

## BiOWiSH<sup>®</sup> Crop Liquid

### Evaluation of BiOWiSH<sup>®</sup> Crop Liquid on Yield and Quality in Walnuts – Year 2

#### Executive Summary

BiOWiSH Technologies, Inc. engaged Helena R&D as a third-party Contract Research Organization (CRO) to conduct a 3-year study to determine the effects of BiOWiSH<sup>®</sup> Crop Liquid on walnut production. The results reported in this study are from year 2 of the trial. The studies compared 2 treatments:

- A regional fertilizer program as the control (Control)
- The same fertilizer program with BiOWiSH<sup>®</sup> Crop Liquid added (Control + BiOWiSH<sup>®</sup> Crop Liquid)

The study determined that the Control + BiOWiSH<sup>®</sup> Crop Liquid program increased several growth, yield, and quality parameters in walnuts which led to higher profit. The study also demonstrated the consistency of BiOWiSH<sup>®</sup> Crop Liquid, as the same data trends were noted in the first-year study.

#### Background

##### About BiOWiSH<sup>®</sup> Crop Liquid

BiOWiSH<sup>®</sup> Crop Liquid is a microbial biostimulant that can be coated onto dry fertilizer or mixed with liquid fertilizers to create an enhanced efficiency fertilizer with industry leading shelf-life and consistent results across a broad range of operating conditions and environments, all at a low cost to farmers. BiOWiSH<sup>®</sup> Crop Liquid stimulates native microbial activity and promotes root development, increasing nutrient uptake and improving plant vigor. BiOWiSH<sup>®</sup> Crop Liquid is proven to enhance the effects of applied fertilizers by increasing yield and soil health.

##### About Helena Chemical Company

Helena Agri-Enterprises is a leading provider of crop production and crop protection products in the United States and worldwide. Headquartered in the USA, the company has been in the agronomic products supply business for more than 50 years and has expanded their contract research services over the last decade. As an independent CRO, Helena R&D is a team of highly trained and experienced study directors, field researchers, and support staff. They are one of several independent CROs that BiOWiSH Technologies, Inc. works with to independently evaluate our agronomy products.

#### Objectives

The objective of this second year research study was to determine the efficacy of BiOWiSH<sup>®</sup> Crop Liquid technology, manufactured in the USA by BiOWiSH Technologies, Inc., in different years on walnut production when added to a fertility program common to the production area in central California. The focus was on BiOWiSH<sup>®</sup> Crop Liquid's impact on soil nutrients, plant vigor, in-shell and nut meat yield, nut grading quality, and the grower economics.

In this trial, the common regional walnut fertility program included commodity in-organic fertilizers, a proprietary liquid potassium fertilizer (Nucleus<sup>®</sup> 0-0-21), and a liquid humic acid product (Hydra-Hume<sup>®</sup>). This program was compared to the same program with BiOWiSH<sup>®</sup> Crop Liquid.

#### BiOWiSH<sup>®</sup> Crop Liquid



- Improves crop yields
- Increases nutrient availability
- Enhances root development
- Improves plant vigor
- Stimulates native microbial activity in the soil
- Improves soil health

##### Available Sizes

- 50 gal/190 L
- 264 gal/1000 L

## Implementation Program

The 3<sup>rd</sup> party CRO conducted the trial on a commercial farm near Live Oak, CA. Pest and disease management techniques were implemented on site when required. The trial site was a 9 year-old Howard block of trees managed in 2 large blocks which were randomly assigned to treatments. The size of each block was 696 ft (212.1 m) x 1018 ft (310.3 m) which was equivalent to 17.5 acres (7.1 ha) per treatment.

All evaluations were conducted on the same marked trees and included leaf color, tree vigor, new shoot growth, length of shoot growth, in-shell yield, nut meat yield, and USDA grades for color and size. The evaluations were used to perform an economic analysis between the treatments.

Representative pre-plant and post-harvest composite soil samples were obtained from each block to evaluate soil nutrient levels and a leaf tissue sample (50 leaves composited from all marked trees in each treatment) was collected to evaluate tissue levels of nutrients.

In this trial, the standard walnut fertility program included CAN-17, UAN-32%, Nucleus® 0-0-21 (used to deliver potassium to the root zone for quick and efficient potassium uptake), a liquid 7-25-5 fertilizer, a liquid 3-0-8 fertilizer, and Hydra-Hume® (used as a fertilizer efficiency aid to help farmers get more use from the fertilizer they apply). Three fertilizer applications were injected through an irrigation system at the volume indicated in Table 1, which details the 2 treatments, fertilizers, and application timings.

Table 1. Fertilizer, Treatments, and Application Timings

Treatment	First Application During "Leaf Out" – April	Fertilizer	Second Application During Growth Stage in May	Third Application During growth stage in June
Control**	CAN-17 31 gal/ac (290 L/ha)	UAN-32%	18.75 gal/ac (175.4 L/ha)	18.75 gal/ac (175.4 L/ha)
		Nucleus® 0-0-21	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
		3-0-8	1 qt/ac (2.3 L/ha)	1 qt/ac (2.3 L/ha)
	3-0-8 1 qt/ac (2.3 L/ha)	7-25-5	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
		Humic Acid	1 gal/ac (9.3 L/ha)	1 gal/ac (9.3 L/ha)
		Water	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
Control + BiOWiSH® Crop Liquid***	CAN-17 31 gal/ac (290 L/ha)	UAN-32%	18.75 gal/ac (175.4 L/ha)	18.75 gal/ac (175.4 L/ha)
		Nucleus® 0-0-21	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
		3-0-8	1 qt/ac (2.3 L/ha)	1 qt/ac (2.3 L/ha)
	3-0-8 1 qt/ac (2.3 L/ha)	7-25-5	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
		Humic Acid	1 gal/ac (9.3 L/ha)	1 gal/ac (9.3 L/ha)
		Water	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)
BiOWiSH® Crop Liquid	Labeled Rate***	Humic Acid	1 gal/ac (9.3 L/ha)	1 gal/ac (9.3 L/ha)
		Water	4 gal/ac (37.4 L/ha)	4 gal/ac (37.4 L/ha)

\*Nucleus® and Hydra-Hume® are registered product names of Helena AgriEnterprises.

\*\*For the Control treatment, untreated nitrogen (CAN-17 or UAN 32%), 3-0-8, 7-25-5, 0-0-21, and(or) humic acid liquid were mixed with water to the volumes indicated in the table. This fertilizer solution was then applied through the irrigation dripper line to the appropriate 17.5-acre block at each application, respectively.

\*\*\*For the Control + BiOWiSH® Crop Liquid treatment, untreated nitrogen (CAN-17 or UAN 32%), 3-0-8, 7-25-5, 0-0-21, and(or) humic acid liquid were mixed with BiOWiSH® Crop Liquid according to label recommendations then mixed with water to the final volume indicated in the table above. This fertilizer solution was then applied through the irrigation dripper line to the appropriate 17.5-acre block at each application, respectively.

The following evaluations were made in order to determine the effects of the BiOWiSH® Crop Liquid on walnut production. Visual evaluations for plant health were conducted 6 times between May 10<sup>th</sup> and August 20<sup>th</sup>. This involved rating each tree for leaf color on a 0-10 scale (0 = pale yellow – 10 = dark green), tree vigor on a 0-5 scale (0 = poor – 5 = excellent), and percentage of shoots showing new growth. The average length of new growth was also determined on July 28<sup>th</sup>.

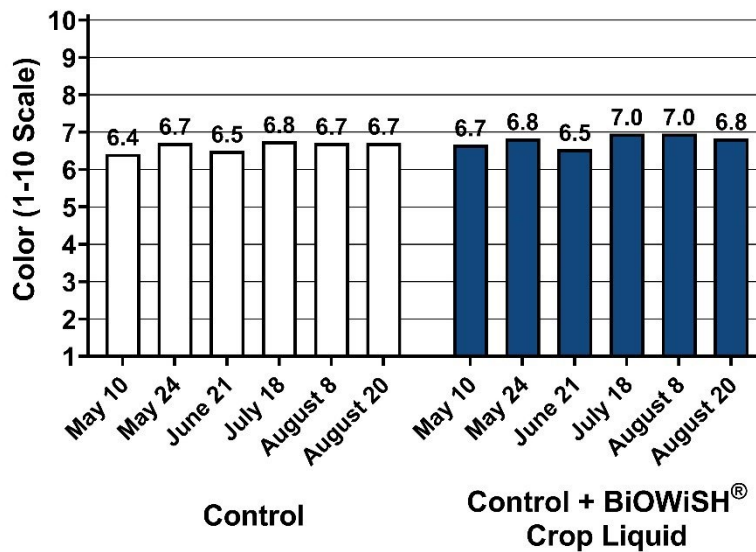
At harvest, all shaken walnuts per tree were raked, collected, and weighed. This data was used to calculate in-shell walnut yields. A 100-nut sample was collected from each tree and stored in a freezer until processing (“crack out”). First, in-shell weights were obtained, then nuts were sized in-shell according to the official USDA size grades for walnuts, including, baby, medium, large, and jumbo (number per 100 nuts) and used to calculate the yield of walnuts within each USDA size class. Walnuts were then cracked and meats were graded for color according to official USDA color grades of extra light, light, light amber, and amber (number per 100 nuts). The meats were then weighed and used to calculate overall meat yield per acre. Fifty leaves were collected from each treatment block on July 28<sup>th</sup> and sent to an accredited third-party laboratory for plant tissue analysis. Yield data and current commodity prices were used to calculate net income and profit change.

## Results

### Tree Health and Growth

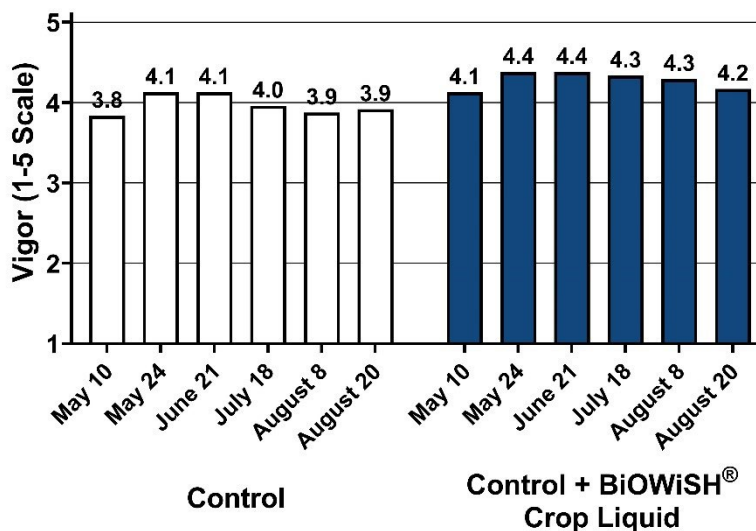
Analysis of leaf color, evaluated 6 times between May and August, suggested that color ratings were higher for Control + BiOWiSH<sup>®</sup> Crop Liquid compared to Control at 5 of the 6 time points evaluated (Figure 1).

Figure 1. Effect of BiOWiSH<sup>®</sup> Crop Liquid on Leaf Color in Walnut Trees



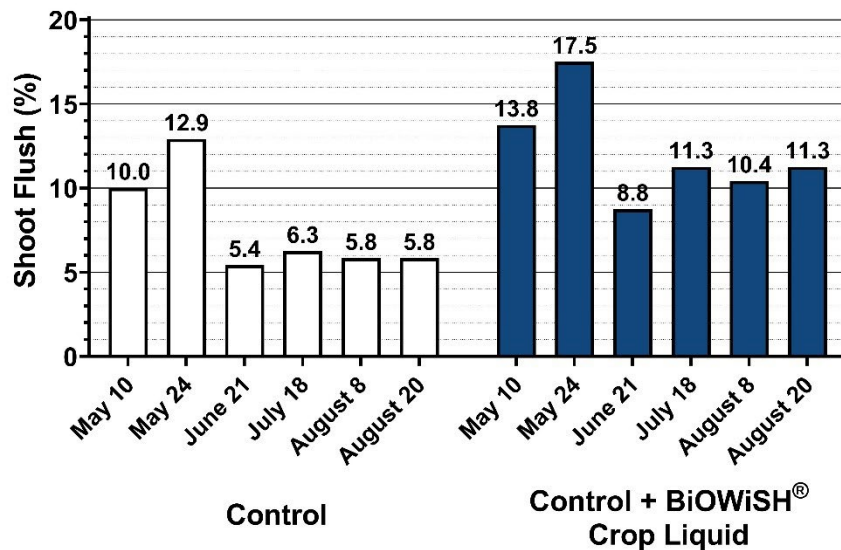
Analysis of tree vigor, evaluated 6 times between May and August, suggested that vigor ratings were higher for the Control + BiOWiSH<sup>®</sup> Crop Liquid at each of the 6 time points evaluated (Figure 2).

Figure 2. Effect of BiOWiSH<sup>®</sup> Crop Liquid on Tree Vigor in Walnut Trees



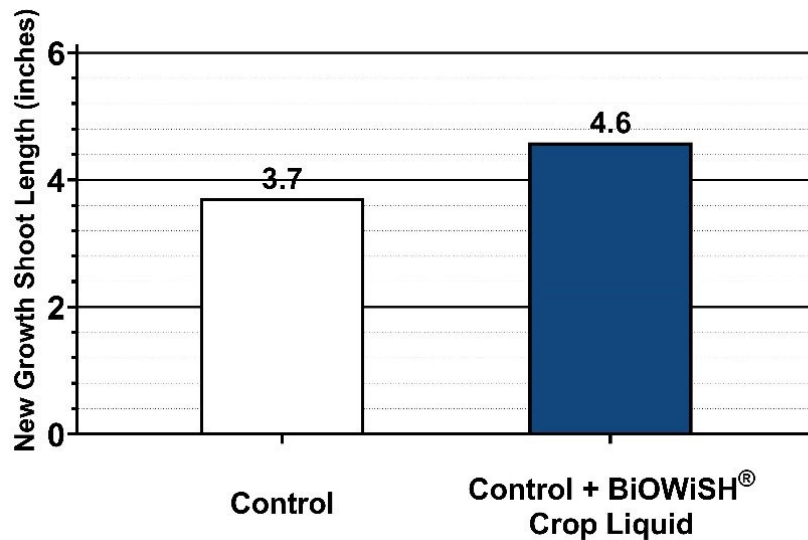
Analysis of new shoot growth, evaluated 6 times between May and August, suggested that the percentage of shoots showing new growth was higher for the Control + BiOWiSH® Crop Liquid compared to Control at each of the 6 time points evaluated (Figure 3).

Figure 3. Effect of BiOWiSH® Crop Liquid on New Shoot Growth in Walnut Trees



Analysis of shoot growth length, evaluated once in July, suggested that average shoot length was greater for the Control + BiOWiSH® Crop Liquid compared to Control (Figure 4).

Figure 4. Effect of BiOWiSH® Crop Liquid on Average Shoot Length Growth in Walnut Trees

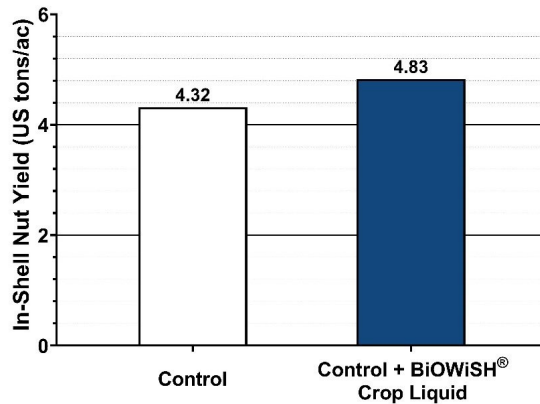


Note: 1 in = 2.54 cm

## Yield Parameters

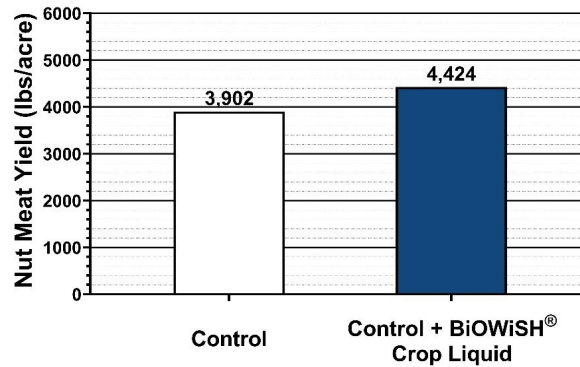
Analysis of walnut yield parameters showed an increase in in-shell nut yield (11.8%, Figure 5) and an increase in nut meat yield (13.4%, Figure 6) in the Control + BiOWiSH® Crop Liquid when compared to the Control, respectively.

Figure 5. Effect of BiOWiSH® Crop Liquid In-Shell Yield of Walnuts



**Note:** 1 US Ton/ac = 2.2 metric tons/ha

Figure 6. Effect of BiOWiSH® Crop Liquid on Nut Meat Yield in Walnuts

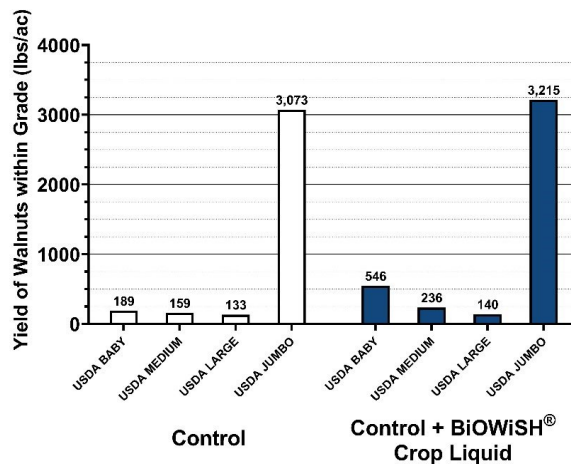


**Note:** 1 lb/ac = 1.1 kg/ha

## USDA Quality and Color Grades

Analysis of USDA quality grades suggested that the Control + BiOWiSH® Crop Liquid treatment program had greater yields within all USDA classes, including baby, medium, large, and jumbo (Figure 7).

Figure 7. Effect of BiOWiSH® Crop Liquid on Yield Within USDA Quality Grades in Walnuts



**Note:** 1 lb/ac = 1.1 kg/ha

Analysis of USDA nut meat color grades showed that nut meat color were similar across all grades when comparing Control and Control + BiOWiSH® Crop Liquid, each having > 88% of nut meats showing the highest color rating of USDA Extra Light (data not shown).

## Laboratory Leaf Tissue Analysis

The laboratory leaf tissue analysis showed the Control had slightly higher overall leaf nutrients, however both the Control and Control + BiOWiSH® Crop Liquid treated trees had comparable ratings of nutrients that are known to promote plant vigor and yield (Table 2).

Table 2. In-season Laboratory Leaf Tissue Analysis

Treatments	N %	P %	K %	Ca %	Mg %
Control	2.96	0.16	2.09	2.32	0.66
Rating	H	S	H	E	E
Control + BiOWiSH® Crop Liquid	2.95	0.15	1.72	2.06	0.55
Rating	H	S-L	H	H-E	H-E

## Laboratory Soil Analysis

Pre-treatment laboratory soil analysis from composite soil samples for each treatment block showed that the Control and Control + BiOWiSH® Crop Liquid treatment blocks had comparable initial levels and ratings of nitrogen, phosphorus, potassium, magnesium, calcium, and organic matter (Table 3).

Table 3. Pre-Treatment Laboratory Soil Analysis

Treatments	Timing	N ppm	P Weak Bray, ppm	P Strong Bray, ppm	K ppm	Mg ppm	Ca ppm	OM %
Control	Pre-Treatment	2	31	70	161	235	1074	1.5
Rating			VH	VH	VH	VH	H	VL
Control + BiOWiSH® Crop Liquid	Pre-Treatment	3	25	61	196	323	1137	1.1
Rating			H	VH	VH	VH	M	VL

Post-harvest laboratory soil analysis from composite soil samples for each treated block showed that the Control + BiOWiSH® Crop Liquid treatment blocks had higher levels of nitrogen, magnesium, calcium, and organic matter, however phosphorus and potassium levels were slightly lower. As with the plant tissue analysis, these slight differences in the soil data may be attributable to normal sampling and laboratory analysis variances. Alternatively, these data combined with the increased yield parameters (Figures 5 and 6, respectively) potentially indicate that more available nutrients were mineralized and/or taken up in the Control + BiOWiSH® Crop Liquid block (Table 4).

Table 4. Post-Harvest Laboratory Soil Analysis

Treatments	Timing	N ppm	P Weak Bray, ppm	P Strong Bray, ppm	K ppm	Mg ppm	Ca ppm	OM %
Control	Pre-Treatment	2	26	62	173	341	1130	0.4
Rating			H	VH	VH	VH	M	VL
Control + BiOWiSH® Crop Liquid	Pre-Treatment	3	16	44	161	533	1581	1.1
Rating			M	H	H	VH	L	VL

## Economic Analysis

Based upon the average in-shell yield increase (11.8%) in the Control + BiOWiSH® Crop Liquid treated blocks, net income increased by 12%, resulting in a profit increase of \$1216 USD/ac.

Table 5. Effect of BiOWiSH® Crop Liquid on Economic Performance in Walnuts\*

Treatments	In-shell Yield US tons/ac [MT/ha]	Yield Increase %	Net Income* USD/ac [\$USD/ha]	Net Income Gain %	Profit Change** USD/ac [\$USD/ha]
Control	4.32 [9.68]	-	\$10,288 [\$25,422]	-	-
Control + BiOWiSH® Crop Liquid	4.83 [10.83]	11.8%	\$11,504 [\$28,427]	12%	\$1216 [\$3005]

\*Calculations for conversions between imperial and metric units are based on the original source data; slight rounding differences may occur within reported publication values.

\*\*Net income gain is the crop value minus the fertility program cost. It does not account for non-fertility expenses.

\*\*\*Profit change is the difference between net income of the respective program and the control.

## Conclusion

In this large-block commercial research trial, the addition of BiOWiSH® Crop Liquid to the Control fertility program increased new shoot growth, walnut yields within each USDA size grade, in-shell walnut yield, and nut meat yield when compared to the Control. When considering the yield increases, the leaf color, tree vigor, leaf tissue analysis, and soil analysis, they potentially indicate that more available nutrients were taken up in the Control + BiOWiSH® Crop Liquid block. All the measured factors interacted to increase net income by 12% and increased profit by \$1216 USD/ac in the Control + BiOWiSH® Crop Liquid treatment. Overall, the study indicates that BiOWiSH® Crop Liquid is a useful addition to a walnut program and offers a significant return on investment opportunity to the grower.



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