

ARDP - Project Report 2015
Evaluating Threat Level, Infestation Behavior, Economic Impact and Potential Measures
for Control of Ambrosia Beetles in NY Apple Orchards

Arthur Agnello¹, Dan Donahue², Anna Wallis², and Kerik Cox³

¹Dept. of Entomology, NYSAES

²ENY Commercial Horticulture Program; ³Dept. of Plant Pathology, NYSAES

Background

The ambrosia beetle *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), also known as the black stem borer, is a serious pest in ornamental tree nurseries and landscapes in North America. A native of Asia (mainly Japan, Korea, Vietnam, China, and Taiwan), it now occurs in central Europe and the US, first documented here in New York, in greenhouse-grown grape stems. Since then, it has become established in much of the United States. It has previously been noted as a pest in ornamental nurseries, with a wide host range including oak, elm, red maple, beech, and other hardwood species. It attacks and bores galleries into the wood of trunks or limbs of apparently healthy plants and those that are stressed, dying or recently dead. Galleries are excavated by the females, and comprise entrance tunnels, brood chambers containing eggs, and branch tunnels where young develop. The species is bivoltine and overwinters as adults, primarily females, in galleries of its host plants.

The term "ambrosia beetle" refers to species that derive nourishment during the larval and adult stages from a mutualistic "ambrosia" fungus carried by the adult female in mycangia (internal pouches) and introduced into host plants during gallery excavation. The ambrosia fungus associated with *X. germanus* is *Ambrosiella hartigii* Batra, visible in the galleries as an abundant grayish-white mycelium growth. It is this fungal growth that the insects feed on, and not the host plant tissue. However, its presence signals the tree that it is under attack, and as the tree walls off its vascular system, symptoms develop including wilting, dieback, tree decline and death.

In 2013, infestations of *X. germanus* were seen for the first time in commercial apple trees, in multiple western NY sites; some affected trees additionally exhibited fire blight symptoms. Indeed, one of the few instances of streptomycin-resistant fire blight in 2013 was obtained from an *X. germanus* infestation. In addition to fire blight, mycelium of *Fusarium* was observed in some heavily infested samples in 2013, and *Nectria haematococca* (*Fusarium solani*) was recovered from several beetles in 2014. By the end of 2013, hundreds of trees were removed in high density apple plantings during the middle of the growing season. In 2014, trapping and inspection efforts were initiated in the general apple-growing region along Lake Ontario. To date, at least 30 additional infestation sites have been documented, extending as far as to Long Island, and it appears that these ambrosia beetles may have been present in the area for some years before first detected, as they are now being found in nearly every orchard showing these tree decline symptoms; several hundred trees have already been destroyed.

Current studies suggest that this species invades from nearby wooded areas, but there is relatively little research on movement of ambrosia beetles from wooded areas into nurseries or orchards. The insects attack stressed (including some apparently healthy) trees, boring into the trunk or limbs to create galleries where young develop. A variety of stressors, including flooding, drought, and freezing exposure have been identified as potential causes of physiological stress that preferentially attract ambrosia beetles. Trees under this type of stress

produce several types of volatiles, among them ethanol, which has been documented to be a strong attractant to the beetles. In commercial ornamental tree nurseries, growers routinely rely on insecticide trunk sprays to prevent new infestation and colonization of trees by ambrosia beetles. For effective protection against these insects, pesticide applications must be closely timed with insect attack, applied repeatedly, and/or have long residual activity. A reliable monitoring system would give growers the ability to coordinate any needed control treatments with beetle activity. Ethanol-baited traps have been demonstrated to be useful for monitoring the flight activity of ambrosia beetles in ornamental nurseries.

Objectives & Results

Objective 1. Evaluate current distribution and impact of black stem borer and other ambrosia beetles in Eastern and Western NY Orchards.

In 2015, we assessed black stem borer (BSB) adult occurrence and distribution in several New York apple growing regions, using ethanol-baited bottle traps hung on metal garden hangers at a 1-m height, placed along the edges of orchards bordered by hedgerows and woods likely to be a source of immigrating beetles. Additional traps were located (in the western NY orchards) adjacent to previously attacked trees, to verify their attractiveness. Traps were checked weekly starting at the end of April, before maximum temperatures of 20°C began to occur, and continuing until the first week of September. Traps were placed on 14 farms in Wayne Co., 11 farms in the Hudson Valley, and 9 farms in the Champlain Valley.

BSB adults were captured at nearly all of the sites, and were most numerous in the western NY locations (Figs. 1A, 1B). First activity was noted in WNY on May 5, and there were higher counts along the orchard edges than in the interiors. June 2 was the peak of beetle emergence from the overwintering sites, and 1st generation adults emerged from July 6-27. On August 5, the 2nd generation adults emerged, with catch continuing into September. In the Champlain and Hudson Valleys (Figs. 2, 3), the traps were not set out early enough to detect the first flight activity, but it was apparent that the beetles were present by at least mid-May in these locations. Lower numbers were captured in all ENY sites than in WNY, but there were sufficient trap catches to confirm BSB distribution throughout the region.

Objective 2. Isolate and characterize bacterial and fungal pathogens from beetles and infestation sites in apples, and characterize the progression of disease and tree decline in affected orchards.

A total of 86 blistered bark and gallery wood samples were examined. From these, 34 *X. germanus* adults were recovered and subjected to fungal and bacteria isolation. Prior to surface sterilization, fungi were recovered from 11 beetles, bacteria were recovered from 17 beetles, and 6 beetles were free of microbes. Following surface sterilization and insect maceration, fungi were recovered from the internal contents of 11 beetles, bacteria were recovered from the internal contents of 14 beetles, and 11 beetles were free of internal microbes.

From exoskeleton and internal contents of *X. germanus*, *Nectria haematococca* was the most frequently isolated fungal species (Figs. 4A, 4B). The expected fungal symbiont, *Ambrosiella xylebori*, was only found in 1 beetle. A variety of miscellaneous fungi were found in the remaining beetle and may not have been fungal symbionts. From blistered bark and gallery cambium samples, 48 bacteria isolates were recovered, with 31 of these from blister samples (Fig. 4C). Of the samples, 16 of the bacteria were *Pseudomonas syringae*, which has been shown to be associated with blister bark disease of apples in Italy. Fungi were predominately

found in blister wood samples, and as expected, *A. xylebori* was predominately found in the *X. germanus* galleries. The second most common species isolated from *X. germanus* galleries was *N. haematococca*. This isn't surprising, given that *X. germanus* is report to cultivate *N. haematococca*. The asexual stage of *N. haematococca* is *Fusarium solani*, which is a vascular wilt pathogen of both perennial and annual plants. It remains to be seen whether *X. germanus* cultivating *N. haematococca* is more devastating to apple than its usual fungal symbiont, *A. xylebori*.

Objective 3. Evaluate the effectiveness of chemical treatments using insecticides or biologicals to control either the insects or their fungal symbiont, which has the primary effect on tree health.

The efficacy and practicality of trunk sprays using chlorpyrifos and two pyrethroid products (lambda-cyhalothrin and gamma-cyhalothrin) was evaluated against infestations of ambrosia beetles on two commercial farms having documented infestations (Sodus, NY and Medina, NY). All treatments were replicated in randomized complete plots at each of the individual test sites. Potted 2-yr old Mutsu trees from the nursery were placed in turn into larger pots, which were then flooded to induce stress and promote ethanol production. These potted trees were placed in the rows between the orchard trees, with 5 pots per replicate, and 4 replicates per treatment at each site. The trunks of the potted trees plus the orchard trees were sprayed using a handgun sprayer (Rears Nifty Pul-Tank) on May 7 and 8, before the start of major BSB flight. The treatments were:

- chlorpyrifos (Lorsban Advanced); 1.5 qt/100 gal
- lambda-cyhalothrin (Warrior II); 2.56 fl oz/100 gal
- gamma-cyhalothrin (Declare); 2.05 fl oz/100 gal
- Untreated Check (potted trees only; orchard trees in Check plots sprayed with chlorpyrifos)
- Grower Standard (Lorsban 1.5 qt/100 gal applied by grower using airblast sprayer)

Treatment efficacy was assessed for evidence of new infestations by preliminary inspection of treated and untreated trees on July 9, after termination of the first flight. A final evaluation of the potted trees was conducted on August 19; these were destructively sampled to document all occurrences of holes, galleries, adults, and brood in the treated trees.

In the Preliminary Evaluations, efficacy of the handgun treatments in the potted trees was not consistent between the two sites, with the Lorsban plots tending to have lower levels of infested trees than the Warrior plots at the Sodus site, but the opposite trend occurring at the Medina site (Table 1). Damage in the Lorsban airblast (Grower Standard) treatment was low at both sites; however, because these plots were situated in a different part of each orchard (to prevent the airblast application from interfering with the handgun treatments), there was almost certainly a site variability factor introduced in regard to BSB population pressure, so it is difficult to make any reliable inference about comparative treatment efficacy as a result. There were no significant treatment differences in percent infested trees in the established orchard trees.

Results of the Final Evaluations varied somewhat between sites (Table 1). At Sodus, there was a slight trend toward lower infestations (infestation holes, presence of galleries, gallery contents) in the sprayed vs. Check treatments; however, there was no real separation among the handgun treatments. The Grower Standard was lower in all categories. At the Medina site, the Lorsban handgun treatment generally had the lowest infestations, with the pyrethroid products not performing as well. The Grower Standard was again lower in all categories.

Objective 4. Determine contributing factors for ambrosia beetle establishment and impact in commercial orchards in Eastern NY.

A pattern of apple tree decline was reported in two blocks at an orchard in Hudson, NY in early 2015. Block 1 comprised only Zestar! on (generally) M9-337, and Block 2 also comprised Zestar!/M9-337, but bordering a block of Empire/M9-337.

The ambrosia beetle *Xylosandrus germanus* was found, but was not suspected to be the primary cause of the decline. In order to assess and track tree decline, individual trees were rated on a scale of 0-4. Trees rated 0 were unaffected. Trees rated 1 showed mild chlorosis, trees rated 2 showed medium chlorosis, trees rated 3 showed severe chlorosis, and trees rated 4 indicated a dead tree. These ratings were displayed spatially, creating a map of the decline of the orchard. This allowed the distribution of declining trees to be better understood.

A rating of the blocks was taken in July, and a second rating was taken three months later in October. Only trees scoring a 3 or a 4 were considered during the second rating in October, as normal senescence precluded the accurate assessment of categories 0-2.

In November, a representative subset of the trees in Block 2 was rated for the presence of Black Stem Borer, *Schizophyllum* fungal fruiting bodies, flaky rootstock bark, and the percent rootstock girdling caused by Dogwood Borer feeding. It is known that Black Stem Borers attack trees that are already experiencing stress. The rating of dogwood borer, *Schizophyllum* fungus, and flaky rootstock bark are investigating what else is contributing to the decline of the trees in the block.

There were a total of 1331 trees in Block 1. Based on the June rating, 349 trees (26.2%) were affected, with ratings ranging from 1-4. When comparing the June rating with the October rating, 27 more trees declined to #3 and 11 more trees declined to #4 (completely dead) (Table 2). There were a total of 1382 trees in Block 2. Based on the June rating, 475 trees (34.4%) were affected, with ratings ranging from 1-4. When comparing the June rating with the October rating, there were an equal number of trees rated #3, however 56 more trees rated #4.

A total of 39 trees were rated in November for Dogwood Borer rootstock girdling, presence/absence of *Schizophyllum commune*, degree of bark flaking off the rootstocks, and presence/absence of Black Stem Borer bodies or holes. Data was categorized by the July 15th decline rating 0, 1, 2, 3, 4, and trees whose health deteriorated from a 2 to 4, and from 3 to 4 by October (Table 3). The appearance of *Schizophyllum* fungal fruiting bodies increased as trees declined, with the highest presence occurring in trees that had been rated 4 (dead) since July. There was not enough variation among the percentage of flaky rootstock bark between the different rating categories to obtain any insight, as flaking bark was common. The trees with the highest percent of girdling cause by Dogwood Borer were those that had declined from 2 to 4 over the course of the summer. The presence of Black Stem Borer was highest in trees rated 3 and 4.

In both of the orchard blocks, the distribution of the affected trees was clustered rather than randomly distributed. In many cases, a tree with a rating of 4 would be bordered by trees rating 3 followed by 2 and 1. The tree decline observed varied considerably, with some affected trees stable, while others declined rapidly, resulting in tree loss. Declining trees appeared to have a clustered distribution, with some degree of edge effect (Block 2 in particular). Apparently healthy trees (category 0) were found to suffer from some degree of Dogwood Borer damage and a high degree of flaking rootstock bark, but to no visually obvious effect. Black Stem Borer has not been observed in “apparently healthy” trees. Trees in this orchard will be rated again next season in order to track the progress of decline. As tree condition worsened, Dogwood Borer,

Black Stem Borer, and *Schizophyllum* incidence increased. What is not clear is the causal agent(s) responsible for the initial decline of trees from category 0 to 1. These blocks will continue to be monitored in 2016.

Summary

These trials will likely need to be repeated for a clearer indication of the most effective measures to take, but although recommendations for controlling this pest are still being formulated, it appears that tree health – avoiding stress to the trees – will be an important factor in BSB management. Current recommendations are for growers to remove and destroy any infested trees detected in a planting, to prevent new infestations in surrounding trees. Trapping and monitoring adults using ethanol lures is a useful and informative tactic, but the fact remains that ambrosia beetles are difficult to control with insecticides. Sprays must be closely timed with beetle attacks, and multiple applications may be necessary. Using a material with long residual activity is a plus, and the best timing is likely against emerging overwintered brood, according to the literature. Because these insects do not feed on the tree tissue, systemic insecticides are not effective. In addition, current regulatory actions suggest that the loss of Lorsban as an option is imminent, which will add to the challenge of finding a suitable control method for this insect.

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Table 1. Ambrosia beetle insecticide control trials, 2015

<u>Preliminary Evals - July 9</u>			<u>Final Evaluations - Aug. 19</u>				
Treatment	% infested trees		Avg # holes/ tree	Avg. # infestation sites with presence of gallery live adults brood dead adults			
	Potted	Orchard					
Sodus							
Check	35.0 a	-	2.25 a	1.30 a	0.45 a	0.25 a	0.05 a
Warrior	40.0 a	95.0 a	1.00 ab	0.80 ab	0.05 bc	0.05 a	0.10 a
Declare	30.0 a	75.0 a	0.95 ab	0.85 ab	0.40 ab	0.30 a	0.05 a
Lorsban	15.0 a	77.5 a	1.30 ab	0.85 ab	0.25 abc	0.20 a	0.05 a
Grower Std	20.0 a	25.0 b	0.25 b	0.20 b	0.0 c	0.05 a	0.0 a
Medina							
Check	45.0 a	-	1.65 ab	1.00 ab	0.10 a	0.25 a	0.0 a
Warrior	5.0 b	25.0 a	2.25 a	1.45 a	0.10 a	0.25 a	0.15 b
Declare	20.0 ab	45.0 a	1.00 bc	0.45 bc	0.0 a	0.05 ab	0.0 a
Lorsban	35.0 a	40.0 a	0.35 c	0.15 c	0.0 a	0.05 ab	0.0 a
Grower Std	5.0 b	20.0 a	0.15 c	0.10 c	0.0 a	0.0 b	0.0 a

For each site, values in the same column followed by the same letter are not significantly different ($P < 0.05$, Student's t-test).

Table 2.

Categorical Totals of Tree Ratings				
Rating Category	Block 1		Block 2	
	7/15/2015	10/13/2015	7/15/2015	10/13/2015
0	982	N/A	907	N/A
1	249	N/A	343	N/A
2	68	N/A	81	N/A
3	24	51	32	32
4	8	19	19	75

Table 3.

Percent of trees affected in Block 2					
Rating	Percent of Girdling by Dogwood Borer	Presence of Black Stem Borer	<i>Presence of schizophyllum</i> Fungal Fruiting Bodies	Presence of Flaky Bark on the Rootstock	Number of Trees in Each Category
0	11%	0%	0%	71%	7
1	36%	11%	0%	89%	9
2	85%	33%	17%	67%	6
3	45%	75%	25%	62%	8
2 to 4	100%	33%	0%	67%	3
3 to 4	45%	75%	25%	62%	8
4 to 4	56%	56%	56%	44%	9

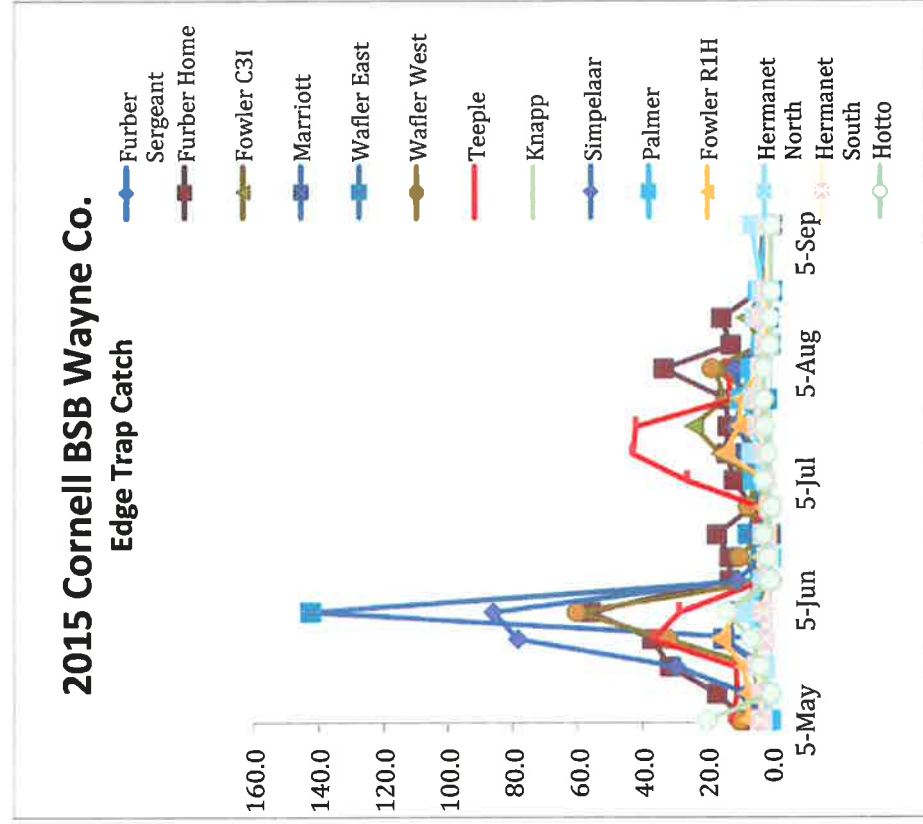


Fig. 1A

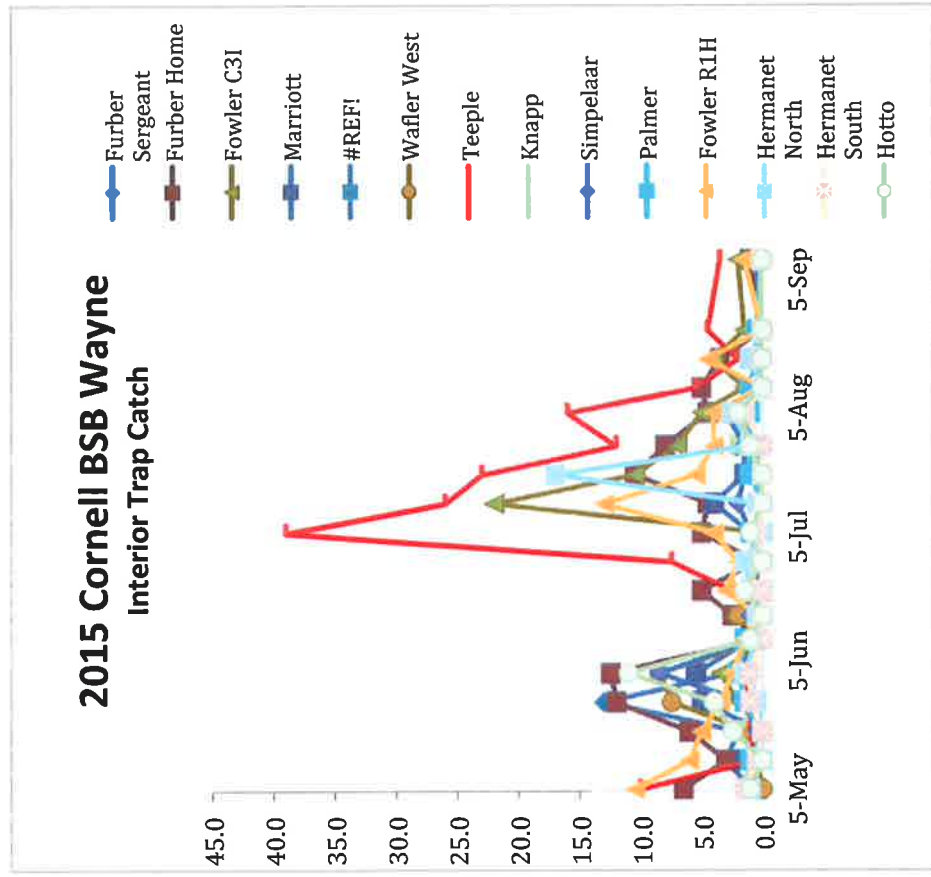


Fig. 1B

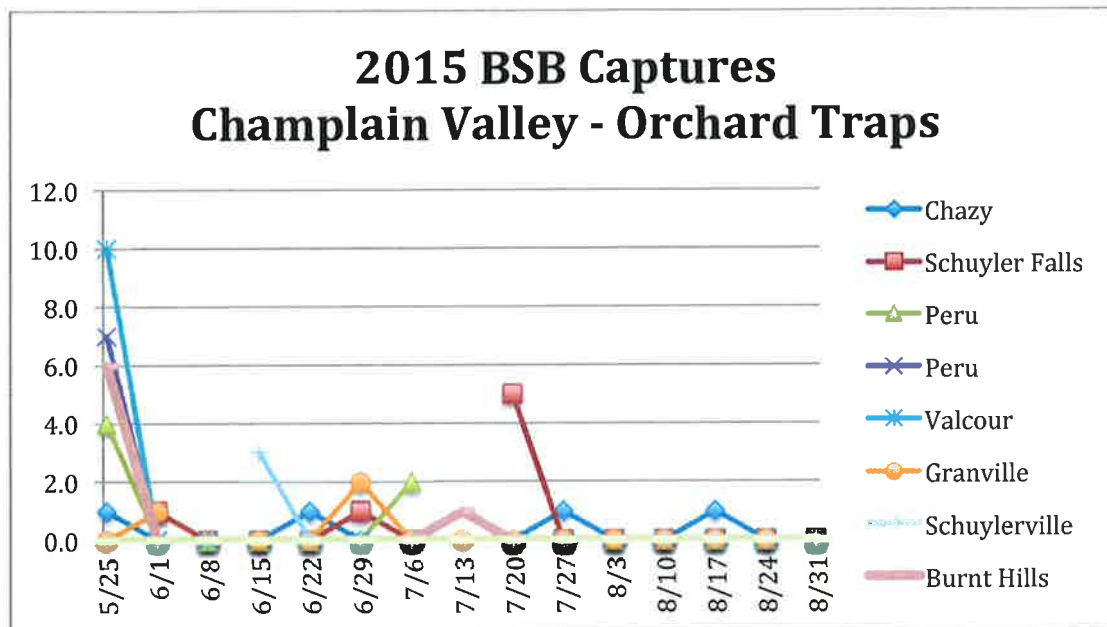


Fig. 2

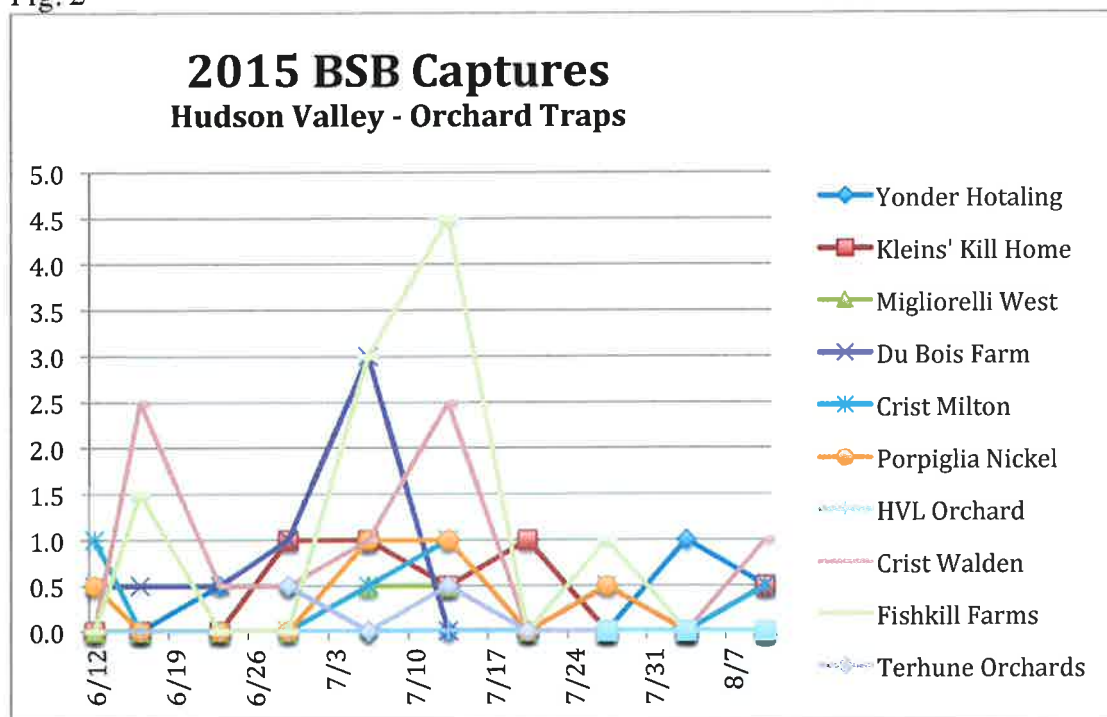


Fig. 3

Fungal species in non-sterile BSB

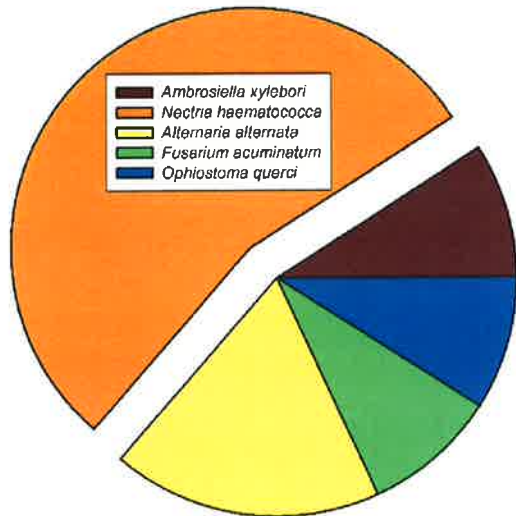


Fig. 4A

Fungal species in surface-sterilized BSB



Fig. 4B

Fungal species in gallery wood

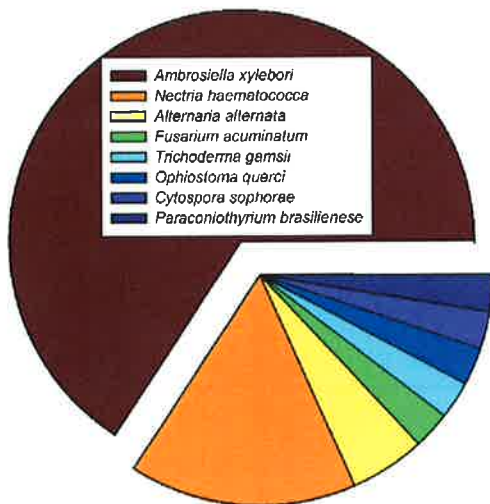


Fig. 4C