Nematode Suppressive Effects of Fluopyram on Zucchini and Cherry Tomato in comparison to Sunn hemp Cover Cropping and Azaractin through Chemigation

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Root-knot nematodes, *Meloidogyne incognita* and *Meloidogyne javanica*, and reniform nematodes, *Rotylenchulus reniformis* are three of the most important plant-parasitic nematodes that parasitize agricultural crops in Hawaii (Schmitt and Sipes, 1998). Root-knot nematode alone can impede cucurbit crop yield by 33%, and tomato (*Solanum lycopersicum*) yield by 38% (Sikora and Fernandez, 2005). Although fumigant nematicides are very effective, they required restricted use pesticide applicator’s license and special equipment to apply which can be challenging for smaller scale farms that are dominating vegetable crop production in Hawaii. Conventional non-fumigant type nematicides such as aldicarb and oxamyl are highly toxic to human and the environment, many of which are not registered for minor crop use in Hawaii.

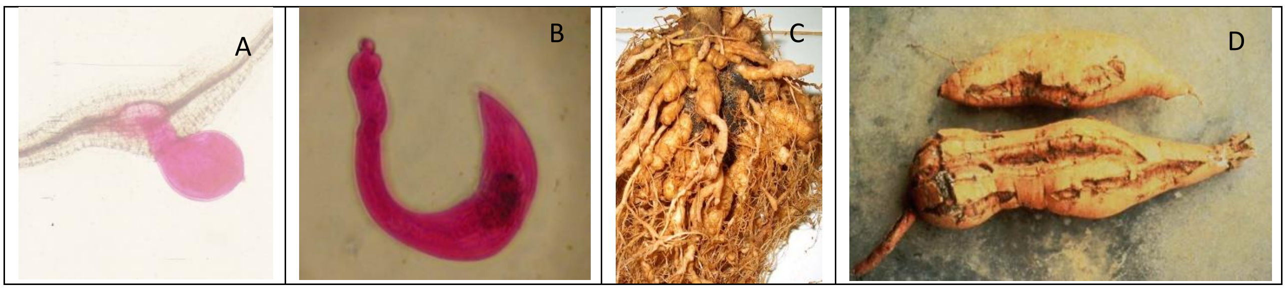

Fig. 1. A) Root-knot and B) reniform nematodes stained with acid fuchsin are commonly found plant-parasitic nematodes on vegetable crops in Hawaii. Damage of C) root-knot nematodes on tomato roots, and D) reniform nematodes on sweet potato swollen roots.

Fig. 2. Sunn hemp.

Sunn hemp (*Crotalaria juncea*) ‘Tropic Sun’ (Fig. 2) has been demonstrated to suppress reniform and root-knot nematodes and release allelopathic compounds against these nematodes (Wang et al., 2001; 2002; 2011) if soil incorporated at preplant with a minimum of 5 tons dry biomass/acre (or 11 Mg/ha, equivalent to 1% w/w) (Wang, 2012). This amount of sunn hemp biomass is easily achievable during summer time in Hawaii but might be insufficient during short day length in the winter and early spring in Hawaii. The other short coming of using sunn hemp alone for nematode management effectively is that it is often used as green manure crop and soil incorporated at preplant, thus the allelopathic effect of the cover crop can only suppress plant-parasitic nematodes at early cash crop planting with a minimal suppressive effect towards the crop harvest especially for longer-term crop. Integrating sunn hemp cover crop with a post-plant organic nematicide application such as neem oil products had been shown to prolong the nematode suppressive effect in a longer-term crop such as sweet potato (Leleiwi et al., 2013). This approach of nematode management had been recommended to organic farmers in Hawaii. However, warm climate conditions along with intensive farming in limited farm lands in Hawaii result in high population levels of plant-parasitic nematodes in many vegetable farms that can exhaust crop production over time. For non-organic farmers, additional options that can manage nematode pest more rigorously might be available.

A newer non-fumigant synthetic pesticide with fungicidal and nematicidal activities, fluopyram, is less toxic to the environment and has shown promising effects against plant-parasitic nematodes (Faske and Hurd, 2015). Fluopyram is a succinate dehydrogenase inhibitor (SDHI) fungicide that is used as a seed treatment and in furrow spray at planting on row crops for management of fungal diseases. Velum® ILeVO (Bayer CropScience, Research Triangle Park, NC) was registered for soybean seed treatment in 2014. A newer formulation that consists of fluopyram + imidacloprid (Velum® Total, Bayer CropScience) has been evaluated as an in-furrow application for suppression of *M. incognita* and *R. reniformis* in cotton and suppressed these nematodes effectively (Lawrence *et al.*, 2014). This formulation was registered only for use against nematodes and insects in cotton and peanut in 2015. Most recently, Velum® One was registered for horticultural crops but only in California and Arizona as a wide-spectrum nematicide that increases root health (Bayer CropScience Press Release, 2017). It's use in Hawaii on horticultural crops are currently under evaluation and are compared to other nematode management practices available to vegetable farmers in Hawaii.

The objective of this research was to evaluate the efficacy of Velumm One chemigation (once or twice) compared to integrating sunn hemp cover cropping with Valem One or Molt-X (a.i. azaractin) against root-knot and reniform nematodes infecting zucchini and cherry tomato in an Oxysol in Hawaii.

Materials and Methods

Two field trials were initiated concurrently at Poamoho Experiment Station in Hawaii to examine nematode suppressive effects of Velum (Bayer CropScience LP, Research Triangle Park, NC) against root-knot nematode (*Meloidogyne* spp.) infection on 'Felix F1' zucchini (Cucurbita pepo) and 'Komohana' cherry tomato (*Solanum lycopersicum*). The soil type at the site is Wahiawa silty clay in the Oxisol order with Tropptic Eutrustox, clayey, kaolinitic, isohyperthermic properties, containing 18.6% sand, 37.7% silt, and 43.7% clay in the top 25 cm soil, with soil organic matter approximately 1.08%. Five treatments examined were 1) Velum One at 6.5 fl oz/acre at crop transplanting (Velum I); 2) Velum One at 6.5 fl oz/acre applied at crop transplanting and 4 weeks after transplanting (Velum II), 3) planting sunn hemp (30 lb seeds/acre) at preplant, soil incorporated followed by Molt-X (10 fl oz/acre, a.i. azaractin) at a 4 week interval after crop transplanting (SH+Molt-X) up to 2 months post planting, and 4) planting sunn hemp followed by Velum One applied at 6.5 fl oz/acre at 4 weeks after transplanting (SH + Velum), and 5) an untreated control. Velum One and Molt-X were applied through drip irrigation using injector. Each field plot was 4×20 ft^2 in size, treatments were replicated 3 times and arranged in randomized complete block design. Zucchini was terminated at 2 months after planting on September 7, 2017 whereas cherry tomato was terminated at 3 months after planting on Oct 12, 2017.

Treatments: Prior to crop transplanting, all treatment plots with sunn hemp (*Crotalaria juncea*) were planted with 'Tropic Sun' sunn hemp seeds on April 29, 2017, whereas the other plots were planted with soybean susceptible to reniform nematodes (*Rotylenchulus reniformis*) and tolerant to root-knot nematodes. Two months after planting sunn hemp or soybean, biomass of crop residues from each plot were recorded, and these crops were terminated on June 29, 2017 by tilling into the soil with a handheld tiller. Sunn hemp generated thick stem tissue that required additional chipping prior to soil incorporation. All field plots were tilled 6 inches deep. Within each plot, 3 zucchini (3-week old) and 3 cherry tomato seedlings (6-week old) were transplanted at 3 ft spacing between plants on both sides of the drip irrigation line on July 6, 2017. The first application of Velum was applied through irrigation lines on the same day as transplanting after the field was irrigated to reach field capacity, with volumetric soil moisture of 35.7% applying at soil temperature fluctuating around 31-32°C. The second application of Velum for Velum II and SH + Velum was conducted on Aug 3, 2017. Velum was injected into the irrigation lines with valve control delivering Velum to the designated field plot using a Docitron injector at a flow rate of 25 ml/min, applied over 60 min, i.e. at 94.38 gal of water per acre. Molt-X was applied at 350 ml/min flow rate over 30 minutes, i.e. at 668.7 gal of water per acre.

Fig. 1. A) Sunn hemp and soybean at preplant, B) valve control for each treatment, C) injecting chemical into irrigation line, D) transplanted tomato and zucchini seedlings.

Nematode assay: Soil samples were taken at the end of sunn hemp or soybean growing period after soil incorporation but prior to zucchini or tomato transplanting from 4 sampling points at 4 inches deep per plot. Soil from each plot was composited into one sampling bag. Soil from each plot were sampled again from the zucchini rhizosphere or tomato rhizosphere separately at one month intervals after transplanting for each crop. Nematodes were extracted from a subsample of 250 cm^3 of soil per sample by elutriation and centrifugal flotation method (Jenkins, 1964). Plant-parasitic nematodes were identified and counted with an inverted microscope. At crop termination, each root system was weighted and rated for root galls using root gall index (RGI) with a scale of 0-10 with 10 being most severely galled according to Netcher and Sikora (1990).

Plant growth and yield: Zucchini canopy width, tomato stem diameter, and chlorophyll content from the third fully matured leaf of each plant were measured biweekly throughout each crop. Tomato canopy width was only measured one time at 1 month after transplanting due to its indeterminate growth. Chlorophyll content was measured using SPAD-502 Chlorophyll Meter (Konica Minolta, Tokyo, Japan 2003). Due to a heavy infestation of melon fly (*Bactrocera cucurbitae*) on zucchini despite standard melon fly management with GF120 and Amulet bait strips along the perimeters of the field, fruit weight was not recorded, but fruit numbers from the three plants per plot were recorded every week for each treatment plot. Cherry tomato fruit was harvested, counted and weighted every week over a 6-week harvesting period from each plant per plot.

Statistical Analysis: All data consisting of one sampling date were subjected to one-way analysis of variance (ANOVA) using PROC GLM in SAS 9.3 (SAS Inc, Cary, NC). Nematode counts were log-transformed, log10 (x+1) to normalize the data prior to ANOVA. Data with multiple sampling dates were examined for treatment and sampling date interaction prior to ANOVA. Yield comparisons from each treatment were based on total harvest throughout each crop. Means were separated by Waller-Duncan *k*-ratio (*k*=100) *t*-test wherever appropriate.

Results and Discussion

Zucchini Trial: Although the zucchini crop was short (2 months), it had lower tolerance to nematode damage, so much so that some plants were dying 2 to 3 weeks after transplanting especially in the control plots (Table 1). Either planting with SH or applying Velum resulted in greater survival of zucchini plants than the control ($P \leq 0.05$). Sunn hemp is well known as an efficient green manure crop despite releasing allelopathic compounds against root-knot and reniform nematodes (Wang et al., 2001). Average wet biomass of SH generated was 10.56 tons/acre (23.67 Mg/ha). Based on Cover Crop Calculator for Hawaii, this would generate 102 lb N/acre and released 58.53 lb of plant-available N/acre. Thus, SH + Velum and SH + Molt-X resulted in higher chlorophyll content and greater canopy width than other treatments ($P \leq 0.05$, Table 1). In particular, integrating SH with Velum produced highest zucchini fruit numbers ($P < 0.05$). None-the-less, application of Velum regardless of one or two times results in similar plant health and growth conditions as reflected in the chlorophyll content, canopy width, root weight, and fruit numbers. RGI was not significantly different among treatments possibly due to the short duration of zucchini test where the root-knot nematodes only completed 1 to 2 life cycles. However, there was a trend that RGI was highest in the control, followed by SH+Molt-X. Based on the root gall index, a second application of Velum within one month after the first application was not warranted partly because after initial infection of the nematodes in the roots, the second generation of root-knot or reniform nematodes might not have been fully matured and hatched out from the roots.

Table 1. Zucchini plant growth, number of plants that survived in the nematode infested field, root-gall index (RGI) and fruit numbers as affected by Velum or sunn hemp cover crop treatments in a field trial at Poamoho, Hawaii.

	No. plant survived	Chlorophyll content (SPAD)	Canopy width (cm)	Root weight (g)	RGI	Fruit number per plant
Velum I [z]	3.00 a[y]	36.08 bc	87.29 b	89.43 b	2.56	50.33 bc
Velum II	3.00 a	37.44 b	88.98 b	101.97 ab	3.00	39.67 c
SH + Velum	3.33 a	44.11 a	116.32 a	155.10 a	3.53	72.00 a
SH + Molt-X	3.00 a	41.96 a	109.32 a	123.97 ab	4.22	53.67 b
Control	2.33 b	33.64 c	68.81 c	71.13 b	6.94	19.67 d
n	-	9	9	9	3	15

[z] Velum I = Velum One applied one time at zucchini planting, Velum II = Velum One applied two times, one at zucchini planting, second at 4 weeks after transplanting, SH + Velum = sunn hemp cover crop followed by applying Velum one time 4 weeks after transplanting, SH + Molt-X = sunn hemp cover crop followed by applying Molt-X monthly, Control = bare ground without nematode management.
[y] Means in each column followed by the same letter were not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

The most abundant plant-parasitic nematodes in the soil were reniform nematodes (*Rotylenchulus reniformis)* and *Meloidogyne* spp. followed by few spiral nematodes (*Helicotylenchus* spp.). No interaction between sampling dates and treatment effect occurred, thus data from the 3 sampling dates were combined in a repeated measure analysis as shown in Table 2. Soil population densities of reniform nematodes were highest in Velum I (one application) treatments ($P \leq 0.05$), and two application of Velum (Velum II) did not suppress reniform nematode abundance on zucchini compared to the control ($P > 0.05$). However, both SH + Velum or SH + Molt-X suppressed reniform nematodes significantly ($P \leq 0.05$). Similar results were observed for root-knot nematodes, where the soil population of *Meloidogyne* were suppressed by SH + Velum or SH + Molt-X but not by Velum I or Velum II compared to the control (Table 2). Numbers of spiral nematodes were higher in Velum I or Velum II than the other treatments ($P \leq 0.05$). Overall abundance of plant-parasitic nematodes in the zucchini rhizosphere were higher in Velum I than the control, whereas SH + Velum or SH +Molt-X were effective in reducing plant-parasitic nematodes in the soil (Table 2). SH + Velum would be less labor intensive than SH + Molt-X as only one Velum application is required, compared to the monthly injection of Molt-X. On the other hand, integrating sunn hemp with Velum provided an additional advantage of reducing the negative impact of Velum (Velum I and Velum II) treatments on soil health as shown in the reduction of bacterivorous and fungivorous nematodes compared to the control (Table 2).

Table 2. Abundance of plant-parasitic, bacteria, fungal and omnivorous nematodes in Velum or sunn hemp cover crop treatments in zucchini rhizosphere at Poamoho, Hawaii.

	Reniform	Root-knot	Spiral	Bacterivores	Fungivores	Herbivores	Omnivores
	----------------------------			Numbers/250 cm3 soil		--------------------------	
Velum I	1857 a	47 ab	137 a	614 b	392 c	2067 a	21 a
Velum II	830 b	66 ab	189 a	827 b	360 c	1119 ab	30 a
SH+Velum	99 c	18 c	8 b	1203 a	722 ab	179 c	23 a
SH + Molt-X	20 d	59 bc	0 b	1538 a	844 a	102 c	40 a
Control	866 b	284 a	16 b	871 ab	416 bc	1178 b	23 a

Means are average of 3 replications repeatedly measured at 3 sampling times (n=9) at 0, 1 and 2 months after zucchini planting. Means in each column followed by the same letter were not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Tomato Trial: Since the tomato trial was longer (3 months) and tomato has greater tolerance to nematode damage, no tomato plants died prior to termination of the experiment. Both treatments with SH had higher tomato stem diameter and chlorophyll content than other treatments ($P \leq 0.05$, Table 3). SH + Velum had higher stem diameter, chlorophyll content than Velum alone but Velum I (one application) resulted in higher cherry tomato fruit numbers and fruit weight than SH + Velum (P ≤ 0.05). However, applying Velum two times resulted in lower tomato yields (fruit number and fruit weight) and root weights than the control ($P \leq 0.05$) even though it did not reduce other plant growth parameters (stem diameter, chlorophyll content and canopy width) of tomato (P > 0.05).

Table 3. Tomato plant growth, root-gall index (RGI) and yield affected by Velum or sunn hemp cover crop treatments in a field trial at Poamoho, Hawaii.

	Stem diameter (mm)	Chlorophyll content	Canopy width (cm)	Root weight (g)	RGI	Fruit number	Fruit weight (g)
Velum I	8.63 c[z]	36.08 bc	122.69 ab	109.67 bc	3.67 b	405.33 a	3524.6 a
Velum II	8.19 cd	37.44 b	123.82 ab	95.00 c	3.22 b	117.00 c	1074.2 c
SH + Velum	9.56 b	44.11 a	123.44 ab	92.33 c	2.34 b	241.00 b	1994.9 b
SH + Molt-X	10.21 a	41.96 a	126.31 a	153.33 ab	5.50 ab	444.33 a	3460.1 a
Control	7.79 d	33.64 c	118.24 b	163.67 a	7.72 a	232.67 ab	1624.6 b
n	15	15	3	9	3	15	15

[z] Means in each column followed by the same letter were not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

None-the-less, all Velum treatments resulted in significant reduction of RGI compared to the control, where SH + Velum was reduced about 70%, Velum I 52% and Velum II 58% of the RGI compared to the Control. As shown in Fig. 3, the suppressive effects of Velum on root-knot nematode damage on zucchini were rather significant.

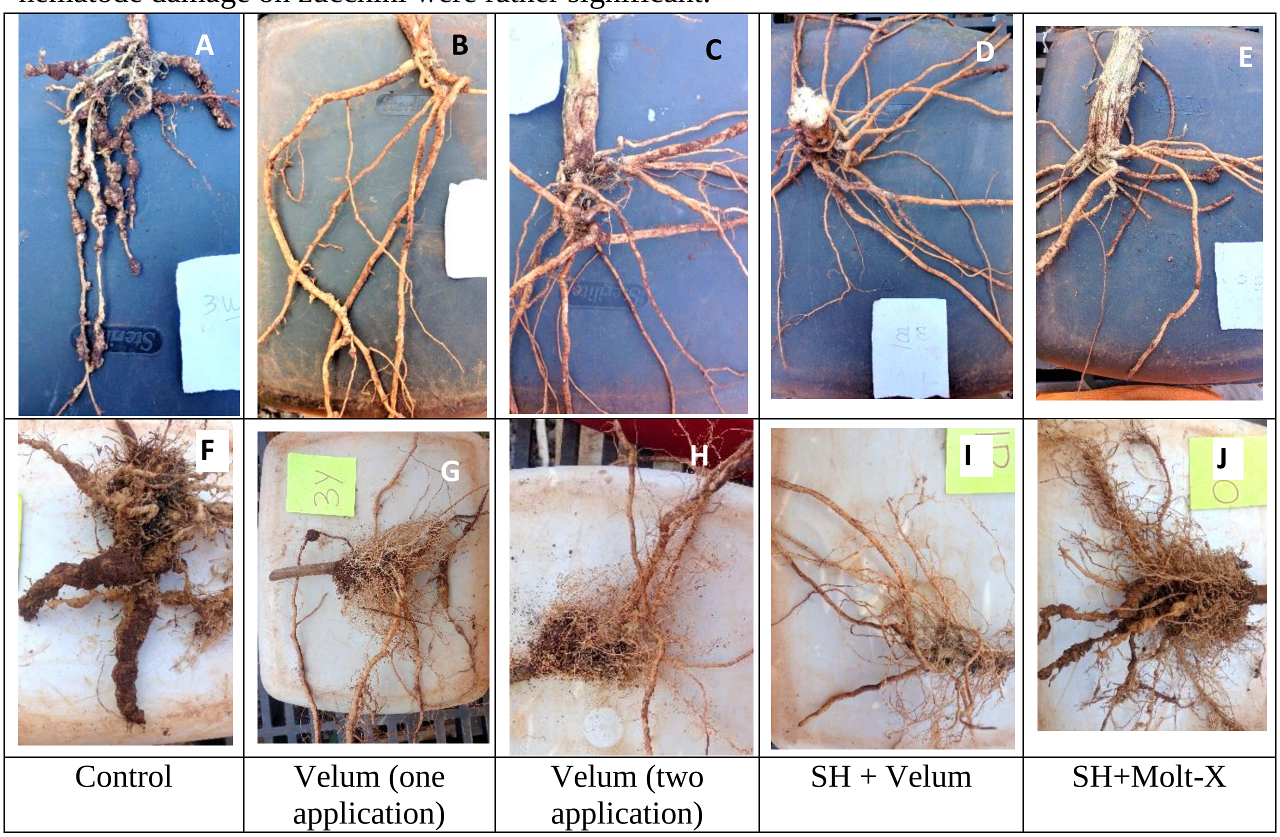

Fig. 3. Effects of Velum or sunn hemp (SH) integrated with Velum or Molt-X on root galls compared to untreated control on zucchini (A-E) and cherry tomato (F-J) at termination of both field trials.

Similar results in suppression of population densities of plant-parasitic nematodes in the soil were observed in the tomato trial as the zucchini trial where application of Velum only at transplanting (Velum I) resulted in highest numbers of reniform nematodes among treatments, and applying another application of Velum (Velum II) still did not reduce reniform nematodes abundance compared to the control ($P > 0.05$, Table 4). However, SH + Velum and SH + Molt-X suppressed reniform nematode abundance compared to the control ($P \leq 0.05$). Only SH + Velum suppressed root-knot nematodes on tomato compared to the control ($P \leq 0.05$).

Table 4. Abundance of plant-parasitic nematodes in Velum or sunn hemp cover crop treatments in cherry tomato rhizosphere at Poamoho, Hawaii.

	Reniform	Root-knot	Spiral
	---------Numbers/250 cm3 soil---------		
Velum I	1841 a	576 ab	129 a
Velum II	873 b	177 ab	170 a
SH+Velum	129 c	219 b	4 b
SH + Molt-X	18 d	760 ab	1 b
Control	814 b	1100 a	38 b

Means are average of 3 replications repeatedly measured over 3 sampling times (n=9) at 0, 1.5 and 3 months after tomato planting. Means in each column followed by the same letter were not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

Due to significant interaction between sampling time and treatment on abundance of the free-living nematodes, these data were presented by sampling date in Table 5. Sunn hemp in either SH+Velum or SH+Molt-X resulted in lower counts of total herbivores (including reniform, root-knot, spiral and other plant-parasitic nematodes) than the Velum I or Velum II ($P \leq 0.05$) at tomato planting (Table 5). However, all Velum treatments (Velum I, Velum II and SH + Velum) resulted in lower counts of total herbivorous nematodes than the control and SH + Molt-X towards the end of the tomato crop ($P \leq 0.05$). While SH+Molt-X suppressed total herbivores during the first 1.5 months after tomato planting, it did not reduce total herbivores at 3 months after planting (Table 5). This could be due to termination of Molt-X injections at 2 months after tomato planting. None-the-less, SH+Molt X had the least negative impact on beneficial free-living nematodes (including bacterivores, fungivores and omnivores) followed by SH + Velum.

Conclusion

Applying Velum One at transplanting of zucchini and cherry tomato reduced root galls caused by root-knot nematodes by 63% and 52%, respectively. In terms of root health, additional treatment of Velum One at 1 month after transplanting did not further reduce root gall formation compared to the one time application at transplanting. None-the-less, applying Velum either once or twice did not suppress soil population densities of root-knot or reniform nematodes on both crops. However, integrating sunn hemp with Velum one month after transplanting provided a synergistic effect against plant-parasitic nematodes while providing a green manure effect, and reducing the negative impact of Velum on free-living nematodes consistently in both trials. SH+Velum treatment also outperformed the standard practice of SH+Molt-X treatment in the tomato trial.

Table 5. Abundance of bacterivorous, fungivorous, herbivorous and omnivorous nematodes in Velum or sunn hemp cover crop treatments in cherry tomato rhizosphere at Poamoho, Hawaii.

	Bacterivores	Fungibores	Herbivores	Omnivores
	------------------------Numbers/250 cm3 soil----------------------			
	At planting			
Velum I	907 a	367 a	2927 a	50 a
Velum II	1407 a	360 a	1193 a	70 a
SH+Velum	1130 a	337 a	193 bc	13 a
SH + Molt-X	1000 a	453 a	107 c	10 a
Control	1157 a	350 a	1353 ab	37 a
	1.5 months after planting			
Velum I	149 d	253 b	1733 a	3 b
Velum II	393 cd	410 b	1130 a	7 b
SH+Velum	2193 a	900 a	243 bc	40 ab
SH+Molt-X	1623 ab	1123 a	30 c	220 a
Control	587 bc	293 b	883 ab	10 b
	3 months after planting			
Velum I	140 c	103 b	3100 b	0 b
Velum II	70 d	127 b	1450 b	0 b
SH+Velum	320 b	407 a	787 b	0 b
SH + Molt-X	817 a	407 a	2217 a	57 a
Control	370 b	180 ab	3043 a	47 a

Means are average of 3 replications. At each sampling date, means in each column followed by the same letter were not different according to Waller-Duncan *k*-ratio (*k*=100) *t*-test.

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