Evaluating Impacts of Brown Marmorated Stink Bug on Non-fruiting Nursery Crops[1]

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Abstract

Halyomorpha halys (Stål), the brown marmorated stink bug or BMSB, has become a major pest and nuisance since it arrived in the U.S. in 1996. This insect can feed on approximately 200 different plant species, many of which are important ornamental crops. BMSB's preference for fruits and the damage they cause is known, but it is not known how their presence on non-fruiting woody ornamentals can impact vegetative growth. The first study confined adult BMSB to branches of established stock block trees of five plant species for one month. Four measurements were taken to assess plant growth: branch length, leaf area, number of leaves, and dry weight. The second study confined adult BMSB on seedlings of eight plant species for one or two months. In this study, the same four measurements were taken as well as the change in trunk diameter. Overall, there were no significant differences in plant growth and only some marginal differences between branches or seedlings caged with BMSB versus no BMSB (control) after one or two months. Adults laid eggs on all plants species in both trials, and nymphs were observed to develop to the 2nd instar stage in the seedling trial on all the plant species except for maple. Confinement of BMSB on branches of well-established nursery plants or on newly planted seedlings had little or no impact on growth over a two month exposure.

Index words: *Halyomorpha halys,* Hemiptera, ornamentals, seedling, vegetative growth.

Species used in this study: Brown marmorated stink bug (*Halyomorpha halys* Stål); Dolgo crabapple (*Malus* sp.); Yoshino cherry (*Prunus yedoensis* Matsum.); Japanese elm (*Ulmus davidiana* Planch.); Siberian elm (*Ulmus pumila* L.); Ginkgo (*Ginkgo biloba* L.); Washington hawthorn (*Crataegus phaenopyrum* (L.f.) Medik); 'Ivory Silk' lilac (*Syringa reticulata* Blume); 'Green Mountain' silver linden (*Tilia tomentosa* Moench); 'Crimson Sunset' maple (*Acer truncatum* Bunge); 'Franksred' maple (*Acer rubrum* L.)

Significance to the Horticulture Industry

The brown marmorated stink bug (BMSB) is an economic pest of many agricultural crops, causing up to 90% loss in stone fruits (Leskey et al. 2012). In nurseries, the BMSB is more often found on woody ornamental plants with mature fruit than without (Martinson et al. 2015) and their feeding causes disfigurement and brown spots in fruit (Leskey et al. 2012). However, nurseries may sell plants before the fruiting stage or prune fruits off larger plants to promote foliage production; it is not known how BMSB might affect the vegetative growth of fruitless plants. This study explored whether vegetative growth was impacted by the presence of BMSB among several common ornamental crops. Little or no changes were observed in growth of all plant species at one to two months, the period when differences might be most apparent before the plant outgrows feeding damage. Growers can use this information to help decide on management when they find BMSB on their non-fruiting nursery crops.

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Introduction

The brown marmorated stink bug (BMSB) arrived in the U.S. in 1996 and has become established in 43 states (StopBMSB 2017). Native to eastern Asia, it causes major crop losses in agriculture (Rice et al. 2014). By 2010, the BMSB became a concern in the nursery industry (Leskey et al. 2012). Nursery fields are often located by other agricultural fields, and high numbers of adult BMSB have been found along nursery field edges that bordered soybean (Venugopal et al. 2015). This suggests movement of BMSB from agronomic crops and into nursery fields. Within nurseries, more BMSB can be found on woody ornamental trees with mature fruit; removal of fruits from *Syringa*, *Malus*, and *Amelanchier* spp. trees suppresses BMSB colonization (Martinson et al. 2015). Thus, BMSB abundance is strongly influenced by fruit presence. This may occur because BMSB survive and develop better on diets of mixed fruit and foliage than foliage alone (Acebes-Doria et al. 2016).

Of the approximately 200 reported host plants of BMSB, many are important nursery crops (Bergmann et al. 2014). Plants from the genus *Syringa*, *Malus*, *Ulmus*, *Acer*, and *Tilia* are some of the preferred ornamental hosts of BMSB (Bergmann et al. 2016). In that study, 123 out of 254 species/cultivars tested are partial hosts. A partial host supports at least one life stage of BMSB but not all, and could be important as BMSB move through a landscape. Since BMSB can feed on a wide range of ornamental hosts, the impacts to the plant are of concern. BMSB feed on fruits of crabapple, hawthorn, and serviceberry, which is associated with fruit disfigurement and overall plant wilting (Leskey et al. 2012). BMSB feeding on the bark of *Malus*, *Ulmus*, and *Acer* results in increased sap flow that attracts ants and wasps (Martinson et al. 2013). The fact that sugar is liberated from the trunks might make the plant vulnerable to pathogens. Lastly, BMSB is known to vector *Paulownia* witches' broom disease (Paulownia Witches'-Broom *Phytoplasma*) in Asia. So far, no cases of *Paulownia* witches' broom have been reported in the U.S. (Rice et al. 2014).

While damage reports exist, there is no information on how BMSB might affect the vegetative growth of non-fruiting nursery plants. Some nurseries focus on foliage production and prune fruits, have ornamental cultivars bred not to produce fruit, or ship plants off before bearing fruit. Given that non-fruiting ornamentals are present, large nymphal and adult BMSB are still found in lower abundance on woody ornamentals without fruit (Martinson et al. 2015), and growers have observed BMSB adults on the shoot tips of seedlings in the field during fall when BMSB populations are high (Jana C. Lee, unpublished data). This is a concern because feeding by other piercing-sucking insects has reduced plant growth. For example, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), will feed on meristematic tissue in the absence of fruits, which results in bud damage and loss of apical dominance to cotton (Strong 1970). Other piercing-sucking insects have affected plant growth for a limited duration. Cotton seedlings recovered lost growth within eight weeks of tarnished plant bug feeding (Strong 1970). Leaf growth on grapevines was reduced three days after leafhopper infestation, but no effects were apparent after 73 days (Lenz et al. 2009).

Before growers decide whether BMSB control methods are needed, it is important to understand the damage BMSB could cause when exposed to leaf, stem, and meristematic tissue. Our objective was to evaluate potential impacts of BMSB by monitoring plant growth on common nursery species. We monitored young seedlings, which are of primary concern to growers, and branches of infrequently sprayed mature plants, which may harbor more of these pests. Impacts were evaluated at one or two months after exposure when differences might be expected.

Materials and Methods

Adult BMSB used in both studies were collected from holly trees in the Willamette Valley of Oregon in May and June. They were maintained at 22 C (71.6 F), 16 hour day length, 60% relative humidity, and held in groups of approximately 50 adults in 29.5 cm by 29.5 cm by 30.5 cm (11.6 in by 11.6 in by 12 in) mesh containers (Bug Dorm, BioQuip, Rancho Dominguez, CA) with carrots, jelly beans, raw shelled peanuts, and water.

Impact in a nursery field. Six common nursery species that are known hosts of BMSB were evaluated for possible damage: Dolgo crabapple, Yoshino cherry, Japanese elm, Washington hawthorn, 'Green Mountain' silver linden, and 'Crimson Sunset' maple. This study used 1.5 to 2.5 m (5 ft. to 8 ft.) tall, established stock block trees, which are used to take scion cuttings for propagation. These trees were not sprayed with pesticides during the experiment and have enough foliage to enable survival of caged BMSB. Trees were irrigated 1 to 3 times a week, and fertilized at least once during the growing season. The study reported here examines how BMSB exposure to the meristem impacts plant growth. Initially, we wanted to test BMSB directly on seedlings planted in the field, but were concerned that BMSB would not survive being caged to a sparsely vegetated seedling exposed to heat, insecticides, and other nursery operations.

Each plant species had six replicate trees with a paired BMSB and control treatment. Each treatment was enclosed in a mesh bag approximately 137 cm long by 42 cm wide (54 in long by 16.5 in), with four branches of apple, hawthorn, or elm, or three branches of cherry, silver linden, or maple. Over roughly 93% of the branches remained unexposed. The number of branches per bag was based on leaf size and foliage density to support BMSB. Each branch was marked 33 cm (13 in) from the tip with a permanent maker. Each tree contained one bag with six adults (three pairs) and a control bag with no BMSB present. Six adult BMSB per tree is in the high end of what has been observed on mature woody ornamental trees in the Pacific Northwest U.S. (Victoria P. Skillman, unpublished data). Inside every mesh bag, a plastic 14 mL (0.5 fl oz) test tube filled with water and plugged with cotton was tied to a branch. All bags were set up on June 7 and 9, 2016. Live BMSB were observed in all bags two weeks later. Since BMSB can hide, they could not be fully accounted for until after one month when all branches were clipped, and bags were taken to the lab.

To evaluate any BMSB damage, each branch was measured for any increase in branch length, leaf area, number of leaves, and dry weight. The change in branch length was determined by re-measuring the branch from the marking to the tip and subtracting the starting length of 33 cm (13 in). Next, each individual leaf after the marking on the branch was removed without its petiole, and area determined using a leaf area meter (LI-3000, LI-COR, Lincoln, NE). All leaves from a branch were counted and the area of each leaf was added together for total leaf area of the branch. Lastly, all leaves, petioles, and stem from a branch were placed in a brown paper bag, dried at 60 C (140 F) for seven days and weighed.

The growth in branch length, leaf area, number of leaves, and dry weight of each branch were averaged for each replicate. A paired t-test compared BMSB and control treatments for each of the four plant growth measurements of each plant species. Tests were considered significant at $\alpha = 0.05/4 = 0.0125$ with a Bonferroni correction (Sheskin 2007) because four measurements were taken from the same sample. Untransformed data met assumptions of a t-test. Analyses were done in R-studio 3.3.1 (Bug in Your Hair, R Development Core Team, 2016).

Impact on nursery seedlings. Eight common nursery plants species were evaluated: Siberian elm, Ginkgo, 'Ivory silk' lilac, 'Franksred' maple as well as the apple, cherry, hawthorn, and linden used in the field study. Bare-root seedlings of each plant species were potted in 4L (#1) pots of Professional Growing Mix (Sun Gro Horticulture, Bellevue, WA) in early May 2016 and maintained in a greenhouse throughout the whole experiment. Trials were initiated once the seedlings had rooted and grown leaves in late June and were roughly 20 to 90 cm (7.8 to 36 in) in height. Then each seedling was enclosed in a mesh bag, from 70 cm long by 30 cm wide (27.5 in long by 11.8 in wide) to 137 cm by 42 cm (24 in by 16.5 in), depending on seedling size. Half of the seedlings received four adult BMSB (two pairs) and the other half had none for a control. Four adult BMSB per plant is on the high end of what has been observed on young seedlings in the field (Jana C. Lee, unpublished data). Each plant species had 10 controls and 10 BMSB replicate pots, except for apple which had 7 and 8 and linden had 8 and 9, respectively. Each ginkgo seedling was exposed to four adult males due to a low number of females available in our BMSB colony. Seedlings were assigned to BMSB or control treatments to keep plant sizes between both treatments similar at study initiation. Seedlings were irrigated three times a week by adding water to a tray under the pot. Since BMSB could drink from the moist soil, no extra water was provided. After two weeks, an average of 1.25 to 3.33 alive adults were visible (not hiding) per seedling under the mesh depending on the plant species. After one month in late July, approximately 5 BMSB and 5 control seedlings per plant species were measured for plant growth and live and dead BMSB were counted. The remaining seedlings were checked after two months in late August.

Seedlings were evaluated for branch length, leaf area, number of leaves, and dry weight as described in the field trial, and also trunk diameter. To assess change in diameter, the trunk was marked 5 cm (2 in) above the soil with a permanent marker and measured with a digital caliper just before it went into the mesh bag. The same point was then re-measured after one or two months and subtracted from the starting diameter for the change in diameter. All branches were removed from the main stem and measured.

Branch length, leaf area, number of leaves, and dry weight were summed together for a given seedling (replicate), while there was only one measurement for change in trunk diameter per seedling. For each plant species, a MANOVA tested the effect of treatment, time (month 1 or 2), and treatment by time interaction on five plant growth measurements as simultaneous dependent variables. If the treatment by time interaction was not significant, it was deleted to simplify the model. Untransformed data met the assumptions of the MANOVA, *i.e.*, independence between BMSB/control samples, and lack of multicollinearity between dependent variables. Analyses were done in in JMP 12.1.0 (SAS 2015).

BMSB survival and post-hoc analyses. Because BMSB survival varied at the end of the experiment, *post-hoc* analyses examined if impacts might be more apparent amongst plants with surviving BMSB. In both field and seedling studies, BMSB treatments were grouped as having: 1) no surviving BMSB, or 2) surviving nymphs or adults after one or two months. An average of 31 to 83% of adults survived per seedling depending on the plant cultivar; reasons for poor survival are in the Results and Discussion section. There were insufficient samples of both "no surviving" or 'surviving" to analyze each plant species separately; all plant species were pooled together for analyses. In the field study, a t-test compared the no surviving versus surviving BMSB groups for each plant growth parameter, $\alpha = 0.0125$. To account for differences in plant size between species, t-tests used matched responses as the dependent variable, *i.e.*, difference in leaf area of BMSB and its control pair for a given replicate. In the seedling study, a MANOVA tested the effect of survival status and time on the five growth measurements; the non-significant status by time interaction term was dropped from the model. To account for different plant sizes, the difference in plant growth was compared with respect to its control (value of no/surviving BMSB seedling – average value of control seedlings of the given month). In the final analysis, the number of surviving BMSB among BMSB-treated seedlings was the independent variable in a multiple regression analysis. The five plant growth measurements with respect to their controls were the dependent variables. Untransformed data met the assumptions of models, and *post-hoc* analyses were done in JMP 12.1.0 (SAS 2015).

Results and Discussion

Impact in the nursery field. Overall, there were no significant differences between branches caged with and without BMSB for one month in the field for dry weight, growth in branch length, leaf area, or number of leaves (Fig. 1). This is not surprising for large trees. The large trees might enact a tolerant or over-compensatory response (Isaacs et al. 2012) to herbivore feeding on a few branches when over 93% of the branches remained unexposed. There were five marginal differences out of 24 comparisons, $0.0125 < P < 0.05$. In apple, the dry weight was marginally higher in BMSB than control treatments (Fig. 1a). In hawthorn (Fig. 1d), the dry weight was marginally higher and number of leaves was marginally lower in BMSB treatments. In linden, branch growth and number of leaves were marginally lower in BMSB treatments (Fig. 1e). When marginal effects appeared, the trends were inconsistent between BMSB and control treatments. There was no visible discoloration damage to leaves in the presence of BMSB, which might occur with a piercing-sucking pest.

Impact on nursery seedlings. There were no significant differences between seedlings with BMSB or control treatments when all five plant growth measurements were taken into account (Table 1). Exposure to BMSB marginally impacted growth in apple ($P = 0.057$) as three out of five measurements were numerically higher in BMSB than control treatments (Fig. 2a). Also, there was a slight treatment by time interaction observed in maple ($P = 0.051$). BMSB impacted maple growth in month 1 ($F_{5,4} = 13.2$, $P = 0.0134$) but not in month 2 ($F_{5,4} = 0.72$, $P = 0.644$). In month 1, four out of five measurements were numerically higher in BMSB than control treatments (Fig. 2b). No obvious visual damage was observed in the presence of BMSB.

Marginal effects only appeared consistently in apple during both the field and seedling studies (Fig. 1a, 2a), but not with hawthorn, linden, or maple. If the apple results are

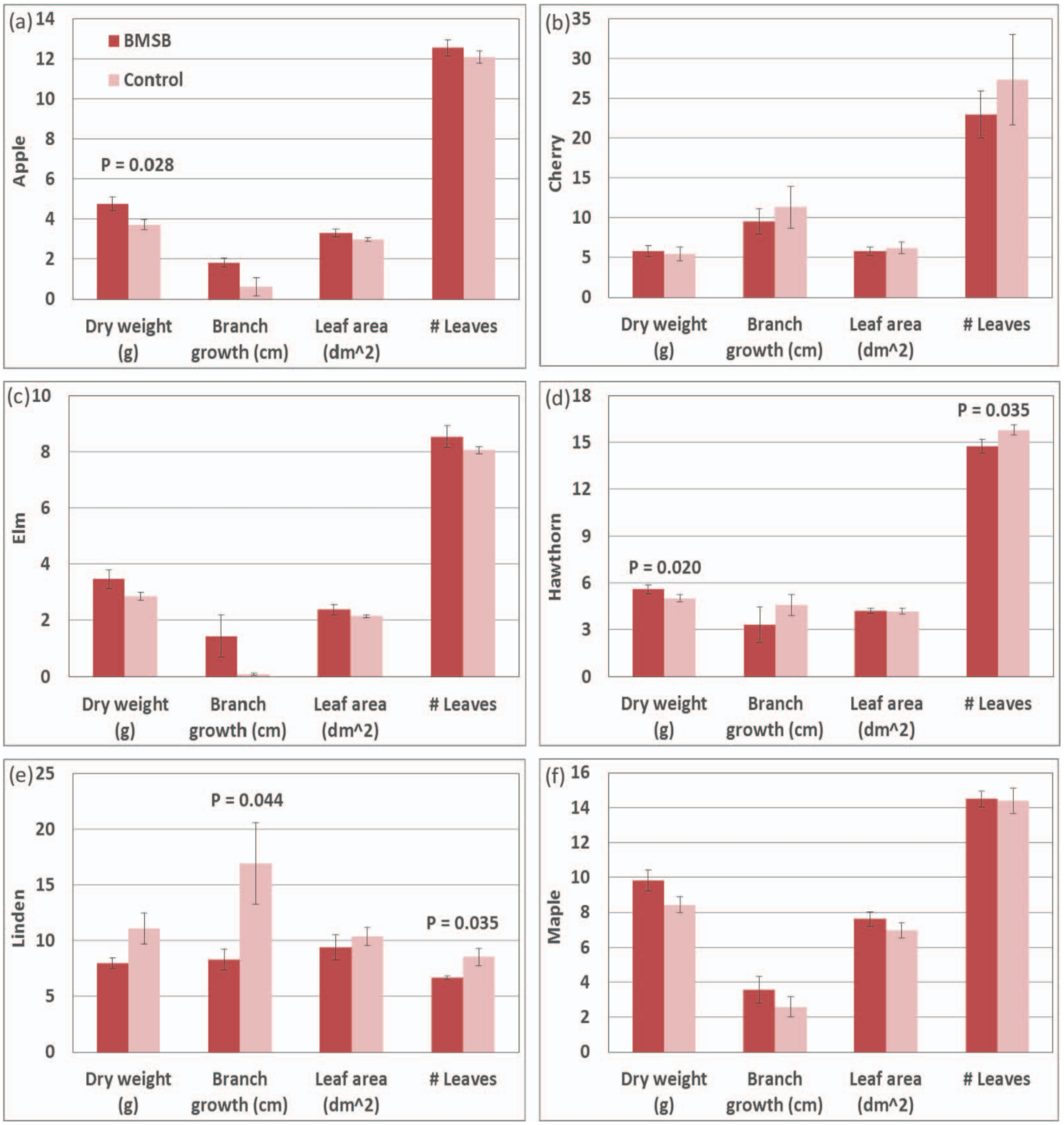

Fig. 1. **The average (± standard error) weight, branch growth, leaf area and number of leaves among brown marmorated stink bug (BMSB) and control treatments in the field trial with (a) apple, (b) cherry, (c) elm, (d) hawthorn, (e) linden, and (f) maple. Marginal P-values from paired t-tests are in graphs, no comparisons were significant at $\alpha = 0.0125$.**

indicative of anything, they may suggest that the presence of BMSB slightly increases vegetative apple growth. This has been observed with another piercing-sucking insect, feeding by leafhoppers stimulated lateral leaf growth in grapevines (Candolfi et al. 1993). Future studies might examine linden more closely, growth was marginally or numerically lower in BMSB than control treatments in the field (Fig. 1e), and numerically lower though not significant in BMSB seedlings for all growth measurements (Table 1).

BMSB survival and post-hoc analyses. Surviving BMSB adults were observed in both the field and seedling trials after two weeks (Materials and Methods section), but few adults survived after one month and none after two months (Table 2). Also, eggs were laid and 2nd instar nymphs were found, indicating that BMSB reproduced on these hosts before dying and some nymphs were able to survive (Table 2). This study exposed plants to BMSB to assess plant growth; adult BMSB likely fed on the plants because they survived for at least two weeks with no other food source. *Post-hoc* analyses further compared plants with greater BMSB survival that possibly experienced more feeding to plants with lower BMSB survival. In the field study, plants with surviving BMSB at the end did not differ from plants with no surviving BMSB in terms of plant growth (dry weight $t_{34} = -1.5$, $P = 0.14$; branch length $t_{34} = 1.73$, $P = 0.091$; leaf area $t_{34} = -1.0$, $P = 0.32$; leaves $t_{34} = 0.19$, $P =$

Table 1. **Average plant measurements and standard error (SE) for brown marmorated stink bug (BMSB)-exposed and control treatments, and MANOVA results for each plant species in a seedling trial.**

		Average ± SE					MANOVA results			
Species	**Treat.**	**Diam. change (mm)**	**Dry weight (g)**	**Branch length (dm)**	**Leaf area (dm^2)**	**# Leaves**	**Effect**	**F**	**df**	**P**
Apple	BMSB	0.95 ± 0.12	9.1 ± 1.1	6.8 ± 0.9	6.4 ± 0.9	39.4 ± 5.0	Treatment	3.49	5,8	0.057
	Control	0.66 ± 0.15	7.7 ± 1.1	8.1 ± 1.1	5.6 ± 0.9	40.9 ± 5.1	Time	1.98	5,8	0.186
Cherry	BMSB	1.11 ± 0.31	14.2 ± 1.3	9.6 ± 0.9	9.6 ± 1.3	55.3 ± 5.9	Treatment	1.18	5,13	0.368
	Control	0.76 ± 0.16	14.9 ± 1.6	12.1 ± 1.3	10.9 ± 1.2	62.9 ± 6.2	Time	1.90	5,13	0.164
Elm	BMSB	1.41 ± 0.29	17.9 ± 1.8	17.6 ± 1.3	12.3 ± 1.2	195.2 ± 19.9	Treatment	0.22	5,13	0.947
	Control	1.41 ± 0.25	19.2 ± 2.1	19.1 ± 1.6	13.7 ± 1.5	216.8 ± 28.7	Time	2.41	5,13	0.093
Ginkgo	BMSB	0.58 ± 0.16	6.9 ± 0.7	2.3 ± 0.3	4.6 ± 0.7	41.5 ± 8.4	Treatment	0.50	5,13	0.773
	Control	0.57 ± 0.13	7.6 ± 0.5	2.8 ± 0.2	3.9 ± 0.4	36.6 ± 8.5	Time	5.47	5,13	0.006
Hawthorn	BMSB	1.18 ± 0.24	8.9 ± 0.6	9.7 ± 0.7	5.1 ± 0.4	39.8 ± 10.9	Treatment	1.36	5,13	0.301
	Control	0.90 ± 0.13	8.9 ± 0.8	10.1 ± 1.1	5.5 ± 0.1	65.8 ± 20.1	Time	12.27	5,13	<0.001
Lilac	BMSB	0.41 ± 0.19	13.9 ± 1.9	12.5 ± 1.3	10.6 ± 1.6	54.8 ± 6.9	Treatment	0.48	5,13	0.788
	Control	0.18 ± 0.06	15.4 ± 1.5	12.2 ± 1.8	8.3 ± 1.3	51.7 ± 7.4	Time	0.67	5,13	0.654
Linden	BMSB	0.47 ± 0.18	4.7 ± 0.8	2.7 ± 0.5	3.4 ± 0.4	10.3 ± 1.1	Treatment	0.22	5,10	0.945
	Control	0.55 ± 0.13	5.5 ± 0.9	2.9 ± 0.5	4.2 ± 0.6	12.0 ± 1.8	Time	0.78	5,10	0.589
Maple	BMSB	0.83 ± 0.12	14.0 ± 1.9	6.9 ± 0.8	13.0 ± 1.1	43.4 ± 7.3	Treatment	0.68	5,12	0.648
	Control	1.19 ± 0.20	14.0 ± 1.4	8.3 ± 1.6	10.8 ± 1.2	38.6 ± 5.4	Time	4.70	5,12	0.013
							Treat*Time	3.09	5,12	0.051

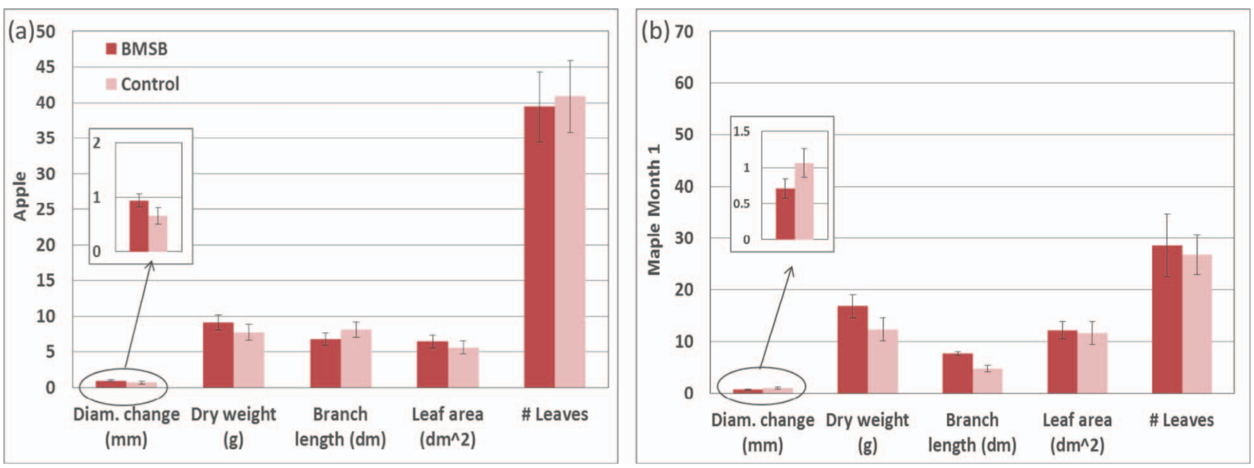

Fig. 2. **The average (± standard error) stem diameter growth, weight, branch length, leaf area and number of leaves among brown marmorated stink bug (BMSB) and control treatments in a seedling trial in (a) apple over both months, (b) maple during month 1.**

Table 2. **The total number of live brown marmorated stink bug (BMSB) adults, egg clusters, and live/dead 2nd instar nymphs present in the field and seedling trials after 1 or 2 months.**[z]

	Field – 1 month			Seedling – 1 month				Seedling – 2 months			
Plant Species	**Live Adults**[1]	**Egg Clusters**	**Live Nymphs**	**Live Adults**[1]	**Egg Clusters**	**Live Nymphs**	**Dead Nymphs**	**Live Adults**	**Egg Clusters**	**Live Nymphs**	**Dead Nymphs**
Apple	18	3	0	0	4	11	0	0	1	0	0
Cherry	9	5	0	3	2	54	0	0	5	52	0
Elm	0	2	0	0	7	9	5	0	7	0	0
Ginkgo	–	–	–	0	–	–	–	0	–	–	–
Hawthorn	18	4	2	0	3	25	0	0	5	0	10
Lilac	–	–	–	0	3	88	0	0	2	1	8
Linden	0	1	0	0	6	38	0	0	5	0	24
Maple	2	0	0	0	3	0	3	0	4	0	0
Total	47	15	2	3	28	225	8	0	29	53	42

[z]Field trials started with 6 adults per replicate and 6 paired replicates. Seedling trials started with 4 adults per replicate and 4-5 replicates per month; ginkgo started with only male adults.

0.851). In the seedling study, seedlings with no/surviving BMSB had similar plant growth outcomes (survival status $F_{5,70} = 1.46$, $P = 0.354$; time $F_{5,70} = 1.27$, $P = 0.287$). The number of surviving BMSB at the end of the trial had no impact on plant growth of a given seedling (Multiple regression $F_{5,71} = 0.74$, $P = 0.596$).

The low survival at one month in the field or greenhouse may be explained by the fact that BMSB nymphs and adults thrive better on mixed diets with fruit rather than single diets and foliage alone (Acebes-Doria et al. 2016, Funayama 2006). While low survival of BMSB may indicate that less feeding occurred on the plant to directly assess feeding damage, the results are helpful for growers. If BMSB does not feed sufficiently on the vegetative parts under no-choice confinement, then BMSB might be expected to have minimal impacts in the field. BMSB are highly mobile (Lee et al. 2014, Wiman et al. 2015), with the ability to select other favorable hosts, and not surprisingly are prevalent on ornamentals with mature fruits in nurseries (Martinson et al. 2015).

In summary, confinement of BMSB on branches of well-established nursery plants during early summer and on newly planted seedlings had little to no impact on growth in one or two months. These results might suggest that branches or seedlings are growing and developing fast enough during the summer to outpace potential damage or that BMSB do not feed sufficiently on vegetative parts of these ornamentals to inhibit growth. While short-term impacts on plant growth do not appear problematic, other concerns with BMSB in nurseries selling non-fruiting crop should be further examined, such as whether feeding makes plants more vulnerable to pathogens or whether shipped plants are likely contaminated with BMSB eggs, nymphs, or adults. Since nurseries may contain patches of ornamentals bearing fruits and are often surrounded by preferred hosts such as hazelnut (Hedstrom 2014), or corn fields (Cissel 2015), BMSB are expected to move through nurseries as they find suitable hosts.

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