Spray Penetration and Natural Enemy Survival in Dense and Sparse Plant Canopies Treated with Carbaryl: Implications for Chemical and Biological Control[1]

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Abstract

Ornamental plant producers often rely on chemical control to manage insect pests. However, cultural practices, such as pruning, can influence plant architecture which may, in turn, affect pesticide penetration. Spray penetration was studied to determine the effect of canopy density on beneficial insect survival following insecticide application and to better understand the implications of canopy density on pest management. Regardless of canopy density or plant species, the interior position of the canopy received less than 8% spray coverage. The middle position of sparse canopies received 288 to 513% more coverage than the middle position of dense canopies. The middle and interior position of dense canopies protected greater than 50% of the convergent lady beetle (*Hippodamia convergens* (Guérin-Méneville) population while only the interior position of dense canopies protected greater than 50% of green lacewing (*Chrysoperla rufilabris* Burmeister) populations.

Index words: beneficial insect, nursery crop, pesticide, plant architecture, woody ornamental.

Species used in this study: China Girl® holly (*Ilex ×meserveae* 'Mesog'); 'Alice' Oakleaf hydrangea (*Hydrangea quercifolia* Bartr. 'Alice'); convergent lady beetle [*Hippodamia convergens* (Guérin-Méneville)]; green lacewing [*Chrysoperla rufilabris* (Burmeister)].

Chemicals used in this study: carbaryl (1-naphthyl N-methylcarbamate, Sevin® SL, Bayer CropScience, Durham, NC).

Significance to the Horticulture Industry

Growers are subject to market pressure to produce plants with dense canopies. Canopy density may affect the efficacy of contact insecticides and the ability to use insecticides and natural enemies simultaneously. Insecticide applications are generally considered more effective on plants with a sparse canopy. In this study, spray coverage within the canopy interior was low regardless of canopy density, indicating that the interior of a plant could serve as a refugium for pest insects but also naturally-occurring biological control organisms during an insecticide application. The canopy interior may also provide a safe place to release natural enemies as part of an augmentative biological control program. The use of natural enemies may be critical to controlling pests, such as scales, that infest the trunk and other interior positions of dense plant canopies, where spray coverage was minimal and in regions or markets implementing insecticide restrictions for pollinator protection.

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Introduction

Market forces, cultural practices, and pest management are inextricably linked during production of ornamental crops. Consumers of woody landscape plants prefer densely-branched plants over ones that are sparse (Glasgow 1999, Jeffers et al. 2009). Therefore, growers endeavor to produce plants with dense canopies through the use of architecture-altering practices such as pruning and plant growth regulators (Cochran and Fulcher 2013, Currey and Erwin 2012, Gilman 2012). However, increasing canopy density can affect pest management. A dense plant canopy can hinder penetration of foliar-applied insecticides to the interior of the canopy (Zhu et al. 2006, 2008). Poor pesticide penetration can lead to problems controlling pests within the plant canopy or directly on the branches, such as scale insects (Hanks and Denno 1993).

Ornamental plants are valued primarily for their aesthetic qualities (Bethke and Cloyd 2009, Sadof and Raupp 1996). Therefore, the economic threshold for an ornamental insect pest is often zero (Klingeman et al. 2000, van de Vrie 1995). For example, a single female bagworm (*Thyridopteryx ephemeraeformis* Haworth) can produce enough offspring to render a plant unmarketable (Horn and Sheppard 1979, Raupp et al. 1989). Conventional chemicals are often the first and only control used in nursery crop production, in part, because they work quickly and can maintain pest populations at acceptable levels with minimal effort from the grower (Bethke and Cloyd 2009, LeBude et al. 2012). Consumer's low tolerance for pest damage often motivates growers to apply pesticides as a preventative with the mindset that they are protecting their crops from pest damage (Briggs et al. 2002, Cho and Ki 1999). If pest populations persist, growers may increase application frequency or the rate of pesticide that they apply (Zhu et al. 2006). However, with adverse public perception of pesticide use, their effects on pollinators and other beneficial insects, and links to secondary pest outbreaks, growers may need to reevaluate their practices (Colin et al. 2004, Falconer 1998, Frank and Sadof 2011, Kher et al. 2013, Montella et al. 2012, Szczepaniec et al. 2011).

It is well documented that natural enemies such as spiders and other predators prefer dense canopies to sparse canopies because dense canopies provide more shelter from heavy rain, other predators, and abundant and diverse food sources (Halaj et al. 2000a, 2000b, Langellotto and Denno 2004). The more complex the plant canopy, the more connectors are available for a predator to move to its prey. Canopy complexity also increases the likelihood that some predators will continue to search for food on a particular plant (Leite et al. 2017, Skirvin 2004). Integrating natural enemies with pesticide application may increase the effectiveness of pest management in dense canopies.

Research on pesticide application to field-grown nursery crops has been conducted, but little information is available on comparing spray penetration into dense and sparse canopies in a container nursery and how natural enemies may be protected from pesticide application in dense canopies (Bache and Johnstone 1992, LeBude et al. 2012, Pan et al. 2016, Sánchez-Hermosilla et al. 2011, Zhu et al. 2006, Zhu et al. 2017a). The objectives of this study were to 1) characterize spray penetration in dense and sparse canopies of select woody ornamental crops and 2) determine if denser canopies protect natural enemies from a foliar-applied contact insecticide.

Materials and Methods

Spray Penetration. Eighteen China Girl® hollies in 11 L (size #3) containers were purchased (SiteOne Landscape Supply, Knoxville, TN) on January 23, 2013 and placed in the North Greenhouse at the University of Tennessee in Knoxville, TN (35°56′46″N 83°56′18″W). On February 18, 2013, branches were counted. To create dense or sparse canopy densities, nine plants were pruned to 35 branches and the other nine were pruned to 75 branches, a 53% disparity, and eight of the most uniform from each treatment group were selected for the experiment.

Twelve 'Alice' oakleaf hydrangeas were grown from 10.2 to 15.2 cm (4.0 to 6.0 in) cuttings taken spring 2012. Plants were potted into size #3 containers filled with 85% pine bark and 15% peat. One week after transplanting, plants were top dressed with 53 g (1.87 oz) of 19N-1.7P-6.6K, 5- to 6-month controlled release fertilizer with micronutrients (Polyon®, Harrell's Inc., Lakeland, FL). In October 2012, plants were placed in a plastic-covered overwintering house until February 4, 2013 when they were placed in a walk-in cooler [~7 C (44 F), intermittent light]. They were watered periodically to prevent desiccation. On March 5, 2013 plants were moved to the North Greenhouse at which time they were pruned to 25.4 cm (10 in) from the substrate surface and again top dressed with 19N–1.7P–6.6K, 5- to 6-month controlled release fertilizer. By March 27, 2013, plants had leafed out and branches were counted. On April 17, 2013, six of the plants were pruned to 11 branches and six were pruned to 19 branches, a 42% disparity, creating sparse and dense canopy densities, respectively. To establish that the disparity in branch number created a disparity in density, hydrangea plant height was measured and then plants were destructively harvested following the experiments. All plant tissue above the substrate surface was oven dried at 61 C (141 F) for 72 hours. Once dry, mass was recorded. Density was calculated as shoot dry mass per unit of height. Hydrangea canopy measurements comparing shoot dry mass to height established that density between dense and sparse plants was different at 1.96:1 and 1.29:1, respectfully (P-value = 0.0001), supporting that branch differences and density are correlated (data not shown). For both the holly and the hydrangea, while the disparity in branch number between dense and sparse canopies was near 50%, both represented plant architecture available in the marketplace.

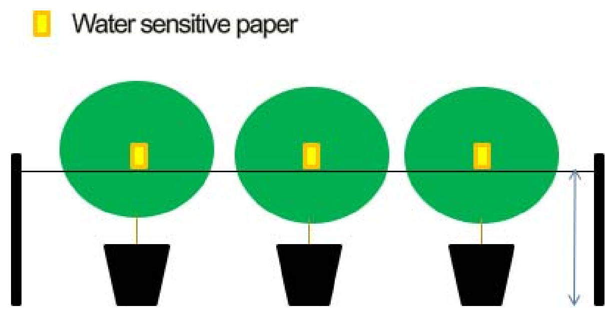

Fig. 1. Water sensitive card placement at exterior canopy position (lateral view).

For each species, the experiment was conducted a total of four times, twice on each date. Spray penetration experiments for holly were conducted on April 19 and April 30, 2013 and for hydrangea were conducted May 17 and May 24, 2013. The same set of plants was used for each experiment.

Plants were placed on the ground in a row to simulate a nursery setting and spaced so that there was no contact between plants. Three 5.1 by 7.6 cm (2.0 by 3.0 in) cards of water sensitive paper (WSP) (Syngenta Crop Protection AG, Basel, Switzerland) were placed on each plant, one per canopy position, with the water sensitive surface perpendicular to the ground. The canopy positions were the exterior, middle and interior of the canopy. The exterior cards were attached in front of each plant with 5.1 cm (2.0 in) alligator clips (Grand Rapids Industrial Products, Wayland, MI) on a wire attached to wooden poles to keep the cards at the same height, 46 cm (18.1 in) above the ground (Fig. 1). A 15.2 cm (6.0 in) long wire with an alligator clip attached to each end was wrapped around the most central stem and held the interior card flush against the stem inside the canopy at 46 cm (18.1 in) above the ground and the middle card half-way between the interior and exterior card within the plant canopy, 46 cm (18.1 in) above the ground (Fig. 2). The exterior position served as the control as spray applied to cards in this position was unimpeded by leaves or branches.

Water was applied to the foliage simulating a pesticide application using a hand held CO_2 sprayer coupled with a

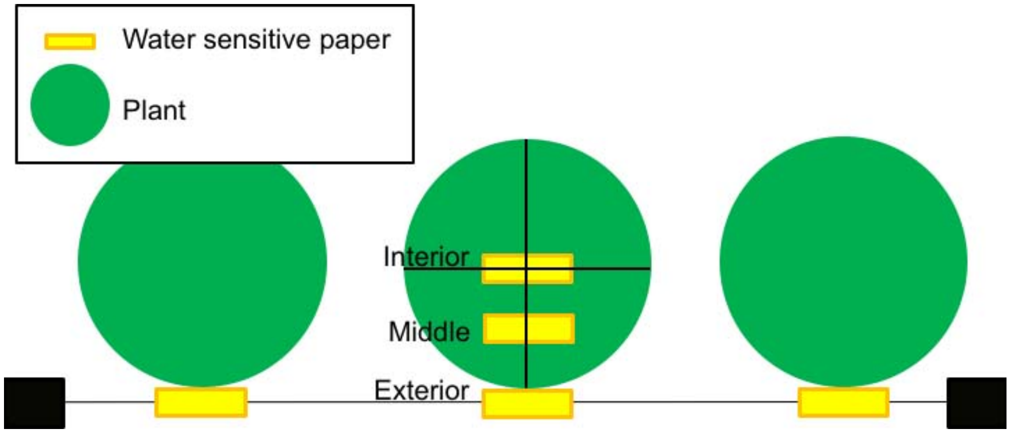

Fig. 2. Water sensitive card placement positions inside the plant canopy from top view.

Teejet® even flat spray tip TP8002E (Spraying Systems Company, Springfield, IL). The sprayer was operated at 30 PSI delivering a 0.64 L·min^{-1} (0.17 GPM) flow rate. Many growers use handheld sprayers due to their ease of use in tight areas such as a greenhouse, and their ability to monitor where they have sprayed in real-time (Derksen et al. 2010). The nozzle was kept 46 cm (18.1 in) above the ground and 0.61 m (2 ft) from the exterior cards and moved at a speed of 1.30 m·s^{-1} (4.7 KPH) [4.25 ft·s^{-1} (2.9 MPH)].

Cards dried on the plants and were immediately collected, labeled, and scanned with a business card scanner (WorldCard Office, Penpower Technology LTD., Fremont, CA). Spray penetration was analyzed using DepositScan scanning software (Zhu et al. 2011). Spray penetration was characterized by coverage (the percentage of WSP surface area that was covered by spray deposits) and droplet density (the number of droplets deposited on the cards per cm^2).

The experiment was arranged as a completely randomized design with eight replications for holly and six replications for hydrangea. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.3S, SAS Institute, Cary, NC). Means were separated using Tukey's HSD at a significance level of 5% ($\alpha = 0.05$). Data for the two plant species were analyzed separately. Data were pooled for each plant species as results were not different in repeated experiments.

Natural Enemy Survival. To determine how spray penetration affected natural enemy survival within dense and sparse plants, adult green lacewing (AGL) and adult convergent lady beetle (ACLB) (Beneficial Insectaries, Redding, CA) were confined to arenas containing hydrangea leaves from the interior, middle, or exterior of either a dense or a sparse plant that was sprayed with water or carbaryl (1-naphthyl N-methylcarbamate, Sevin® soluble liquid, Bayer CropScience, Durham, NC) at 0.95 L per 0.38 kL (1 qt per 100 gal). Each arena contained 10 insects each of either AGL or ACLB. Carbaryl was chosen because it was shown in previous research to be highly toxic to both AGL and ACLB populations (Yeary et al. 2015).

Arenas were built from 90 mm (3.54 in) petri dishes (Fisher Scientific, Pittsburgh, PA) by removing a 7.6 cm (3 in) diameter opening in the lid and replacing it with organdy fabric. A single 90 mm filter paper was placed in each arena to absorb excess moisture. For each arena, a hole was drilled in the lids of a 0.7 ml (3.0 oz) microcentrifuge tube (Costar®, Corning, Corning, NY), plugged with cotton, and filled with a honey water solution (5% v/v) to serve as a food source. Once prepared and provisioned, arenas were held in a walk-in cooler [~7 C (44 F), intermittent light] until spray applications were made, approximately 3 hours.

Oakleaf hydrangea was selected for this objective because the insects would not be able to avoid contact with the large leaf surface and any associated water or insecticide residue. To determine if the hydrangea leaves or the arena environment affected natural enemy survival, 12 oakleaf hydrangeas pruned to create dense and sparse treatments as described above (six with dense and six with sparse canopies) were sprayed using the same method as described in the spray penetration experiment.

On June 7, 2013, water and carbaryl were applied to their respective plants. First, water was applied to the six dense and six sparse plants, and leaves were collected as described below. The back half of each plant was bagged to protect leaves intended for the repeat of this experiment from pesticide residue. Carbaryl was then applied to the six dense and six sparse plants.

After each application, leaves were allowed to dry on the plant and then were collected from the exterior, middle, and interior of each plant canopy and placed in separate plastic bags prior to transfer to an arena. Each petiole was placed in a water pick and placed in its respective arena, one leaf per arena. While leaves were added, arenas remained in the cooler to prevent insects from warming and becoming active. Arenas were then moved to an insect rearing room with daytime temperatures maintained at 21 C (69.8 F). Insect survival and presence or absence of twitching were recorded 24, 48, 72, 96 and 120 hours of exposure (HOE). Insects were counted as twitching if lying on their side (AGL) or back (ACLB) with legs sporadically jerking. On June 8, 2013, plants were rotated 180 degrees and water and carbaryl applications described above were applied to the previously untreated side of each plant in order to repeat the experiment.

Table 1. **Coverage and droplet density ± standard error in the exterior, middle, and interior of China Girl® holly with dense or sparse branch architecture.**

Canopy Density	Canopy Position	Coverage (%)	Droplet Density (Deposits/cm^2)
Dense	Exterior	28.3 ± 1.9[z] a[y]	32 ± 2 a
	Middle	3.3 ± 1.9 c	8 ± 2 d
	Interior	2.7 ± 1.8 c	6 ± 2 d
Sparse	Exterior	33.9 ± 2.0 a	33 ± 2 a
	Middle	12.8 ± 1.8 b	17 ± 2 b
	Interior	7.2 ± 2.0 b	11 ± 2 c
	Num DF	2	2
	Den DF	54.12	52.36
	Significance	***[x]	***
	P-value	0.0008	0.0003
	F Statistic	8.16	9.43

[x]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

[y]Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)

[z]Means are followed by standard errors

Table 2. **Coverage and droplet density ± standard error in the exterior, middle, and interior of oakleaf hydrangea 'Alice' with dense or sparse branch architecture.**

Canopy Density	Canopy Position	Coverage (%)	Droplet Density (Deposits/cm^2)
Dense	Exterior	33.5 ± 4.5 a[z]	62 ± 6 a
	Middle	0.8 ± 4.7 cd	2 ± 6 d
	Interior	0.5 ± 4.7 d	1 ± 6 d
Sparse	Exterior	45.3 ± 4.7 a	49 ± 6 a
	Middle	4.9 ± 4.7 b	15 ± 6 b
	Interior	1.8 ± 4.5 bc	8 ± 6 bc
	Num DF	2	2
	Den DF	54.12	52.36
	Significance	***[y]	***
	P-value	0.0008	0.0003
	F Statistic	8.16	9.43

[z]Means followed by the same letter within a column were not significantly different (Tukey α = 0.05)

[y]Significance at *P*=0.01 (*), *P*=0.001 (**), *P*=0.0001(***)

Each spray product (water and carbaryl) and each insect (AGL or ACLB) were a separate experiment. Experiments were arranged in a randomized complete block design (blocked on experiment) in a 3 (canopy positions) by 2 (canopy densities) factorial arrangement with six arenas per treatment combination and analyzed with repeated measures over time using the GLIMMIX procedure of SAS. Means were separated using Tukey's LSD, α = 0.05. Data were pooled for each insect species and treatment as results were not different in repeated experiments.

Results and Discussion

Spray Penetration. Hydrangea density measurements demonstrated that different levels of plant density were achieved. Densely branched hydrangea plants were 52% denser than those pruned to create the sparsely branched plants (data not shown). Spray coverage was not different for the exterior positions of dense and sparse holly or hydrangea plants, indicating that spray applications were made consistently (Tables 1 and 2). As in related studies, penetration was reduced in the middle and interior compared to the exterior canopy of each species (Derksen et al. 2001, 2008, Zhu et al. 1997). Within the dense holly canopy, as spray penetrated, spray coverage was reduced 88% at the middle position and 90% at the interior position when compared with the coverage on the exterior of the plant (Table 1). Within the sparse holly canopy, coverage was decreased by 62% and 79%, at the middle and interior positions respectively, when compared with the exterior position. Within the dense hydrangeas, a large-leaved species, almost all spray penetrating the canopy was obstructed by foliage and branches; the middle and interior had less than 1% coverage (Table 2). Even within the sparse hydrangea canopy, coverage was decreased by 89% and 96%, at the middle and interior positions respectively, compared with the exterior position. Regardless of plant density, the interior canopy of holly plants had less than 8% coverage and hydrangea canopies had less than 2% coverage (Tables 1 and 2). The sparse holly plants received 288% and the hydrangea 513% more coverage in the middle of the canopy than their dense counterparts, indicating that some pest insects may be easier to control within sparse canopies due to greater insecticide penetration and coverage. Contact insecticides with 36 to 62% coverage were sufficient dependent upon insecticide used to manage all stages of California red scale [*Aonidiella aurantii* (Maskell)] (Garcera et al. 2011). With the interior coverage in both holly and hydrangea at less than 8%, the application may not be sufficient for a contact insecticide to effectively control scales, borers and certain other insects in the interior of the plant canopy.

Droplet density results were similar to coverage for both plant species (Tables 1 and 2). Droplet density on the exterior card was not different among the dense and sparse plants for both holly and hydrangea. In both species, deposits were lower in the middle and interior position than the exterior position regardless of plant architecture. Within the dense canopy, droplet density was reduced from 32 deposits·cm^{-2} in the exterior position to 8 deposits/cm^2 in the middle and 6 deposits·cm^{-2} in the interior position, a 75% and 81% reduction, respectively, in holly and from 62 deposits·cm^{-2} on the exterior position to 2 deposits·cm^{-2} in the middle position and 1 deposit·cm^{-2} in the interior position, a 97% and 98% reduction, respectively, in hydrangea (Tables 1 and 2). Similar decreases in penetration have been documented in hardy hydrangea (*Hydrangea paniculata* 'DVPpinky' Siebold) where only 5% of the deposits found on the exterior of the canopy reached the interior (Derksen et al. 2012). Because of small droplet size, 2 deposits·cm^{-2} may not be enough to achieve adequate control of many insect species. The manufacturer of WSP recommends 20 to 30 deposits·cm^{-2} for contact insecticides [Syngenta Crop Protection Ag (https://www.syngenta.com.au/awri)]. However, this recommendation may be pest and pesticide dependent. Within the sparse canopy, droplet density decreased by more than 65% from the exterior to the interior for both species (67% for holly and 84% for hydrangea). Regardless of density, the interior of holly and hydrangea canopies had 11 or fewer deposits·cm^{-2} ; the middle of sparse plants received a greater droplet density than the middle of dense plants.

Table 3. Adult green lacewing survival at exterior, middle and interior positions of dense and sparse oakleaf hydrangea 'Alice' canopies sprayed with water.

	Survival (% alive)					Functional (% not twitching)				
	Hours of exposure					Hours of exposure				
Canopy position and density	24	48	72	96	120	24	48	72	96	120
Exterior	98	98	96	91	84	98	98	95	90	83
Middle	98	96	89	86	80	98	96	89	86	80
Interior	99	97	93	90	84	98	95	92	90	84
Dense	98	97	91	87	82	98	96	90	87	82
Sparse	99	98	95	91	83	98	96	94	91	83
	DF	Significance	*P*-value	F Statistic		DF	Significance	*P*-value	F Statistic	
Position x T	260.2	NS[z]	0.7809	0.60		260.4	NS	0.6477	0.75	
Density x T	257.5	NS	0.4852	0.87		257.5	NS	0.1282	1.81	
Position x Density x T	260.2	NS	0.2473	1.29		260.4	NS	0.2256	1.34	

[z]Means were not significantly different, $\alpha = 0.05$.

Spray penetration may be greater in sparse canopies, yet still does not meet the 20 to 30 deposits·cm^{-2} recommended for contact insecticides. Particularly for pests that are living within the interior of plant canopies, such as scale insects, pruning to create a sparse canopy may not be enough to ensure adequate coverage.

The differences in canopy coverage between holly and hydrangea could be due to size or leaf morphology. China Girl® holly leaves are waxy, smooth, and convex (Dirr 2009). The waxy surface repels droplets, allowing for more spray to deflect either away from the plant or further into the canopy (Kirkwood 1999). The hydrangea leaves have trichomes, which are also water repellant, but spray droplets are more likely to fall off the leaf rather than deflect (Xu et al. 2011). Hydrangea leaves are also much larger than holly leaves, 2.5 by 3.2 cm (1 by 1.3 in) versus 7.6 to 20.3 cm (3 to 8 in) long and wide, allowing them to shield the interior more effectively. The disparity in branch number between dense and sparse holly (53%) and hydrangea (42%) was due to leaf size as well as leaf spacing; fewer branches were removed in hydrangea because removing one branch removed substantial canopy surface area, whereas with holly, several branches had to be removed to achieve a reduction in canopy surface area.

Foliage and branches inhibited the spray from penetrating into the canopy. To achieve better spray penetration, many landscape pesticide applicators place the wand within the canopy; however this is not feasible in a large nursery where thousands of plants are sprayed. Other studies found that spraying plants from the bottom of the canopy at a 45° angle upwards towards the plant's crown increased spray penetration (Lee et al. 2000, Tunstall et al. 1965). In this study, the effects of an angled application method were not tested. Other improvements to spray penetration have been made by changing the sprayer design. Using an air-assisted sprayer helped increase droplet density within hardy hydrangea 'DVPpinky' canopies and increasing the spray volume (from 187 L·ha^{-1} to 374 L·ha^{-1}) improved canopy penetration (Derksen et al. 2012). However, other studies have shown that for trees with a dense canopy, as spray volume increases, penetration decreases (Miranda-Fuentes et al. 2015). Several studies have reported the ability of air-assisted sprayers to increase deflection of spray droplets off leaf surfaces, allowing spray to better penetrate the canopy when compared with other sprayers (Derksen et al. 2008, Derksen and Sanderson 1996, Ozkan et al. 2006, Piché et al. 2000, Womac et al. 1992). An air-assisted sprayer with a novel five-port nozzle design improved spray penetration and droplet density uniformity within yew (*Taxus* sp.) canopies (Zhu et al. 2008). Other improvements on spray technology include an intelligent sprayer that uses a laser to detect plant presence and density in real time. The laser-guided sprayer can acheive greater spray penetration with reduced spray volume and still effectively control pests (Chen et al. 2012, Jeon and Zhu 2012, Zhu et al. 2017b). Applications that can be applied to deciduous trees in winter or before plants have leafed out in the spring would not have the same penetration issues and consequently, products like dormant oils will achieve better coverage and provide efficacy for managing difficult to control pests, like scale insects, within the canopy interior.

Natural Enemy Survival. No interaction between position and density on survival during the water control experiments for either AGL or ACLB was documented. Survival remained high ($\geq$ 80% in AGL and >60% for ACLB) over the 120 hours of observance, indicating that the experimental environment had limited effect on AGL and ACLB survivability (Tables 3 and 4). Twitching was observed in both species, but was rare and mainly occurred toward the end of the experiment. Therefore, the experimental conditions were considered acceptable and unlikely to influence the outcome of the experiments in which carbaryl was sprayed.

When carbaryl was applied, an interaction between position and density on survival of both insect species occurred (Tables 5 and 6). For both insect species, plant architecture influenced survival potential. AGL confined with treated leaves taken from the middle of dense plants had greater survival than those exposed to leaves taken from the middle of sparse plants (Table 5), and leaves from the interior of dense plants yielded higher survival than when taken from the interior of sparse plants for both species (with the exception of ACLB at 24 HOE) by as

Table 4. **Adult convergent lady beetle survival at exterior, middle and interior positions of dense and sparse oakleaf hydrangea 'Alice' canopies sprayed with water.**

	Survival (% alive)					Functional (% not twitching)				
	Hours of exposure					Hours of exposure				
Canopy position and density	**24**	**48**	**72**	**96**	**120**	**24**	**48**	**72**	**96**	**120**
Exterior	89	83	80	77	70	89	82	79	76	69
Middle	93	83	77	71	63	92	83	77	71	63
Interior	94	85	80	77	72	93	85	80	76	71
Dense	93	84	78	74	68	93	84	78	74	68
Sparse	91	83	79	75	69	91	83	79	75	68
	DF	**Significance**	***P*-value**	**F Statistic**		**DF**	**Significance**	***P*-value**	**F statistic**	
Position x T	265.8	NS[z]	0.6231	0.78		266	NS	0.7501	0.63	
Density x T	264.5	NS	0.9143	0.24		264.6	NS	0.8431	0.35	
Position x Density x T	265.8	NS	0.7894	0.59		266	NS	0.8233	0.54	

[z]Means were not significantly different, $\alpha = 0.05$.

much as 533% and 613% for AGL and ACLB, respectively (Tables 5 and 6).

AGL on treated leaves from the middle position of dense plants had a higher survival than those caged with leaves from the middle position of sparse plants by 141%, 760%, 1,233% and 1,700%, at 24, 48, 72 and 96 HOE, respectively (Table 5). The middle position offered no protection to ACLB populations regardless of plant density (Table 6). When predator species were confined with leaves taken from the interior position of dense plants, AGL had higher survival than when exposed to leaves taken from the interior position of sparse plants by 163%, 361%, 367%, 408%, and 533% at 24, 48, 72, 96, and 120 HOE, respectively, and 100%, 319%, 377%, and 613% at 48, 72, 96, and 120 HOE, respectively, in ACLB (Tables 5 and 6). Additionally, survival of both species confined with carbaryl-treated leaves taken from the dense interior position never dropped below 50%, which is consistent with survival in the water application experiments.

AGL and ACLB survivors were rated for either twitching behavior (incapable of normal mobility or activity) or functionally normal behavior (Tables 3, 4, 5 and 6). Although some twitching insects appeared to eventually regain functionality, the insects would probably not be able to function as predators while immobilized and twitching. In carbaryl experiments, not all surviving insects exhibited functionally normal behavior; their behavior generally followed the same pattern as survival (Tables 5 and 6). AGL and ACLB confined to carbaryl-treated leaves from the interior and middle of dense canopies remained significantly more functional than those confined to leaves from sparse plants ($P<0.0001$ and $P=0.006$, respectively) (Tables 5 and 6). At 72 HOE, only 13% of AGL with exposure to leaves from the interior were functional and just 3% were functional on leaves from the middle position of sparse plants; 9% of ACLB on leaves from the interior were functional and 17% of those exposed to leaves from the middle positions were functional. However, greater than 50% of both AGL and ACLB were still functional on leaves from the interior and 40% or more were functional on leaves from the middle of the dense canopy at 72 HOE. While not recorded in the study, we also observed carbaryl-exposed ACLB walking in circles and walking into arena walls; neither behavior was observed in the water control ACLB.

The interior and middle positions of both dense and sparse hydrangea plants received less than 5% insecticide coverage (Table 2), but only the interior position of dense plants protected greater than 55% of AGL and ACLB from both debilitating behavior and death over the course of the study. The low survivability and lower functionality even in areas with limited penetration indicates that just a small amount of carbaryl can harm some natural enemies, but may also be effective against pest insects. Using a less toxic, more targeted insecticide may have led to a different outcome, possibly with greater survival in the interior of the canopy.

This study was conducted in an unnatural environment where insects were confined to arenas with treated leaves. In a natural setting, insects move around the canopy searching for prey, making them more likely to come into contact with residue from other canopy positions. It is also possible that in a natural setting, natural enemies may avoid insecticide residue. Tomato leafminers [*Tuta absoluta* (Meyrick)] avoided laying eggs where the insecticide azadirachtin (Azamax®, DVA Brasil, Campinas, SP, Brasil) was present (Tomé et al. 2013). Tawny mole cricket (*Scapteriscus vicinus* Scudder) avoided tunneling in areas where bifenthrin [Talstar EZ® (FMC, Philadelphia, PA)], chlorantraniliprole, Acelepryn® (DuPont, Wilmington, DE), and fipronil (Chipco Choice, Bayer Environmental Science, Montvale, NJ) had been applied (Silcox et al. 2012). However, Ranos et al. (2018) found that the parasitoid wasp *Copidosoma truncatellum* (Dalman) remained longer in areas treated with insecticides (acephate and chlorfenapyr) than in areas that were untreated. Moreover, a field study conducted on cotton found that pests were effectively controlled by insecticides, yet lacewings and ladybeetles remained equally abundant in both sprayed and unsprayed fields (Sarwar and Sattar 2016).

Understanding the nexus of plant architecture and pest management in nursery production is important for both traditional and integrated pest management. Intra-canopy pesticide penetration as well as residue levels and degradation rates are critical facets of both conservation

Table 5. **Interaction of canopy position and plant density within oakleaf hydrangea 'Alice' canopies sprayed with carbaryl on adult green lacewing survival and ability to function.**

		Survival (% alive)					Functional (% not twitching)				
		Hours of exposure					Hours of exposure				
Position	Density	24	48	72	96	120	24	48	72	96	120
Exterior	Dense	36 efghij[z]	6 klmn	3 lmno	1 lmno	0 lmnop	13 ghij	4 ijk	3 jk	1 jk	0 jk
Exterior	Sparse	27 ghijln	6 kmo	2 mp	1 mp	1 mp	5 ijk	4 ijk	2 jk	1 jk	1 jk
Middle	Dense	70 abc	43 defgh	40 efghi	36 efghij	23 ghijklmn	51 bcf	40 degh	40 cdefgh	33 eghi	22 jk
Middle	Sparse	29 fghijklm	5 nop	3 nop	2 nop	0 nop	11 ghij	3 jk	3 jk	2 jk	0 jk
Interior	Dense	92 a	83 ab	70 bcd	61 cde	57 cdef	84 a	80 ab	67 cd	60 cde	57 cde
Interior	Sparse	35 efgk	18 hijklmno	15 hijklmno	12 ijklmno	9 jlmno	25 fgij	13 hk	13 ghij	11 ghij	9 ijk
		Position (P) *P*-value	**Density (D) *P*-value**	**P*D DF**	**P*D *P*-value**	**P*D F Statistic**	**Position (P) *P*-value**	**Density (D) *P*-value**	**P*D DF**	**P*D *P*-value**	**P*D F Statistic**
		<0.0001	<0.0001	69.72	<0.0001	16.28	<0.0001	<0.0001	67.3	<0.0001	14.46

[z]Means followed by the same letter within a column were not significantly different (Tukey $\alpha = 0.05$)

Table 6. **Interaction of canopy position and plant density within oakleaf hydrangea 'Alice' canopies sprayed with carbaryl on adult convergent lady beetle survival and ability to function.**

		Survival (% alive)					Functional (% not twitching)				
		Hours of exposure					Hours of exposure				
Position	Density	24	48	72	96	120	24	48	72	96	120
Exterior	Dense	72 abde[z]	48 cfghjklno	24 impqrst	15 mqrst	8 qrst	28 efghij	18 ghij	11 hij	9 i	8 i
Exterior	Sparse	58 abcdfghi	39 ejklmnopqr	8 stu	2 stu	8 stu	21 ghij	14 ghij	4 i	2 i	7 i
Middle	Dense	79 ac	67 abcdef	54 bdefhij	48 bdefghijklm	32 gklmnopqrs	65 a	56 abcef	46 bcdefg	44 abcdefgh	29 dij
Middle	Sparse	63 abcefg	43 dhijlmop	28 hiklmnopqrs	19 knqrst	13 nqrst	30 cdefghi	23 fghij	17 ghij	14 ghij	9 i
Interior	Dense	82 ab	76 abcd	67 abcdef	62 abcdefgh	57 abcdefghi	76 ab	70 ab	64 abcd	60 abcde	56 abcdef
Interior	Sparse	63 abcdefg	38 fhikmnpqs	16 lort	13 ort	8 ru	25 fghij	20 ghij	9 i	8 i	7 i
		Position (P) *P*-value	**Density (D) *P*-value**	**P*D DF**	**P*D *P*-value**	**P*D F Statistic**	**Position (P) *P*-value**	**Density (D) *P*-value**	**P*D DF**	**P*D *P*-value**	**P*D F Statistic**
		0.0009	<0.0001	68.63	0.0172	4.31	<0.0001	<0.0001	68.27	0.006	8.29

[z]Means followed by the same letter within a column were not significantly different (Tukey $\alpha = 0.05$)

and augmentative biological control. In future studies, additional insecticides should be tested to determine how different insecticide modes of action affect natural enemy survival within dense canopies. Also, residue persistence at different canopy positions should be investigated to determine natural enemy survival when release follows a pesticide application. Additionally, research conducted in a natural environment is needed to ascertain the distribution and movement of natural enemy species within the plant canopy in order to further evaluate the significance of canopy density on conservative and augmentative biological control. By better understanding the role of plant canopy on spray penetration and survival of both destructive and beneficial insects, nursery managers can improve pest management practices and advance sustainability in the Green Industry.

Literature Cited

Bache, D.H. and D. Johnstone. 1992. Microclimate and Spray Dispersion. Ellis Horwood, New York, NY. p. 147–155.

Bethke, J.A. and R.A. Cloyd. 2009. Pesticide use in ornamental production: What are the benefits? Pest Mgt. Sci. 65:345–350.

Briggs, J., T. Whitwell, R.T. Fernandez, and M.B. Riley. 2002. Effect of integrated pest management strategies on chlorothalonil, metalaxyl, and thiophanate-methyl runoff at a container nursery. J. Amer. Soc. Hort. Sci. 127:1018–1024.

Chen, Y., H. Zhu, and H.E. Ozkan. 2012. Development of a variable-rate sprayer with laser scanning sensor to synchronize spray outputs to tree structures. Trans. ASABE. 55:773–781.

Cho, S. and N. Ki. 1999. Autonomous speed sprayer guidance using machine vision and fuzzy logic. Tran Trans. ASAE. 42:1137–1143.

Cochran, D.R. and A. Fulcher. 2013. Type and rate of plant growth regulator influence vegetative, floral growth, and quality of Little Lime^{TM} Hydrangea. HortTechnology 23:306–311.

Colin, M.E., J.M. Bonmatin, I. Moineau, C. Gaimon, S. Brun, and J.P. Vermandere. 2004. A method to quantify and analyze the foraging activity of honey bees: Relevance to the sublethal effects induced by systemic insecticides. Arch. Environ. Contamination Toxicology 47:387–95.

Currey, C.J. and J.E. Erwin. 2012. Foliar applications of plant growth regulators affect stem elongation and branching of 11 kalanchoe species. HortTechnology 22:338–344.

Derksen, R., J. Altland, and J. Rennecker. 2012. Fate of preemergence herbicide applications sprayed through containerized hydrangea canopies. J. Environ. Hort. 30:76–82.

Derksen, R.C., J. Frantz, C.M. Ranger, J.C. Locke, H. Zhu, and C.R. Krause. 2008. Comparing greenhouse handgun delivery to poinsettias by spray volume and quality. Trans. ASABE. 51:27–33.

Derksen, R., S. Miller, H. Ozkan, and D. Fox. 2001. Spray deposition characteristics on tomatoes and disease management as influenced by droplet size, spray volume, and air-assistance. ASAE Annual International Meeting, ASAE paper 011120.

Derksen, R.C. and J. Sanderson. 1996. Volume, speed, and poinsettia foliar deposits. Trans. ASAE 39**:**5–9.

Derksen, R., C. Ranger, L. Canas, J. Locke, H. Zhu, and C. Krause. 2010. Evaluation of handgun and broadcast systems for spray deposition in greenhouse poinsettia canopies. Trans. ASABE. 53:5–12.

Dirr, M.A. 2009. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Stipes Publishing, Champagne, IL. 1,325 p.

Falconer, K.E. 1998. Managing diffuse environmental contamination from agricultural pesticides: An economic perspective on issues and policy options, with particular reference to Europe. Agr. Ecosyst. Environ. 69:37–54.

Frank, S.D. and C.S. Sadof. 2011. Reducing pesticide volume and nontarget effects of ambrosia beetle management in nurseries. J. Econ. Entomol. 104:1960–1968.

Garcera, C., E. Molto, and P. Chueca. 2011. Effect of spray volume of two organophosphate pesticides on coverage and on mortality of California red scale *Aonidiella aurantii* (Maskell). Crop Protection 30:693–697.

Gilman, E.F. 2012. An Illustrated Guide to Pruning. Cengage Learning, Clifton Park, NY. 476 p.

Glasgow, T. 1999. Customer perceptions of plant quality, North Carolina State Univ., Raleigh. Ph.D. Diss.

Halaj, J., A.B. Cady, and G.W. Uetz. 2000a. Modular habitat refugia enhance generalist predators and lower plant damage in soybeans. Environ. Entomol. 29:383–393.

Halaj, J., D.W. Ross, and A.R. Moldenke. 2000b. Importance of habitat structure to the arthropod food-web in Douglas-fir canopies. Oikos 90:139–152.

Hanks, L.M. and R.F. Denno. 1993. Natural enemies and plant water relations influence the distribution of an armored scale insect. Ecology 74:1081–1091.

Horn, D.J. and R.F. Sheppard. 1979. Sex-ratio, pupal parasitism, and predation in two declining populations of the bagworm, *Thyridopteryx ephemeraeformis* (Haworth) (Lepidoptera: Psychidae). Ecol. Entomol. 4:259–265.

Jeffers, A.H., M.A. Palma, W.E. Klingeman, C.R. Hall, D.S. Buckley, and D.A. Kopsell. 2009. Assessments of bare-root liner quality and purchasing decisions made by green industry professionals. HortScience 44:717–724.

Jeon, H.Y. and H. Zhu. 2012. Development of a variable-rate sprayer for nursery liner applications. Trans. ASABE 55:303–312.

Kher, S.V., J. De Jonge, M.T.A. Wentholt, R. Deliza, J.C. de Andrade, and H.J. Cnossen. 2013. Consumer perceptions of risks of chemical and microbiological contaminants associated with food chains: A cross-national study. Intl. J. Consumer Studies. 37:73–83.

Kirkwood, R.C. 1999. Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. Pesticide Sci. 55:69–77.

Klingeman, W.E., S.K. Braman, and G.D. Buntin. 2000. Evaluating grower, landscape manager, and consumer perceptions of azalea lace bug (Heteroptera : Tingidae) feeding injury. J. Econ. Entomol. 93:141–148.

Langellotto, G.A. and R.F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: A meta-analytical synthesis. Oecologia 139:1–10.

LeBude, A.V., S.A. White, A. Fulcher, S. Frank, W.E. Klingeman, J.-H. Chong, M.R. Chappell, A. Windham, K. Braman, F. Hale, W. Dunwell, J. Williams-Woodward, K. Ivors, C. Adkins, and J. Neal. 2012. Assessing the integrated pest management practices of southeastern US ornamental nursery operations. Pest Mgt. Sci. 68:1278–1288.

Lee, A., P. Miller, J. Power, J. Cross, A. Gilbert, C. Glass, W. Taylor, P. Walklate, and N. Western. 2000. The application of pesticide sprays to tomato crops. Aspects Appl. Biol. 57:383–390.

Leite, G.L.D., R.V. Veloso, J.C. Zanuncio, A.M. Azevedo, J.L. Silva, C.F. Wilcken, and M.A. Soares. 2017. Architectural diversity and galling insects on *Caryocar brasiliense* trees. Scientific Rpts. 7:16677

Miranda-Fuentes, A., A. Rodríguez-Lizana, E. Gil, J. Agüera-Vega, and J.A. Gil-Ribes. 2015. Influence of liquid-volume and airflow rates on spray application quality and homogeneity in super-intensive olive tree canopies. Sci. Total Environ. 537:250–259.

Montella, I.R., R. Schama, and D. Valle. 2012. The classification of esterases: An important gene family involved in insecticide resistance - A Review. Memorias Do Instituto Oswaldo Cruz 107:437–449.

Ozkan, H., H. Zhu, R. Derksen, H. Guler, and C. Krause. 2006. Evaluation of various spraying equipment for effective application of fungicides to control Asian soybean rust. Aspects Appl. Biol. 77:423.

Pan, Z., D. Lie, L. Qiang, H. Shaolan, Y. Shilai, L. Yande, Y. Yongxu, and P. Haiyang. 2016. Effects of citrus tree-shape and spraying height of small unmanned aerial vehicle on droplet distribution. Intl. J. Agric. Biol. Eng. 9(4):45–52.

Piché, M., B. Panneton, and R. Thériault. 2000. Field evaluation of air-assisted boom spraying on broccoli and potato. Trans. ASAE. 43:793–799.

Ranos, R.S., V.C.R. de Araújo, R.R. Pereira, J.C. Martins, O.S. Queiroz, R.S. Silva, and M.C. Picanço. 2018. Investigation of the lethal and behavioral effects of commercial insecticides on the parasitoid wasp *Copidosoma truncatellum*. Chemosphere 191:770–778.

Raupp, M.J., J.A. Davidson, C.S. Koehler, C.S. Sadof, and K. Reichelderfer. 1989. Economic and aesthetic injury levels and thresholds for insect pests of ornamental plants. Florida Entomol. 72:403–407.

Sadof, C.S. and M.J. Raupp. 1996. Aesthetic Thresholds and Their Development, pp. 203–226. *In*: L.G. Higley and L.P. Pedigo (eds.), Economic Thresholds for Integrated Pest Management. University of Nebraska Press, Lincoln, NE.

Sarwar, M. and M. Sattar. 2016. An analysis of comparative efficacies of various insecticides on the densities of important insect pests and the natural enemies of cotton, *Gossypium hirsutum* L. Pakistan J. Zool. 48(1):131–136.

Sánchez-Hermosilla, J., V.J. Rincón, F. Páez, F. Agüera, and F. Carvajal. 2011. Field evaluation of a self-propelled sprayer and effects of the application rate on spray deposition and losses to the ground in greenhouse tomato crops. Pest Mgt. Sci. 67:942–947.

Silcox, D.E., C.E. Sorenson, and R.L. Brandenburg. 2012. Quantifying efficacy and avoidance behavior by tawny mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus vicinus*) to three synthetic insecticides. Florida Entomol. 95:63–74.

Skirvin, D.J. 2004. Virtual plant models of predatory mite movement in complex plant canopies. Ecol. Modelling 171:301–313.

Szczepaniec, A., S.F. Creary, K.L. Laskowski, J.P. Nyrop, and M.J. Raupp. 2011. Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. PloS One 6:e20018.

Tomé, H.V.V., J.C. Martins, A.S. Corrêa, T.V.S. Galdino, M.S. Picanço, and R.N.C. Guedes. 2013. Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tutu absoluta*. Crop Protection 46:63–69.

Tunstall, J., G. Matthews, and A. Rhodes. 1965. Development of cotton spraying equipment in Central Africa. Emp. Cott. Gr. Rev. 42:131–145.

van de Vrie, M. 1995. Control of Tetranychidae in crops: Greenhouse ornamentals, pp. 76–90. *In*: W. Helle and M.W. Sabelis (eds), Spider Mites: Their Biology, Natural Enemies and Control, vol. 1. Elsevier Science Publishers, Amsterdam, The Netherlands.

Womac, A., J. Mulrooney, and W. Scott. 1992. Characteristics of air-assisted and drop-nozzle sprays in cotton. Trans. ASAE. 35:1369–1376.

Xu, L.Y., H.P. Zhu, H.E. Ozkan, W.E. Bagley, and C.R. Krause. 2011. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. Pest Mgt. Sci. 67:842–851.

Yeary, W.M., A. Fulcher, W. Klingeman, J. Grant, and X. Sun. 2015. Responses of three natural enemy species to contact and systemic insecticide exposures in confined assays. J. Entomol. Sci. 50:35–46.

Zhu, H., R.D. Brazee, R.C. Derksen, R.D. Fox, C.R. Krause, H.E. Ozkan, and K. Losely. 2006. A specially designed air-assisted sprayer to improve spray penetration and air jet velocity distribution inside dense nursery crops. Trans. ASABE. 49:1285–1294.

Zhu, H., R.D. Brazee, R.D. Fox, R.C. Derksen, and H.E. Ozkan. 2008. Development of a canopy opener to improve spray deposition and coverage inside soybean canopies: Part 1. Mathematical models to assist opener development. Trans. ASABE. 51:1905–1912.

Zhu, H., C. Krause, R. Fox, R. Brazee, and R. Derksen. 1997. Techniques for assessing spray drift and canopy penetration in nurseries. ASAE Paper No. 975006.

Zhu, H., H. Liu, Y. Shen, and R. Zondag. 2017a. Spray deposition inside multiple-row nursery trees with a laser-guided sprayer. J. Environ. Hort. 35:13–23.

Zhu, H., R. Rosetta, M.E. Reding, R.H. Zondag, C.M. Ranger, L. Canas, A. Fulcher, R.C. Derksen, H.E. Ozkan, C.R. Krause. 2017b. Validation of a laser-guided variable-rate sprayer for managing insects in ornamental nurseries. Trans. ASABE 60:337–345.

Zhu, H.P., M. Salyani, and R.D. Fox. 2011. A portable scanning system for evaluation of spray deposit distribution. Computers Electronics Agr. 76:38–43.