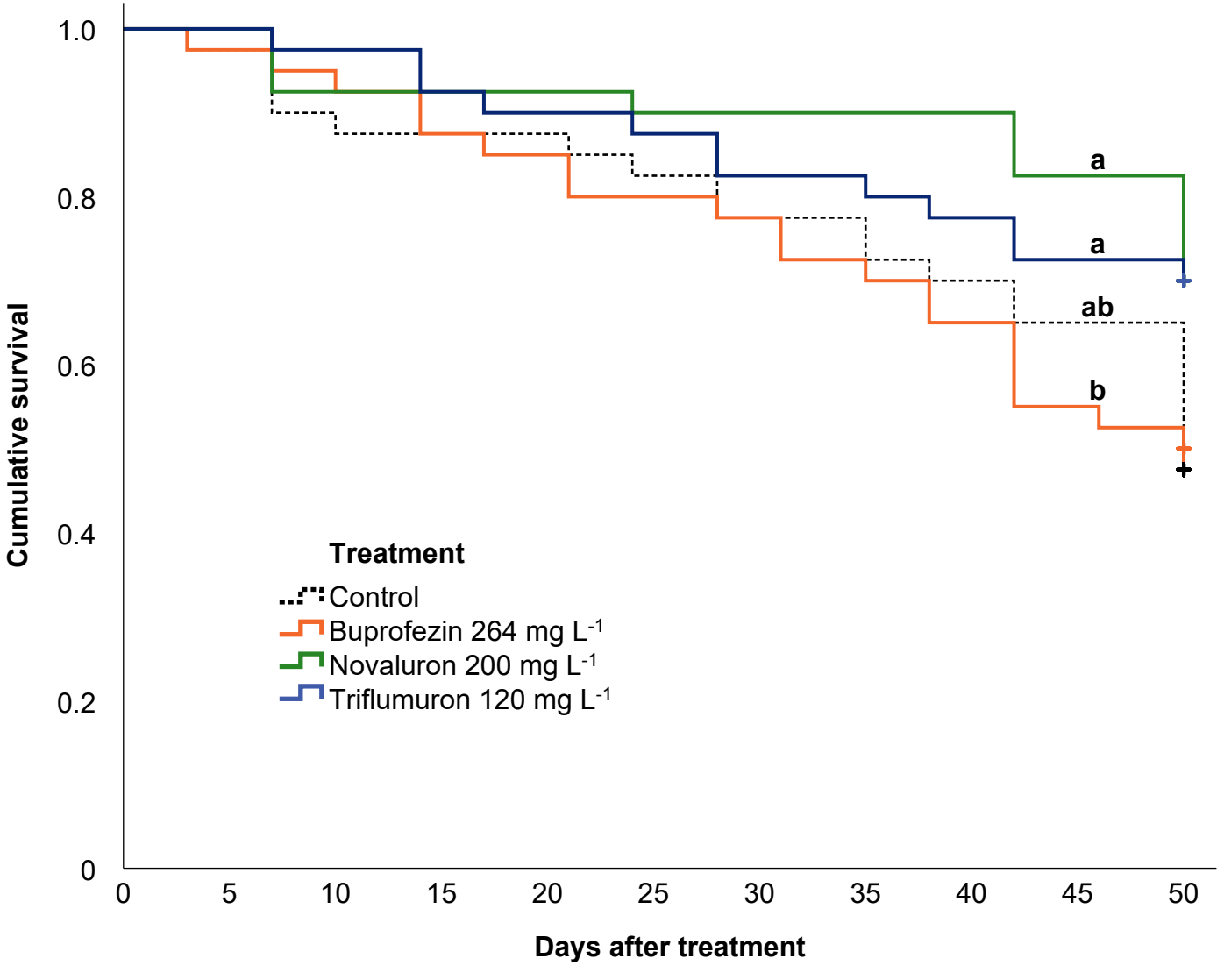
Treatment
Control
Buprofezin 264 mg L-1
Novaluron 200 mg L-1
Triflumuron 120 mg L-1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Treatment
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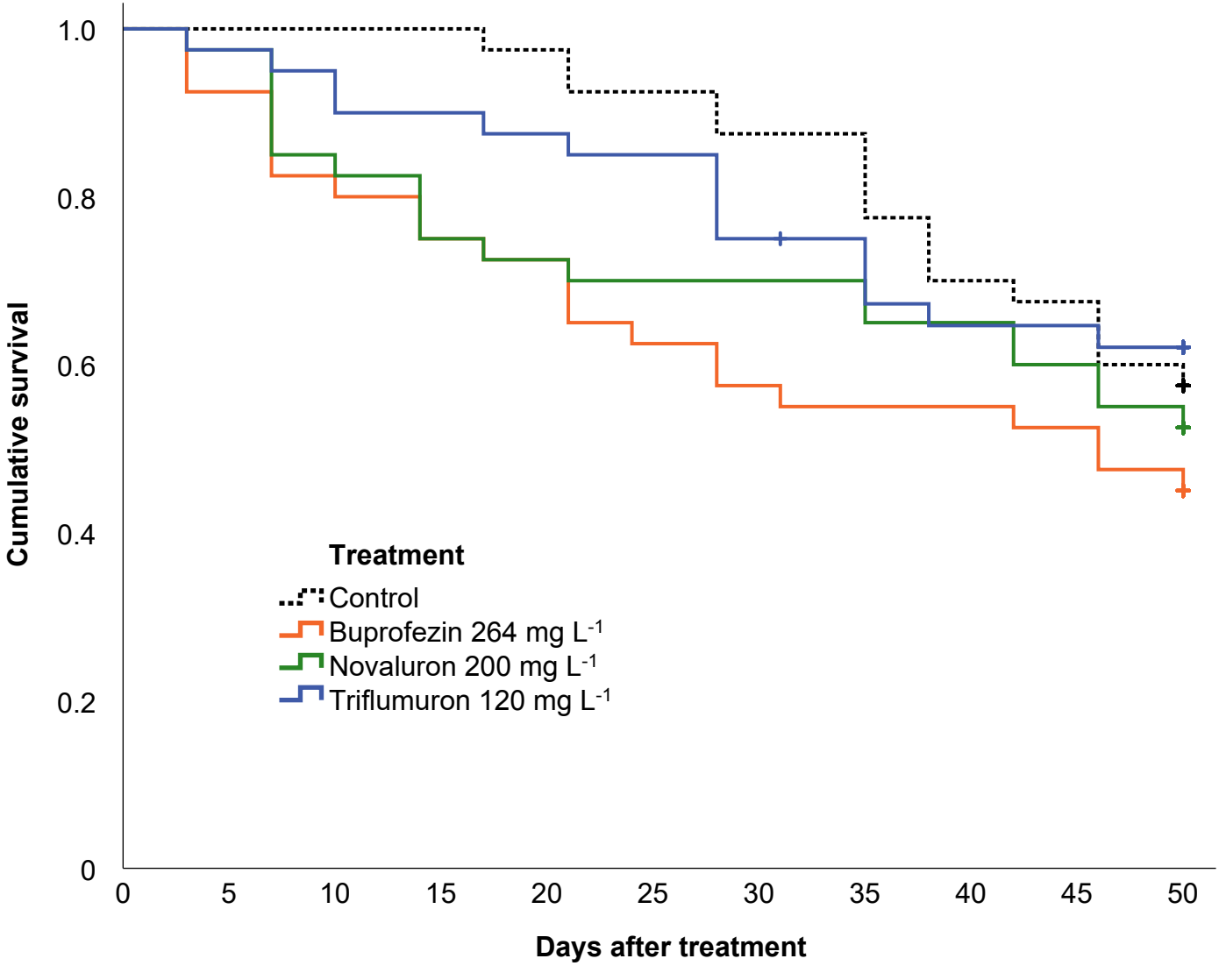
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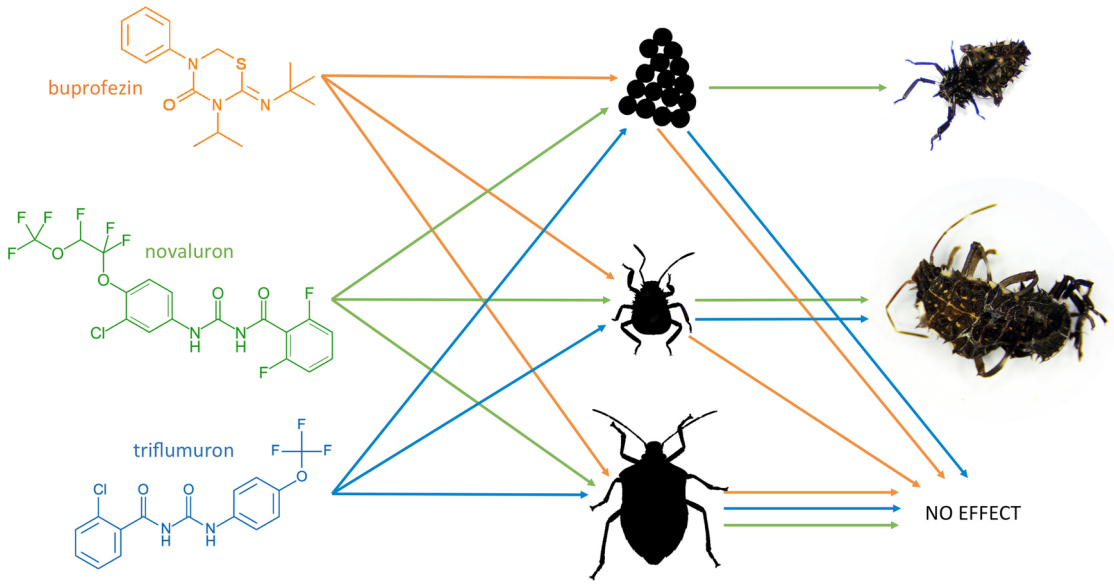
Days after treatment
Treatment
Control
Buprofezin 264 mg L-1
Novaluron 200 mg L-1
Triflumuron 120 mg L-1



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Effects of three chitin synthesis inhibitors on egg masses, nymphs and adults of *Halyomorpha halys* (Hemiptera: Pentatomidae)

Graphical abstract text

The activity of the chitin synthesis inhibitors buprofezin, novaluron and triflumuron was investigated on egg masses, 3rd instar nymphs and adults of *Halyomorpha halys*. Novaluron caused high mortality on nymphs emerged from sprayed egg masses or exposed to residues on potted peach plants. When sprayed on BMSB adults, none of the chitin synthesis inhibitors affected survival, fecundity or egg hatching.

Table 1. Insecticides used for the bioassays.

Active ingredient	Commercial name	Active ingredient concentration in the commercial products	Concentrations tested	
Buprofezin	Applaud plus (Sipcam Italia, batch no. TA1601)	250 g kg^{-1}	264 mg L^{-1} (= 106 g Applaud Plus/100 L)	132 mg L^{-1} (= 53 g Applaud Plus/100 L)
Novaluron	Rimon 10 EC (Adama Makhteshim, batch no. 40191262)	100 g L^{-1}	200 mg L^{-1} (=200 ml Rimon 10 EC /100 L)	100 mg L^{-1} (=100 ml Rimon 10 EC /100 L)
Triflumuron	Alsystin 48 SC (Bayer Crop Sciences, batch no. EZ01929602)	480 g L^{-1}	120 mg L^{-1} (=25 ml Alsystin 48 SC / 100 L)	60 mg L^{-1} (=12.5 ml Alsystin 48 SC/100 L)