

**Metalized Polyethylene Mulch to Repel Asian Citrus Psyllid, Slow Spread of
Huanglongbing and Improve Growth of New Citrus Plantings**

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Abstract

BACKGROUND: Greening or huanglongbing (HLB) is a debilitating disease of citrus caused by *Candidatus Liberibacter asiaticus* and transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri*. HLB now occurs worldwide in all major citrus growing regions except the Mediterranean and Australia. Management relies principally on insecticidal control of the ACP vector, but is insufficient, even for young trees which are most susceptible to the disease. We tested the ability of metalized polyethylene mulch to repel adult ACP as well as effects on incidence of HLB and early tree growth.

RESULTS: Metalized mulch significantly reduced ACP populations and HLB incidence compared to whiteface mulch or bare ground. In addition, metalized mulch, together with the associated drip irrigation and fertigation system, increased soil moisture, reduced weed pressure, and increased tree growth rate.

CONCLUSION: Metalized mulch slows spread of ACP and therefore HLB pressure on young citrus trees. Metalized mulch can thereby augment current control measures for young trees based primarily on systemic insecticides. Additional costs could be compensated for by increased tree growth rate which would shorten time to crop profitability. These advantages make a compelling case for large scale trials using metallized mulch in young citrus plantings threatened by HLB.

Key words: Asian citrus psyllid, *Diaphorina citri*, Huanglongbing, citrus greening disease, fertigation

1 INTRODUCTION

Huanglongbing (HLB), also known as citrus greening, is a bacterial disease caused by *Candidatus Liberibacter asiaticus* (Las). Considered the most devastating disease of citrus worldwide, HLB has been found in over 40 citrus producing countries in Asia, Oceania, and America.^{1,2,3} Control of the vector, *Diaphorina citri*, the Asian citrus psyllid (ACP) is key to management of the disease, although the best and most economical strategies for vector control are still active research objectives. The task is complicated by an apparent need to control ACP populations to extremely low levels to slow disease spread sufficiently to maintain economically viable production. This is especially true for trees soon after planting when exposure time, economic potential, and susceptibility to the disease are all maximal. Trees flush most frequently when young and are therefore most attractive to ACP which requires developing shoots for oviposition.⁴ Systemic neonicotinoid insecticides have been the primary means of protecting young trees from ACP, but results are not completely satisfactory and reliance on a single mode of action increases the likelihood of insecticide resistance.⁵ Therefore, additional tactics are needed to protect new plantings from ACP and ultimately HLB.

Reflective mulch has long been used to repel aphids, thrips and whiteflies from colonizing a variety of young crop plants, presumably by disorienting the insects during flight.⁶

Aluminum foil was first tested in the 1950's as mulch for various vegetables and was shown to repel green peach aphids in 1968.^{7,8,9} Since those studies, various reflective mulches have been developed for a wide range of crops and shown to significantly increase crop yields.⁶ Possible repellence of bees could be a concern in many crops on which they are

needed for pollination, but Moore et al. 1965 found more honey bees, as well as a reduction in agromyzid leafminer damage, in squash growing on aluminum mulch in Florida compared to black mulch or bare ground.¹⁰ Polyethylene mulch covered with aluminum paint eliminated the need of pesticides to control cucumber beetles in cucumber.¹¹ Truly metalized mulch has an aluminum coating vacuum deposited on polyethylene which affords a superior degree of reflectivity to painted film.

It is commonly accepted that early young plant development is the most critical growth stage to protect plants from many viruses and diseases; as the plant matures it is better able to cope with the infections. Summers et al. 1995 concluded that virus infection at first harvest is a better indicator of mulch effectiveness than insect counts.¹² In addition, it has been shown that reflective mulch combined with imidacloprid enhanced control of whitefly and apterous aphids in zucchini.¹³ Therefore, we tested the ability of metalized polyethylene mulch to protect a new citrus planting from the visually oriented, day-flying ACP, and consequently HLB. Black plastic mulch has been shown to reduce weed pressure over traditional bare ground methods but did not show an increase in citrus tree growth over a two year period.¹⁴ Therefore, we also assessed soil moisture and weed cover as factors affecting tree growth and consequently, the economic benefit of starting young citrus in the field on plastic mulch.

2 EXPERIMENTAL METHODS

2.1 Planting 'Hamlin' orange trees grafted to US-802 rootstock, a hybrid of pummelo (*Citrus grandis*) with trifoliolate orange (*Poncirus trifoliata*), were transplanted 18 May 2010 at the Southwest Florida Research and Education Center located in Immokalee, FL. The ground was leveled and raised beds, (45.7cm = 18 inches) prepared above ground level separated

by a 46.7 cm deep drainage swale. Soil was Immokalee fine sand supplemented with 50 tons/ha (20 tons/ac) incorporated horticultural compost. Tree density was 2.44m (8ft) within rows (2 per bed) and 5.5m (18ft) between rows. Micro-sprinklers (MaxiJet Max-14 360° Fill-In, Dundee FL) rated at 60.6 L (16 gal) per hour were installed, one for every two trees.

Plots consisting of ten trees each were assigned in a randomized complete block design to one of three treatments: whitefaced polyethylene mulch, metalized polyethylene mulch, and a bare-ground control. Four replicates were distributed in alternate tree rows with an untreated row between each as buffer. Micro-sprinklers were removed in the plots and a drip irrigation system installed. Two Toro Turbo-Key® drip emitters (The Toro Company, Bloomington, MN), each providing 7.6 L (2 gal) per hour, were placed within 15cm (6 in) of the trunk on the east and west side of each tree. Liquid fertilizer (8-2-8 NPK) was applied using Mazzei Injector model #287 (Mazzei, Bakersfield, CA) weekly at the equivalent recommended rate of 0.34 kg (0.75 lbs) 8-2-8 granular fertilizer applied every six weeks. Ten-tree plots established in buffer rows, irrigated with micro-sprinklers and fertilized according to industry standards were also evaluated for comparison. Each year, granular fertilizer applications were terminated early October in the standard to reduce new growth and therefore the potential impact of a freeze. Liquid fertilizer was cut off in drip irrigated plots six weeks later, thus maintaining total nutrients applied equal among all trees.

2.2 Mulch The first reflective mulch used was 15.2 μ (0.6 mil) metalized low density polyethylene (Intergrow, Clearwater, Fla., USA) laid on 10 May 2010 with a Kennco vegetable bedder (Kennco, Ruskin, FL) at a width of 1.5 m (5 ft) and flush with the soil line. Whitefaced black 31.8 μ (1.25 mil) low-density polyethylene mulch (Ginegar Plastic Products

Ltd., Kibbutz Ginegar, Israel) was laid using the same method. Holes were melted into the plastic for planting using a metal ring 15.2 cm, (6 in) in diameter heated on a gas grill. The metalized product was not sufficiently durable and was replaced on 9 Jul 2010 with a 31.8 μ (1.25 mil) metalized low-density polyethylene (Pliant Corp. Schaumburg IL) laid by hand at an exposed width of 1.8 m (6 ft) with one piece of 1.2m (4 ft) width placed on either side of the tree and held down in the middle by 14.4 cm (6 in) galvanized wire ground staples and the outer edges secured with soil. Both metalized and whitefaced mulches were replaced on 28 January 2011 and again on 12 January 2012 by hand as described above.

2.3 Pesticides Neonicotinoid insecticides normally applied as a drench to young trees for ACP and citrus leafminer control were not used to insure that populations of ACP were adequate for evaluation. However, insecticides zeta-cypermethrin (17.1%) (Mustang[®], FMC, Philadelphia, PA) 314 ml/ha (4.3 fl oz/A) carbaryl (44.1%) (Sevin XLR Plus[®], Bayer CropScience LP, Research Triangle, NC) 4.7 L/ha (2 qts/A), and 703 ml/ha (10 fl oz/A) spirotetramat (22.4%) (Movento[®] Bayer CropScience LP, Research Triangle, NC) were applied to the foliage of all trees on 10 June, 2 October, and 21 November 2011 respectively, after approximately three quarters of total flush inspected were found to be infested with ACP. During the 2012 growing season (17.1%) (Mustang[®], FMC, Philadelphia, PA) 314 ml/ha (4.3 fl oz/A) carbaryl (44.1%) (Sevin XLR Plus[®], Bayer CropScience LP, Research Triangle, NC) 4.7 L/ha (2 qts/A) were sprayed on 18 May and 14 September 2012 respectively. In addition, methoxyfenozide (Intrepid[®] 2F, DowAgrosciences, Indianapolis, IN), an ecdyson agonist that has no impact on ACP or aphids, was applied approximately once a month to control citrus leafminer.

Weed control in buffer rows consisted of three applications of bromacil (40%) + diuron (40%) (Krovar[®], DuPont, Wilmington, DE) 4.5 kg/ha (4 lbs/acre) plus glyphosate (41%) (Roundup Pro[®] Monsanto Company, St. Louis, MO) at 1.5% concentration on 3 March, 29 July, and 13 September 2011. Mulch plots were treated at the same time with minimal spot sprays of glyphosate.

2.4 Sampling Flush observation, yellow sticky traps, and tap sampling were used to monitor ACP populations.^{15,16,17} Young flush shoots were inspected on all trees every other week throughout the growing season for ACP and other citrus pests as well as beneficial insects. Up to twenty shoots per tree, if available, were inspected each time, noting presence or absence of any ACP stage. Aphids were recorded for each shoot as present or absent. Citrus leafminer damage was monitored on two different occasions during the growing season and rated on a scale of 1 to 10, with 1 being less than 10% of the surface damaged and 10 being the entire surface damaged. Lacewing larvae, spiders and lady beetle adults or larvae were also noted. Corn root worm yellow sticky cards (Great Lakes IPM, Vestaburg, MI) 22.9x27.9cm (9x11in) were placed at canopy height between the 3rd – 4th, 5th – 6th, and 7th – 8th trees of each plot to monitor ACP movement within the plots. Sticky cards were changed every other week.

Leaf samples (five leaves) were taken from the north, east, south, west, and top of all trees on 5 Aug and 13 Dec 2011. Samples were analyzed by qualitative PCR to determine the presence of Las DNA. Total plant DNA was extracted from 100 mg of petiole tissue using the Promega Wizard[®] 96 DNA Plant isolation kit (Promega, USA). Briefly, tissues were flash frozen under liquid nitrogen prior to pulverization to a fine powder using a Mini-beadbeater (Bio Spec Products Inc., Bartlesville, OK). Samples were then processed as per

manufacture's instruction, DNA eluted in 50 μ L AE Buffer and stored at -20°C . Primers and probes were obtained for *Candidatus Liberibacter asiaticus* (HLBas/HLBr and HLBP)¹⁸.

Primers and probes for the plant cytochrome oxidase, COX gene (COXf/COXr and COX-p) were used for an internal control to check the extraction.¹⁸ The internal probe COX-p was labeled with 6-carboxy-4', 5'-dichloro-2', 7'- dimethoxyfluorescein (JOE) reporter dye at the 5'-terminal nucleotide and with BHQ-2 at the 3'-terminal nucleotide. The positive control was DNA from known positive citrus trees located in the SWFREC grove and negative controls were obtained from citrus grown under screen-house conditions at SWFREC and tested annually.

Five central trees in each plot were checked weekly from 9 June through 3 November 2011 for soil moisture at a depth of 20 cm, using a HydroSense (Campbell Scientific Australia, Garbutt, QLD Australia) soil moisture probe placed 10.2cm, (4in) south of the trunk. Tree growth was evaluated by estimating trunk cross sectional area on all trees in experimental and grower standard plots 7 June, 12 Sep, and 14 Dec 2011. A Max-Cal digital calipers (Fowler, Newton, MA) was used to take two measurements of trunk diameter, one in a north-south and the other an east-west orientation at 7.2 cm (3 in) above the graft union. The measurement location was marked with white latex paint for future measurements to insure continuity. Cross sectional area was calculated using the formula for an ellipse $A = \pi \left(\frac{d_1}{2}\right) \left(\frac{d_2}{2}\right)$ where d_1 and d_2 were the two measured diameters.¹⁹

Weed biomass was estimated once in early August 2011 to compare weed pressure among all test plots including the grower standard. All weeds in experimental plots and an equivalent area of the buffer rows were harvested at ground level, placed in labeled paper bags, dried in an oven for 72 hours at 49°C , (120°F) and weighed.

2.5 Analysis Soil moisture, soil temperature, flush inspection, sticky card, and trunk measurement data were analyzed using repeated measures analysis of variance (RMAOV) with date as the covariate and means separation determined upon obtaining a significant treatment F value by LSD t-test (SAS 9.3, SAS Institute, Cary, NC). Weed biomass and HLB sample data was analyzed using one way analysis of variance (ANOVA) with LSD t-test for means separation.

3 RESULTS

3.1. ACP and other arthropods Neither citrus pests nor beneficials were observed for the first 11 months after planting. A significant treatment effect was observed over all dates from April 2011 through November 2012 in percentage of shoots infested with ACP (Table 1). Less than half as many shoots were infested with ACP on trees growing on metalized mulch compared to trees on whitefaced mulch or bare ground, with no significant difference between these latter two. These trends of reduced ACP were consistent whenever ACP were present throughout the entire study (Figure 1).

ACP movement in the plots as indicated by sticky card captures showed even greater dissimilarities among treatments, with almost two times more ACP captured by traps placed between trees in bare ground compared to metalized mulch (Table 1). Captures on whiteface mulch were intermediate and significantly different from either of these other two treatments. Differences among treatments were most marked during peak population periods, especially early in the trial before increasing tree canopy size began shading out the mulch (Figure 2). Aphid populations, as indicated by percentage infested flush, were low and showed no differences among treatments by flush observation (whitefaced $0.652 \pm 0.129\%$, metalized $0.513 \pm 0.100\%$, bare ground $0.534 \pm 0.088\%$ (mean \pm std.err.)), nor did leafminer

by rating damaged flush (whitefaced 5.313 ± 0.234 , metalized 5.025 ± 0.186 , bare ground 5.763 ± 0.182 (mean \pm std.err.)), or ladybeetles on sticky cards (whitefaced 0.152 ± 0.017 (mean \pm std.err.), metalized 0.136 ± 0.016 , bare ground 0.141 ± 0.017). Spider and lacewing populations were too low for evaluation of treatment effects.

3.2 HLB Incidence Results of PCR analysis mirrored those for ACP, with lowest HLB incidence among trees on metalized mulch (Table 2). Incidence of PCR positive trees sampled 5 August 2011 was 30% on bare ground and whitefaced mulch, six times higher than on metalized mulch with the grower standard intermediate. By the second sample date of 13 December 2011, incidence had doubled to 60% in bare ground and whitefaced mulch plots, still 3 times greater than on metalized mulch with the grower standard intermediate. On the third sample date, 12 September 2012, incidence of PCR positive trees in metalized mulch plots had reached 70% but was still significantly less than all other treatments.

3.3 Tree growth The system of plastic mulch, drip irrigation, and fertigation altered growing conditions in ways that had positive effects on young tree development. Higher soil moisture levels were maintained under metalized mulch and whitefaced mulch. Bare ground was similar to whitefaced mulch, with lowest levels measured in the grower standard (Table 3, Figure 3). No statistical differences in weed biomass were found among drip-irrigated treatments, although 7 times more weeds were harvested from bare ground plots (Table 3). However, significantly more weeds were found on bare ground irrigated with micro-sprinklers (grower standard) compared to all drip treatments.

Thirty three months after planting, tree growth as indicated by trunk cross section area was greatest for the metalized mulch treatment followed by bare ground and whitefaced mulch

treatments (Table 3, Figure 4). Last was the grower standard irrigated with micro-sprinklers where trunks had grown little more than half the girth of trees on metalized mulch.

4 DISCUSSION

Reflective mulch repelled ACP just as has been reported in other crops for other day-flying herbivores including aphids, whiteflies, thrips, leaf mining agromyzids, and cucumber beetles.^{4,5,6,7,8,9,10} The twilight/night-flying citrus leafminer was not repelled. Psyllids are probably similar to aphids with three types of photoreceptors in the compound eye and are most sensitive to the green region (c. 530 nm), followed by the blue-green region (490 nm) and finally the near UV (330–340 nm).²⁰ Wavelengths in the UV region influence insect behavior, such as orientation, navigation, host finding and feeding.²¹ It has been proposed that, while aphids are strongly attracted to UV light when taking off, they later enter into an alighting or searching phase and are repelled by UV light from the sky while at the same time attracted to green light reflected from plants.²² Metalized mulch disorients the insect by creating a strong source of sunlight emanating from below the host plant. Adult ACP were more readily observed in trees on metalized mulch during extended cloudy/overcast weather than under sunny conditions. Reflective mulch might also serve to repel the insect once alighted, by continued disorientation or higher canopy temperature. Further additional research is being conducted to ascertain whether the mulch merely prevented ACP from locating young shoots, or if it also repelled them after landing.

ACP nymphs do not move from the oviposition shoot, so vector transmission of HLB among trees is due exclusively to the activity of adult. Metalized mulch reduced incidence of HLB infection, presumably by reducing alighting and feeding by ACP adults. Nymphs are primarily responsible for acquisition of the pathogen, and thus key to secondary spread.²³

Consequently, reduction of colonization by ACP slowed both primary and secondary spread of HLB. Nymphs may also contribute to the overall pathogen load in the plant through replication, and therefore increase the likelihood and intensity of HLB infection.²⁴

Additionally, although the *C. Liberibacter asiaticus* is considered to be heat-tolerant, a drastic reduction in titer has been observed experimentally at 38°, suggesting that metalized mulch may also reduce HLB infection rates by raising canopy temperatures during the day through reflected light.²⁵ The theory of increased canopy temperatures inhibiting the spread of HLB is being evaluated in a separate study.

The use of metalized polyethylene mulch in new citrus plantings required a change from standard micro-sprinkler irrigation and dry fertilizer application to drip irrigation and liquid fertigation. Thus, the addition of metalized polyethylene mulch provides yet another element to an advanced citrus production system that includes directed drip irrigation and nutrient injection to accelerate early tree growth and production.²⁶ Soil moisture was improved by the drip system due to placement of emitters which concentrated water near the tree as opposed to the more dispersed pattern of micro-sprinklers. Polyethylene mulch further improved moisture levels, probably by reducing evaporation, an effect enhanced by metalized mulch, a virtually impermeable film (VIF). Reduction in weed competition for resources such as water and fertilizer provided yet another advantage to the mulch system. A further enhancement in growth rate may have resulted from increased photosynthesis in response to light reflected upward by the metalized film. Thus, we observed successive improvement in tree growth as indicated by trunk diameter with the addition of critical elements from micro-sprinkler to drip, to whitefaced mulch, to metalized mulch.

Problems encountered during this study included durability of the first thin-film mulches used, break-down of the aluminum layer due to ultraviolet light, and its solubility in low pH nutrient sprays which removed the aluminum and exposed the black plastic layer underneath. These problems are being addressed by increasing the thickness of the film to $76\ \mu$ (3 mil) and adding a clear coat of UV protected polyethylene over the aluminized layer (Imaflex, Montreal, Canada) since replacing the mulch by hand each year would be too labor intensive for most commercial operations. With these improvements, we hope to increase the life expectancy of metalized mulch to 3 years, at which time the mulch would be largely shaded out by the tree canopy.

The young tree production system described here includes some additional costs but also considerable savings as well as the possibility of earlier profitability compared to conventional practices. On the cost side is the mulch itself and its installation, the nutrient injection system, and possibly a dual irrigation system with micro-sprinklers added for cold protection. On the savings side are reduced water use and costs for weed control and pesticides, as well as labor saved through use of fertigation and chemigation. On the benefit side is more rapid tree growth and reduced incidence of HLB with consequent earlier and greater production. These economic considerations and other effects of scale are being more fully explored commercial trials presently underway.

5 CONCLUSION

Metalized mulch decreased colonization by ACP and thereby slowed the advance of HLB infection. This together with drip irrigation and fertigation almost doubled growth rates of young trees compared to the current standard. Our experiment was conducted without

recourse to systemic insecticides that are typically used to control ACP and reduce HLB spread in young citrus.¹⁶ It is likely that progress of the HLB epidemic would be much slower yet were metalized mulch used in concert with systemic insecticides to further reduce psyllid populations. Effectiveness of these tactics should be improved by scaling up from small plot to larger blocks as previously hypothesized for thrips by Reitz et al. 2003.²⁷ Given these advantages, metalized mulch at planting could become a standard feature of future citrus production systems, especially in HLB endemic environments.

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FIGURE LEGENDS

Figure 1. Percent young shoots infested with ACP during the 2011 and 2012 growing seasons observed every 14 days. Insecticides sprayed on dates: 10 June, 2 October, and 21 November 2011, 20 May and 15 September 2012.

Figure 2. Average ACP per sticky card from 21 April 2011 through 29 November 2012. Insecticides sprayed on dates: 10 June, 2 October, and 21 November 2011, 20 May and 15 September 2012.

Figure 3. Soil moisture content measured at 20 cm depth weekly from 9 June to 3 November 2011.

Figure 4. Trunk area estimated from 2 diameters measured 3 inches above the graft union on 7 June, 12 September, 14 December 2011, 19 June 2012, and 5 February 2013.

Table 1. Mean (\pm SEM) proportion infested shoots and average number of *D. citri* observed per sticky card at 14 day intervals from April 2011-Nov 2012.

Treatment	Infested shoots ^a	<i>D. citri</i> /Card
Metalized	0.34 \pm 0.01 b	4.03 \pm 0.26 c
Whitefaced	0.68 \pm 0.02 a	5.95 \pm 0.32 b
Bare Ground	0.66 \pm 0.02 a	7.59 \pm 0.45 a

^aMeans within columns with different letters are statistically different at $\alpha=0.05$ (F=66.18; DF=42,2038 P<0.0001) (shoots) (F=40.21; DF=53,1491; P<0.0001) (cards).

Table 2. Percentage plants PCR positive for HLB 5 Aug 2011 , 13 Dec 2011 , and 12 Sept 2012

Treatment	5 Aug 2011 (%) ^a	13 Dec 2011 (%)	12 Sep 2012 (%)
Metalized	5 ± 3.5 b	15 ± 5.7 c	70 ± 7.3 b
Whitefaced	30 ± 7.3 a	60 ± 7.8 a	90 ± 4.8 a
Bare Ground	30 ± 7.3 a	60 ± 7.8 a	88 ± 5.3 a
Grower Standard	15 ± 5.7 ab	38 ± 7.8 b	90 ± 4.9 a

^aMeans within columns with different letters are statistically different at $\alpha=0.05$ ($F=9.65$; $DF=3, 12$; $P=0.0016$) (early) ($F=3.93$; $DF=3, 12$; $P=0.0097$) (middle) ($F=2.86$; $DF=3, 12$; $P=0.0389$) (late).

Table 3. Mean (\pm SEM) weed biomass harvested from all experimental plots Aug 2011, percent soil moisture measured at a 20cm depth over all dates from 9 June through 26 Oct. 2011, and trunk area measured 7.2 cm above the graft union 5 Feb 2013.

Treatment	Weed Biomass (kg) ^a	Soil Moisture (%)	Trunk Area (mm ²)
Metalized	0.045 \pm 0.01 b	5.52 \pm 0.16 a	1028.37 \pm 44.07 a
Whitefaced	0.035 \pm 0.01 b	4.92 \pm 0.13 ab	837.01 \pm 34.48 b
Bare Ground	2.790 \pm 1.57 b	4.47 \pm 0.13 b	811.25 \pm 29.66 b
Grower Standard	6.250 \pm 1.07 a	3.64 \pm 0.13 c	542.18 \pm 23.61 c

^aMeans within columns with different letters are statistically different at $\alpha=0.05$ (F=8.60; DF=3, 12; P<0.0001) (weeds) (F=160.65; DF=36, 1723; P<0.0001) (moisture) (F=114.98; DF=19, 774; P<0.0001)(trunk).







