The Effects of Anaerobic Soil Disinfestation on Weed and Nematode Control, Fruit Yield, and Quality of Florida Fresh-market Tomato

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Abstract. Anaerobic soil disinfection (ASD) is considered a promising sustainable alternative to chemical soil fumigation (CSF), and has been shown to be effective against soilborne diseases, plant-parasitic nematodes, and weeds in several crop production systems. Nevertheless, limited information is available on the effects of ASD on crop yield and quality. Therefore, a field study was conducted on fresh-market tomato (Solanum lycopersicum L.) in two different locations in Florida (Immokalee and Citra), to evaluate and compare the ASD and CSF performances on weed and nematode control, and on fruit yield and quality. In Immokalee, Pic-Clor 60 (1,3-dichloropropene + chloropicrin) was used as the CSF, whereas in Citra, the CSF was Paldin™ [dimethyl disulfide (DMDS) + chloropicrin]. Anaerobic soil disinfection treatments were applied using a mix of composted poultry litter (CPL) at the rate of 22 Mg·ha⁻¹, and two rates of molasses (13.9 (ASD1) and 27.7 m³·ha⁻¹ (ASD2)) as a carbon (C) source. In both locations, soil subjected to ASD reached highly anaerobic conditions, and cumulative soil anaerobiosis was 167% and 116% higher in ASD2 plots than in ASD1 plots, in Immokalee and Citra, respectively. In Immokalee, the CSF provided the most significant weed control, but ASD treatments also suppressed weeds enough to prevent an impact on yield. In Citra, all treatments, including the CSF, provided poor weed control relative to the Immokalee site. In both locations, the application of ASD provided a level of root-knot nematode (Meloidogyne sp.) control equivalent to, or more effective than the CSF. In Immokalee, ASD2 and ASD1 plots provided 26.7% and 19.7% higher total marketable yield as compared with CSF plots, respectively. However, in Citra, total marketable yield was unaffected by soil treatments. Tomato fruit quality parameters were not influenced by soil treatments, except for fruit firmness in Immokalee, which was significantly higher in fruits from ASD treatment than in those from CSF soil. Fruit mineral content was similar or higher in ASD plots as compared with CSF. In fresh-market tomato, ASD applied using a mixture of CPL and molasses may be a sustainable alternative to CSF for maintaining or even improving marketable yield and fruit quality.

Soilborne fungal pathogens, nematodes, and weeds represent some of the most important biotic factors limiting vegetable crop production and profitability in the world. After the phaseout of methyl bromide, although other chemical soil fumigants (CSF) are available, there is still a pressing need for effective, viable, and more sustainable options (Shennan et al., 2014). Among the nonchemical alternatives, ASD, also known as “biological soil disinfection” (Blok et al., 2000) or “reductive soil disinfection” (Shimamura et al., 1999), is considered as one of the most promising methods. Anaerobic soil disinfection has proved to be effective against several soilborne fungal and bacterial plant diseases, and plant-parasitic nematodes and weeds, across a wide range of crops and environments (Butler et al., 2012a, 2012b; Lamers et al., 2010; Momma, 2008; Rosskopf et al., 2015; Shennan et al., 2014). Developed independently in Japan (Shimamura et al., 1999) and in the Netherlands (Blok et al., 2000), for both open field and protected crops, the technique is gaining interest in the United States, China, and other countries (Kim et al., 2007; Meng et al., 2015; Shennan et al., 2014). Suitable also for raised-bed crops, ASD does not require the use of chemicals and may be applied even in organic production systems. The current approach to ASD treatment in Florida recommends application 3 weeks before crop transplan ting and consists of creating temporary anaerobic (reducing) conditions by 1) amending the soil with a readily decomposable C source to initiate rapid soil microbial growth and respiration, 2) covering the bed with oxygen impermeable polyethylene mulch to minimize gas exchange, and 3) irrigating the soil to saturate the pore space, which, besides creating anaerobic conditions, enhances the diffusion of by-products through the soil solution within the volume of soil that will host the crop root system (Butler et al., 2014; Shennan et al., 2014).

The growth of aerobic microorganisms stimulated by the organic amendment causes a rapid decline in oxygen content in the soil with consequent decrease of the redox potential (Eh) and the development of anaerobic conditions that promote the growth of facultative and obligate anaerobic microorganisms over the aerobic microbial community (Mowlick et al., 2012, 2013a, 2013b; van Agtmaal et al., 2015). Under reducing conditions, the organic matter is subject to fermentation with consequent production of short-chain fatty acids (acetic, butyric, and propionic acids), aldehydes, alcohols, and volatile organic compounds (VOC) that are toxic and/or suppressive for several soilborne pathogens, plant-parasitic nematodes, and weeds (Bonanomi et al., 2007; Momma et al., 2006; Momma, 2008; Oka, 2010; van Agtmaal et al., 2015). Momma et al. (2011) reported that in presence of anaerobic conditions the generation of ions such as Fe⁺² and Mn⁺² may contribute to the suppression of soilborne pathogens like Fusarium oxysporum. Although the mechanism of pest suppression by ASD is not fully understood and
requires further research, an aspect that needs particular attention is the ASD application technique. It is critical for the adoption of ASD at the commercial level, to define and validate a feasible field-scale ASD application procedure. The main factors affecting the level of an aerobicosis and low pH achievable, as well as the microbial type and population growth, and the maintenance of reducing conditions overtime are the soil type, the initial water volume applied, and the type and rate of organic matter applied (Butler et al., 2012b, 2014). A good C source should be locally and abundantly available, low cost, homogenous, easy to apply, and effective in supporting microbial growth. Depending on local availability and costs, the C source may be constituted by cover crop residues, ethanol, molasses, rice, or wheat bran, or a combination of organic materials (Rosskopf et al., 2015; Shennan et al., 2014).

Although temporary, the reduction of Eh and the lowering of pH caused by the ASD treatment (Momma, 2008), may have a substantial impact on the pests and the entire soil-microorganism-plant system (Husson, 2013; Strauss and Kluepfel, 2015; van Agtmael et al., 2015). Combined with solarization and using molasses as a C source, the ASD treatment provided equivalent or great- er marketable yields than the methyl bromide treatment (Momma, 2008; Shennan et al., 2014), little is known about the effects of the ASD per se on tomato (S. lycopersicum) crop yield and fruit quality.

Therefore, a field study was carried out in two different locations in Florida, which leads the United States in the production of fresh-market tomato, accounting for 34% of the U.S. fresh-market tomato harvested area and 39% of the national crop value in 2014 (USDA-NASS, 2015), the majority of which is produced using soil fumigation as the basis for pest management. The study was conducted in an open-field, fresh-market tomato production system to evaluate ASD in comparison with the reference CSF treatments for weeds and nematodes control as well as for influence on fruit yield and quality.

**Materials and Methods**

**Experimental sites and treatments.** During the spring season of 2015, two experiments were conducted in open-field fresh-market tomato in southwestern (Immokalee) and northern Florida (Citra). The conventional CSF was compared with two ASD treatments, which consisted of amending the soil with 22 Mg·ha⁻¹ of CPL and two rates of molasses [13.9 (ASD1) and 27.7 m³·ha⁻¹ (ASD2)] as a C source. The first experiment was established on 2 Feb. 2015 at the University of Florida (UF)/Institute of Food and Agriculture Science (IFAS)/South West Florida Research and Education Center (SWFREC) in Immokalee, FL. The second experiment was established in North Florida on 25 Mar. 2015, at the UF Plant Science Research and Education Unit in Citra, FL.

In Immokalee, the soil was a Spodosol classified as Immokalee fine sand (sandy, siliceous, hyperthermic Arenic Haplaquods), and the experimental field was previously characterized as having moderate weed and root-knot nematode pressure. In Immokalee, weed species homogeneously infesting the experimental field included yellow nutsedge (Cyperus esculentus L.), the monocotyledonous goosegrass (Eleusine indica (L.) Gaertn.), southern crabgrass [Digitaria sanguinalis (L.) Koel.], large crabgrass [Digitaria sanguinalis (L.) Scop.], and smooth crabgrass [Digitaria ischaemum (Schreb.) Muhl.], and the dicotyledonous pigweed (Amaranthus retroflexus L.), false daisy (Eclipta prostrata L.), and old world diamond (Hedyotis corymbosa L.), which represent some of the most common weeds infesting tomato crops in the area. In Citra, the soil was a Gainesville loamy sand (hyperthermic, coated typic quartzipsamments) and the experimental field had high weed pressure, consisting predominantly of yellow nutsedge. At this site, root-knot nematode pressure was also high based on previous observations.

At each location, treatments (CSF, ASD1, and ASD2) were arranged in a randomized complete block design with four replications. A nontreated control was not included because the ASD method has been proven to have efficacy against multiple soilborne pest species when compared with untreated soil (McCarty et al., 2014), while the comparison with the current commercial fumigants available in each area is critical for the adoption of ASD at commercial level. In Immokalee, each of the four blocks, was constituted by one raised bed, 0.90 m wide, 0.20 m high, and 60 m long, and treatments were applied to 15-m-long sections of the bed, with 3-m space between plots. In Citra, each of the four blocks consisted of three beds 0.90 m wide and 15-m long. In both locations, before treatment application, soil was rototilled and a starter fertilizer mix including nitrogen (N), phosphorous (P), and potassium (K) was applied at the rate of 34, 49, and 37 kg·ha⁻¹ at the Immokalee site, and 56, 22, and 42 kg·ha⁻¹ at the Citra site, respectively. In both locations, the starter fertilizer mix was broad-cast applied to the soil surface on a band of 60 cm wide. Then, rounded false beds were formed hilling the soil from a depth of 10 cm, and ASD beds were amended with CPL at the rate of 22 Mg·ha⁻¹, and with a 1:1 (v:v) water dilution of sucarglute molasses (agricultural carbon source, Terra Feed, LLC, Plant City, FL). The CPL contained 23.0% of C, 2.6% of N, 1.4% of P, and 2.5% of K. Molasses had a density of 1420 kg·m⁻³, 22% of water content, a pH of 49.5–5.2, 1.23% of N and 34.25% of C. The molasses–water mix was applied to ASD1 and ASD2 plots at the rate of 27.7 and 55.4 m³·ha⁻¹.

After CPL and molasses application, the soil was tilled to a depth of 15 cm with a rotary cultivator, beds were formed and covered with a 0.33-mm black/white VaporSafe® TIF (Raven Industries Inc., Sioux Falls, SD) polyethylene mulch containing an ethylene vinyl alcohol barrier layer. Simultaneously, two drip irrigation lines [20 cm emitter spacing, 0.9 kg·h⁻¹·m⁻¹ emitter rate (Jain Irrigation Inc., Haines City, FL)] were installed under the mulch in each bed, \( \approx 2.54 \) cm below the soil surface and 20 cm apart from the center of the bed.

ASD plots were then irrigated for \( \approx 4 \) h at the rate of 5 cm of water (based on raised-bed area only) to saturate air-filled pore space in the top 10 cm of the bed and enhance the development of anaerobic conditions (Butler et al., 2012a).

On the same day, the control plots were fumigated by shank injection with Pic-Clor 60 (Soil Chemical Corporation, Hollister, CA) containing a mixture of 1,3-dichloropropene (39.0%) and chloropicrin (59.6%) at the rate of 224 kg·ha⁻¹ in Immokalee, and with Paladin™ (Arkema Inc., King of Prussia, PA) composed of DMDS (79%) and chloropicrin (21%) at the rate of 496 L·ha⁻¹ in Citra. In Immokalee, CSF was performed to a depth of 23 cm with three shanks; external shanks were 56 cm apart, and a third shank was placed in the middle off-centered, 23 and 33 cm apart from the external shanks. In Citra, CSF was performed to a depth of 25 cm with two shanks placed 30 cm apart. In both locations, fumigated plots were mulched right after CSF.

**Soil pH and redox potential.** Before initial irrigation, two oxidation–reduction potential sensors (Pt combination electrodes, Ag/AgCl reference; Sensorex, Garden Grove, CA) were installed to a depth of 15 cm in each plot, to measure the redox potential (Eh), and thus, to evaluate the level of anaerobiosis achieved in the soil during the first 3 weeks after treatment application (WATA) application.

Electrodes were continuously monitored using an automatic data logging system (CR-1000 with AM 16/32 multiplexers; Campbell Scientific, Logan, UT) and the collected data were used to calculate the cumulative number of hours under anaerobic conditions. Raw soil redox potential values were corrected to relate to the redox potential of a standard hydrogen electrode (Fiedler et al., 2007). Hourly average soil redox potential values below the calculated critical redox potential (CEh) were considered to be indicative of anaerobic conditions. Critical redox potential was considered as an indicator of reduced (anaerobic) soil conditions for the US National Resources Conservation Service (NRCS) (Rabenhorst and Castenson, 2005; USDA-NRCS, 2010), and was calculated using the formula:

\[
\text{CEh} = \left[ 595 (\text{v}) \right] – 60 (\text{n}) \times \text{soil pH}
\]

Soil pH was measured three WATA to calculate CEh. For values below CEh, the
absolute value of the difference between a given value and C/Eh were summed over the 3-week treatment period to provide a measure of cumulative soil anaerobic conditions. Using a soil probe (1.75-cm internal diameter), six soil cores were taken randomly from each plot at 20-cm depth and combined in a bulk sample for each plot. Soil pH was measured directly in each soil sample (without an extraction) using a FieldScout SoilStik pH meter (Spectrum Technologies, Inc., Aurora, IL). 

**Crop transplanting and growing conditions.** Tomato plants of ‘Skyway 687’ (Enza Zaden, Salinas, CA) and ‘Immokalee and Tributte’ (Sakata, Morgan Hill, CA) in Citra, both large, round fresh-market commercial tomato varieties, were transplanted at the third true leaf stage, three WATA on 24 Feb. and 17 Apr. 2015 in Immokalee and Citra, respectively. Both varieties were selected for their intermediate resistance to Tomato spotted wilt virus and Tomato yellow leaf curl virus. ‘Skyway 687’ is also resistant to Tomato apex necrotic virus, fusarium wilt, verticillium wilt, and has intermediate resistance to root-knot nematodes, whereas ‘Tribute’ is resistant to fusarium wilt, verticillium wilt, and alternaria stem canker, with intermediate resistance to gray leaf spot. In both experiments, tomato plants were planted in single rows, at a distance of 0.45 m within the row and 1.8 m between rows, establishing a density of 12,000 plants/ha. Each of the 12 plots contained 34 plants.

In both locations, plants were trellised using the stake and weave method. In Immokalee, the crop was managed using a hybrid seepage drip irrigation system, whereas in Citra, the crop was watered by drip irrigation. In Immokalee, irrigation volumes were defined daily based on the Florida Automated Weather Network (FAWN) recommendations for tomato. While in Citra, irrigation volumes were targeted controlled with a run time of 30 min twice a day. Soil moisture in the root zone was monitored by tensiometers installed in each replication. In Immokalee, the water table was regulated on a daily basis by monitoring wells installed at both ends of the external beds. The water table was maintained between 40 and 80 cm below the bed surface. The monitoring wells were constructed of 1.2-m-long 10-cm-diameter polyvinyl chloride (PVC) pipes screened 20 cm from the bottom (Smajstrla and Munoz-Carpensa, 2011). A float was attached to one end of a PVC pipe to serve as the water level indicator. Permanent marks were made every 25 cm to indicate the water table depth below the polyethylene mulch bed.

Fertigation was started 3 weeks after planting following the UF/IFAS fertilizer recommendations (FDACS, 2005). At the Immokalee site, N and K were applied twice a week by fertigation using potassium nitrate (13–0–48) and ammonium nitrate (34–0–0). In Citra, fertiler was applied by fertigation once per week using a 6–0–8 plus micro blend by Mayo Fertilizer Inc. (Mayo, FL). Total in-season fertilizer rates applied by fertigation were 180 and 268 kg·ha⁻¹ of N and 263 and 265 kg·ha⁻¹ of K in Immokalee and Citra, respectively. Total N, P, and K fertilizer rates were 214, 49, and 300 kg·ha⁻¹ in Immokalee, and 323, 22, and 307 kg·ha⁻¹ in Citra, respectively.

Weather data were obtained by the FAWN (http://fawn.ifas.ufl.edu/) stations located in Immokalee and Citra. Pests and diseases were managed following the UF/IFAS recommendations based on weekly scouting (Freeman et al., 2014). In Citra, the crop was uniformly attacked by early blight (Alternaria solani); despite the regular application of fungicides, the disease severely affected the crop, thereby affecting fruit yield.

**Weed evaluation, soil sampling, and nematode analysis.** The number of emerging weeds and the percentage of weed coverage of each plot were evaluated from four subsamples (0.25 m²) at 9, 22, 37, 50, 69, and 80 DAP in Immokalee, and at 20, 81, and 91 DAP in Citra. For nematode analysis, soil samples were collected, as previously described for soil pH analysis, before transplanting, and at the end of the crop season in both locations (80 DAP in Immokalee, and 91 DAP in Citra). A subsample of 100-cm² soil was used to extract nematode second stage juveniles (J2) using the Baermann funnel technique. Nematodes were counted and identified as root-knot Meloidogyne sp. or nonparasitic using inverted microscopes. At the end of the season, three randomly selected plants were removed from each plot and the roots were rated for galling and root disease. Galling was assessed on a scale of 0 to 10, with 10 representing severe (100%) galling (Bridge and Page, 1980). A subjective scale of 0 to 5 was used to assess root disease, with 0 representing healthy roots with no apparent signs of damage and 5 representing completely diseased and degraded roots. Root-knot nematode J2 were extracted from plant root tissue by placing a subsample of root tissue into funnels for 60 h, after which root-knot nematode J2 were identified and counted microscopically, as described previously.

**Fruit yield and grade distribution.** Yield was measured from one 4.5-m long (10 plants) representative section in each experimental unit. Fruits ranging from marketable mature green to ripe were harvested three times on 29 Apr., 13 and 26 May 2015 [64, 78, and 91 d after planting (DAP)], and on 26 June, 6, and 17 July 2015 (70, 80, and 91 DAP) in Immokalee and Citra, respectively. Fruits were graded into size categories large (greater than 7.00 cm), large (6.35 to 7.00 cm), medium (5.72 to 6.43 cm), and unmarketable fruit according to USDA grade standards (USDA, 1991) and weighed. Total soluble solids (TSS) and pH analysis were measured directly in each soil sample (without an extraction) using a FieldScout SoilStik pH meter (Spectrum Technologies, Inc., Aurora, IL). All means were separated using Duncan’s multiple range test at P = 0.05. Weed cover percentage data were transformed by arcsine transformation before ANOVA to obtain a normal distribution.

**Results and Discussion**

**Weather conditions.** In Immokalee, mean daily air temperature was on average 16.1 °C during the 3-week treatment and 23.9 °C during the crop season (from transplanting until final harvest). During the first three WATA, daily minimum and maximum air temperatures ranged from –0.9 to 15.5 °C and 15.5 to 29.6 °C, respectively. During the cropping season, daily minimum and maximum air temperature ranged from 8.2 to 22.2 °C and 18.5 to 34.6 °C, respectively.
ing, during the cropping season, daily mini-
respectively (Fig. 1). No freezing events were
shown consistent results and can assure a com-
the experiment conducted by Butler et al. (2012a)
anaerobiosis was significantly influenced by
the soil is significantly influenced by the amount of C
Soil redox potential. In both locations, plots
higher cumulative anaerobic conditions as
on average 230.2 W m⁻² and cumulative
318.3 mm. A leaching rainfall event with 83 mm of rainfall also occurred in
Citra and was recorded on 8 July (82 DAP).
Cumulative precipitation recorded in both
locations was within the range of mean cumu-
lative rainfall typically recorded in Florida
during the spring season (Fraisse et al., 2010).

Soil treatment effects on weeds. After the
phaseout of methyl bromide, weed control
became one of the major issues in the
vegetable industry, as none of the soil disin-
estation alternatives currently available has
shown consistent results and can assure a com-
plete weed control in all the application
conditions. The effectiveness of several CSFs,
currently available is in fact, influenced by
the specific application conditions (soil type, soil
moisture, infestation level, bed geometry,
application equipment). Therefore, also for
the ASD technique, it was critical to eval-
uate the efficacy of control against weeds, at
field level, under specific application
conditions, and in comparison with differ-
ent CSF reference standards. In Immokalee, soil treatments exerted a significant effect on both weed number and coverage percentage (Table 1). Since the first assessment at 9 DAP and during the entire crop cycle, CSF assured a near complete control of all weeds. Plots subject to ASD1 showed the highest weed number, ranging on average from 9.25 to 8.25 shoots/m² at 9 and 80 DAP, respectively, as well as the highest weed coverage percentage, ranging from an average of 0.03% at 9 DAP to 16.25% at 80 DAP. However, no differences were observed between plots treated with ASD1 and ASD2 throughout the season, both in terms of weed number and percent coverage. Weeds devel-
oped mostly through planting holes and only a few yellow nutsedge shoots were able to penetrate the TIF polyethylene mulch. More-
over, it was observed that the application of
ASD especially at higher molasses rate
(ASD2) substantially reduced the emergence of weeds in the alleys among beds, which
remained clean until first harvest. This could
be explained by the partial movement of the
mollases outside the bed. At the end of the
season, considering the percent of weed cov-
ereage, CSF displayed an efficacy approaching
100%, while ASD treatments showed an average efficacy of 85%.

In Citra, all soil treatments including the
CSF resulted in a very low level of weed
control although there were no significant
differences among treatments (Table 1). The number of emerging weeds ranged from an
average of 13.2 shoots/m² at 20 DAP to 37.7
and 26.3 shoots/m² at 81 and 91 DAP, re-
spectively (Table 1). Weed percent coverage
ranged from 14.7% at 20 DAP to 59.8% at 91
DAP, and was not influenced by soil treat-
ments, except at 81 DAP, when ASD2 plots
showed a percent weed coverage lower than
ASD1, 31.3% vs. 54.4% (P = 0.05). In plots with
CSF, the percent weed coverage was equivalent to that of ASD plots regardless of
the applied molasses rate.

In presence of high nutsedge pressure,
such as that observed in Citra, neither ASD
nor DMDS provided acceptable weed con-
trol. After the loss of methyl bromide, in-
creased research on alternative fumigants has
established the need for an herbicide partner
with the majority of alternatives, particularly
where nutsedge is the principal weed prob-
lem (Noling et al., 2006). However, where
grass weeds dominate or under relatively low
weed pressure, as observed in Immokalee, the
application of ASD can provide adequate
weed control, as reflected in increased crop
yield (see below). Considering the limited weed
control efficacy provided by the ASD in Citra, it
may be interesting to investigate in future
studies the possibility to combine the ASD
 technique with the application of herbicides.

Soil treatment effects on plant-parasitic and nonparasitic nematodes. To evaluate the
impact of soil disinfestation treatments on soil nematode populations, both plant-parasitic
and nonparasitic nematodes were analyzed immediately before treatment application,
at three WATA and at the end of the crop
season. Before treatment application, the
plant-parasitic nematode population was low
in Immokalee, where the root-knot nematode
population was on an average of 2.84 J2 per
100 cm² of soil, while it was relatively high in
Citra with an average root-knot nematode
population of 10.40 J2 per cm² of soil. The
number of nonparasitic nematodes was on
average 238 per cm² of soil in Immokalee and
491 per cm² of soil in Citra (Table 2). All soil
treatments reduced the root-knot nematode
population to zero at three WATA in both
locations (Table 2), demonstrating the effec-
tiveness of ASD in controlling root-knot nem-
atodes. These results were consistent with the
findings of Butler et al. (2012a) who observed a
decrease of root-knot nematodes in plots
amended with mollases and/or CPL in a peper–eggplant double crop system. The
control of root-knot nematodes in ASD plots
may be explained by the nematotoxic effect
of organic acids and ammonium released
during the decomposition of the organic
matter (Katase et al., 2009; Thoden et al.,
2011). At the same time, in Immokalee, the
nonparasitic nematodes population increased
up to 2840 per cm² of soil in ASD2 plots, and
was significantly higher (P = 0.004) in soil
treated with ASD at both molasses rates than in
CSF soil, where even the nonparasitic nemat-
ate population was reduced to zero. Whereas
in Citra, all tested treatments reduced the
population of nonparasitic nematodes, and
although ASD plots showed higher levels of
nonparasitic nematodes, no significant differ-
ce (P = 0.07) was observed between ASD and CSF plots (Table 2). The increase of
nonparasitic nematodes observed in Immo-
 kalee only in ASD plots, may be due to the soil
amendment with CPL, and was consistent
with the findings of several authors, who have
observed that the number of free-living bacterial- and fungal-feeding nematodes mark-
edly increases after the addition of any form of
organic soil amendment (Thoden et al., 2011).

At the end of the season, in Immokalee, the
root-knot nematode population remained low,
ranging from an average of 0 J2 per cm² of soil in ASD2 plots to 17 J2 per cm² of soil
in plots subject to ASD1, but was not significantly (P = 0.36) different among the
treatments. Also in Citra, the end-of-season
root-knot nematode population was not sig-
nificantly (P = 0.54) different among the
treatments; however, in this case, the para-
sitic nematode population increased in all
 treatments and was on average of 44.4 J2 per
cm² of soil (Table 2). The root-knot nematode
population increase observed in Citra at the
end of the season could be at least in part due
to the low weed control obtained in this lo-
cation, as weeds may host parasitic nematodes
(Rich et al., 2009). Such results suggest the
importance of controlling both weed and nematodes at the same time. In both locations, the
end-of-season nonparasitic nematode population
was not significantly ($P = 0.50$ and $0.12$) different among the treatments.

At the end of the crop season, an assessment of the nematode population and root condition ratings were also performed on the tomato roots (Table 3). While the number of root-knot nematode J2 per gram of roots was not significantly different among the treatments ($P = 0.12$ in Immokalee and $0.31$ in Citra), the root gall index was significantly lower ($P = 0.01$) from roots subjected to ASD1 than from those subjected to ASD2 or CSF in Immokalee. In Citra, the root gall index was significantly lower ($P = 0.04$) on roots from ASD1 plots than in those subjected to CSF. As expected, based on initial numbers in Immokalee, the number of root-knot nematodes per gram of root was low ranging from a minimum average of $0.56 \text{ J2/g}$ of root in ASD1 to $2.05 \text{ J2/g}$ of roots in CSF plots. In Citra, the number of root-knot nematodes per gram of root was higher and ranged from a minimum average of $5.45 \text{ J2/g}$ of roots in ASD1 to a maximum average of $13.24 \text{ J2/g}$ of roots in ASD2 plots. In both locations, the number of nonparasitic nematodes per gram of roots was not significantly different among the treatments. It follows that ASD can assure a level of control of the root-knot nematode population equivalent or higher than that provided by the CSF. Moreover, regardless of the molasses rate, the ASD has a lower impact on the nonparasitic nematode population in soil compared with the CSF, thus maintaining...
a higher level of soil biodiversity, even though it did not last for the entire cropping season.

**Soil treatment effects on fruit yield and grade distribution.** In Immokalee, the soil disinfection treatments tested showed no influence on total marketable yield at first harvest and first and second harvest combined (FSHC). However, total season marketable yield was significantly \( P = 0.03 \) higher in ASD than CSF plots (Table 4). With a total season marketable yield of 62.1 Mg·ha\(^{-1}\), plots subject to ASD2 provided the highest tomato fruit yield, which did not differ from the fruit yield obtained at lower molasses rate (ASD1). Compared with the CSF, ASD2 and ASD1 plots provided 26.7% and 19.7% higher total season marketable yield, respectively. Total marketable yields obtained in Immokalee were within the range of those reported by Ozores-Hampton et al. (2012, 2015) for the cultivar Florida 47 R grown in southwestern Florida during the spring (51.8–109.5 Mg·ha\(^{-1}\)). Extra-large fruits declined overtime from 82.5% at first harvest to 70.2% of the total season marketable yield; however, soil disinfection treatments had no significant effect on the fruit size distribution either at first harvest, FSHC, or total season harvest. Soil treatments did not influence the amount of total unmarketable fruit, which averaged 7.6% of the total season fruit yield.

Given the relatively low weed and nematode pressure observed in the Immokalee site, the higher total season marketable yield measured in ASD plots as compared with CSF may be explained by the higher water and nutrient-holding capacity conferred to the soil by the CPL (Butler et al., 2014; Ozores-Hampton et al., 2011), rather than by improved control of root-knot nematodes and weeds.

In Citra, total marketable yield was on average 11.8 Mg·ha\(^{-1}\), resulting in lower than normal yield range (55–78 Mg·ha\(^{-1}\)) for the same area and season (Zotarelli et al., 2009), and was not significantly \( P = 0.19 \) affected by soil disinfection treatment (Table 4). Total marketable yield was not significantly influenced by soil treatments also at first harvest \( P = 0.47 \) and at FSHC \( P = 0.30 \). At first harvest, extra-large, large, and medium sized fruits accounted on average for 48.9%, 38.1%, and 12.9% of the total marketable yield, respectively. At the end of the season, 36.8% of the marketable fruits were large, and extra-large and medium sized fruit accounted for 31.6% and 31.7% of the total season marketable yield. However, also in Citra, fruit size distribution was not significantly affected by treatments either at first harvest, FSHC, or total season harvest. The amount of unmarketable fruits was on average 28.8% of total season fruit yield and was not influenced by soil disinfection treatments (Table 4).

The low total marketable yield obtained in Citra, regardless of soil treatment, may be attributable to the combined effect of the high weed and root-knot nematode pressure, as well as to the later than average planting date and to the occurrence of early blight that had a detrimental impact in all of the treatments in all of the replications. As Stall and Morales-Payan (2003) found that season-long interference of 25 yellow nutsedge plants per meter square resulted in a 10% reduction in marketable tomato yield, probably over 10% of the yield reduction observed in Citra could be due only to the nutsedge competition.

In fresh-market tomato, the use of ASD can provide equal or higher marketable yields as compared with a standard CSF, which is consistent with the findings of Butler et al. (2014) who observed good yield performance of bell pepper and eggplant.

**Soil treatment effects on fresh-market tomato fruit quality and mineral content.** In Immokalee, tomato fruit color, TSS, pH, and DM content were not influenced by the soil disinfection treatments, while a significant
Table 2. Soil root-knot and nonparasitic nematode juveniles (J2) counted before and after treatment in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015. a

| Assessment timing | Treatmentsa | Root-knot nematodes (J2/cm² soil) | Nonparasitic nematodes (number/cm² soil) | P value |
|------------------|-------------|----------------------------------|------------------------------------------|---------|
| Immokalee, FL    |             |                                  |                                          |         |
| Pretreatment     |             | 2.84                             | 238.14                                   |         |
| Posttreatment (21 DAT)a |             | 0.00                             | 0.00 b                                   | 0.05    |
| ASD1             |             | 0.00                             | 2,098.00 a                               |         |
| ASD2             |             | 0.00                             | 2,840.80 a                               |         |
| Harvest          |             | 2.84                             | 209.75                                   |         |
| CSF              |             | 17.01                            | 572.75                                   | 0.004   |
| ASD1             |             | 0.00                             | 303.25                                   |         |
| ASD2             |             | 0.36                             | 0.50                                     |         |
| Citra, FL        |             | 10.40                            | 491.40                                   |         |
| Pretreatment     |             | 0.00                             | 27.00                                    |         |
| Posttreatment (21 DAT)a |             | 0.00                             | 138.50                                   |         |
| ASD1             |             | 0.00                             | 129.25                                   |         |
| ASD2             |             | 0.00                             | 0.07                                     |         |
| Harvest          |             | 28.35                            | 164.43                                   |         |
| CSF              |             | 62.37                            | 172.94                                   |         |
| ASD1             |             | 42.53                            | 725.76                                   |         |
| ASD2             |             | 0.54                             | 0.12                                     |         |

aReported values are averages of four replications. Means followed by different letters within a column for each experiment and assessment timing are significantly different at P = 0.05 by Duncan’s multiple range test.

ASD1 (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter (CPL)), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of CPL).

Table 3. Soil treatment effects on plant stem diameter, root weight and condition, root infesting root-knot juveniles (J2), and nonparasitic nematodes and nematode gall index of fresh-market tomato plants grown in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015. a

| Treatmentsa | Stem diam (mm) | Root wt (g) | Root conditionb (0–5 scale) | Root-knot nematodes (J2/g root) | Nonparasitic nematodes (number/g root) | Gall indexc (0–10 scale) | P value |
|-------------|----------------|-------------|----------------------------|---------------------------------|----------------------------------------|--------------------------|---------|
| Immokalee, FL |                |             |                            |                                 |                                        |                          |         |
| CSF         | 21.26          | 48.29       | 2.93 a                     | 2.05                            | 11.56                                  | 1.13 a                   |         |
| ASD1        | 20.57 a        | 45.45       | 2.05 b                     | 0.56                            | 7.71                                   | 0.63 b                   |         |
| ASD2        | 19.07          | 45.36       | 2.46 ab                    | 1.02                            | 9.96                                   | 1.26 a                   |         |
| P value     | 0.10           | 0.65        | 0.03                       | 0.12                            | 0.40                                   | 0.01                     |         |
| Citra, FL   |                |             |                            |                                 |                                        |                          |         |
| CSF         | 15.08 b        | 29.56       | 2.46                       | 10.59                           | 11.20                                  | 5.48 a                   |         |
| ASD1        | 20.57 a        | 36.56       | 2.63                       | 5.45                            | 14.32                                  | 3.52 b                   |         |
| ASD2        | 22.17 a        | 33.15       | 2.74                       | 13.24                           | 12.68                                  | 5.05 ab                  |         |
| P value     | 0.01           | 0.79        | 0.52                       | 0.31                            | 0.88                                   | 0.04                     |         |

aReported values are averages of four replications. Means followed by different letters within a column are significantly different at P = 0.05 by Duncan’s multiple range test.

ASD1 (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of CPL).

bRoot weight on fresh weight basis.

cRoot condition: 0 = clean, white roots, 5 = completely rotted and discolored roots.

cNematode gall index: 0 = no galling, 10 = complete galling (Bridge and Page, 1980).

A significant effect (P = 0.02) was observed on the fruit firmness (Table 5). The fruit firmness was higher (lower deformation) in ASD1 and ASD2 than in CSF plots, which may be explained by the greater vigor and improved nutritional status of ASD plants as compared with CSF plants. Greater fruit firmness, without consequent negative effects on other commercial quality parameters (color, TSS, pH, DM) results in a longer shelf life (Meli et al., 2010) and is highly desirable considering that most of the Florida tomato production is shipped to the North part of the United States. In Citra, none of the fruit quality parameters were significantly affected by soil disinfection treatments (Table 5).

Soil treatment had a significant impact on the tomato fruit mineral content in Immokalee (Table 6). In Immokalee, K, Ca, and Mg content was significantly higher in fruit from ASD plots than from CSF. Total N was higher in fruit from CSF plots than in those from ASD1 plots (P = 0.01), whereas treatment had no effect on the fruit P content. Among the micronutrients analyzed, fruit Fe content was significantly higher in CSF plots than in those subject to ASD2, and Mn fruit content was higher in CSF plots as compared with ASD fruits from both treatments, regardless of the molasses rate. No significant differences were observed for the content of B, Cu, Zn, Na, Mo, and Ni. In Citra, the fruit macro- and micronutrient content was not significantly influenced by soil treatments, except the case of Zn that was significantly higher (P = 0.003) in fruit from plots subjected to ASD1 and ASD2 than in those of CSF plots.

From these findings, we conclude that the application of ASD does not negatively affect the commercial tomato fruit quality, and that the quality and the mineral content of fruit produced with ASD is comparable or higher than that of fruit produced in CSF plots. Nevertheless, further studies should validate these results and consider the potential effects of ASD on other quality aspects, including the content of secondary metabolites that contribute to the health-promoting properties of tomatoes.

Conclusions

The results of this study, conducted on fresh-market tomato in two Florida locations (Immokalee and Citra), indicated that ASD, applied using a mixture of CPL and molasses as C source, may be potentially used as a sustainable alternative to conventional CSF for the control of plant-parasitic nematodes and weeds, without causing negative effects on tomato fruit yield and quality. The use of totally impermeable film, rather than transparent solarization mulch, did not hinder the development of anaerobic conditions, and avoided the need to substitute the solarization mulch with a second film. Although, cumulative redox potential was higher in ASD2 (27.7 m³ ha⁻¹ of molasses) as compared with ASD1 (13.9 m³ ha⁻¹ of molasses) plots, both treatments reached highly anaerobic conditions in both locations. In Immokalee, where the weed pressure was relatively low, ASD showed adequate herbicidal effect. However, in Citra, under relatively high nutsedge pressure, all treatments including the CSF resulted in an unacceptable level of nutsedge control. In both locations, the application of ASD resulted in root-knot nematode control similar to or greater than the CSF control. In Immokalee, total marketable yield was 26.7% and 19.7% higher in ASD2 and ASD1 plots than in CSF, respectively. While in Citra, regardless of the molasses rate, ASD provided a total marketable yield equivalent to the CSF. In both locations, the fruit quality was not influenced by soil treatments and was similar in ASD and CSF plots, except for the fruit firmness that in Immokalee was higher in ASD than in CSF plots. In terms of nutritional value, the macro- and micronutrient content of fruit produced in ASD plots was similar or higher than that of fruit produced in CSF plots.
Overall, the results of the two locations demonstrate that the ASD technique may be a valid and sustainable alternative to the conventional CSF, and could be transferred at commercial level. As tested, molasses rates showed similar performance in terms of root-knot nematode and weed control, yield, and fruit quality; therefore, the lower molasses rate could be suggested to reduce the cost of the ASD treatment. However, further research is needed to validate the results of this study, consider other commercial crops, enhance the herbicidal activity, and test other potential C sources, to improve the ASD application technique for field production of vegetables and minimize the soil treatment costs.

Table 4. Soil treatment effects on tomato fruit size (scored by weight within each category) distribution from first, second and third harvest combined, and season total harvest (three harvests combined) in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and in Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015.3

Table 5. Soil treatment effects on tomato fruit firmness (expressed as fruit deformation), skin color, Brix*, pH, and dry matter content at first harvest in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and in Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015.3

Table 6. Soil treatment effects on tomato fruit macro and micronutrient content at first harvest in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and in Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015.3

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