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Organic farming research project report submitted to the Organic Farming Research Foundation:

Project Title:

Intercropping with resistant varieties for management of plant diseases in organic tomato production

FINAL PROJECT REPORT

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1. Project Summary

The rate of early blight increase and lesion expansion was reduced on early blight-susceptible tomato cv. 'Brandywine' when intercropped with resistant variety 'Juliet' compared to a monoculture under field conditions. Tomatoes were planted either in monoculture plots or in plots alternating plants of 'Brandywine' and 'Juliet'. A suspension of *Alternaria solani* spores was sprayed on corner plants in replicate plots in early July. Disease progression was monitored at 7-day intervals on 'Brandywine' throughout the season. Early blight defoliation over the season was 23.2% greater on 'Brandywine' in monoculture than when intercropped. Rate of disease increase was linear and r-values were greater in monoculture than in intercropped (1.13 versus 0.90) plots ($P=0.006$). Average lesion expansion on middle-aged leaves on 'Brandywine' in the monoculture was 0.035 cm/day compared to 0.053 cm/day when intercropped with 'Juliet' ($P=0.094$). Yield from 'Brandywine' plants was 21% greater when intercropped with 'Juliet' than when grown in monoculture. Results indicate that less susceptible leaf material in the plot reduced spread of the pathogen. Reduction in lesion expansion on 'Brandywine' when intercropped with the resistant variety 'Juliet' suggests an interaction initiating a defense response in 'Brandywine'. Early blight resistance was compared on 16 tomato varieties in small plots (five replicates of four plants each) in 2001 and 2002. 'Brandywine', 'Daybreak', 'Johnnys 361', and 'Valley Girl' were among the most susceptible both years, while 'Matts Wild Cherry', '99197', '99203', 'Juliet', 'Red Currant' and 'Sun Gold' showed significantly less disease in both years. The response of 'Green Zebra' and 'Prudens Purple' varied substantially from one year to the next.

2. Introduction to Topic

Many problems of modern agriculture, such as the overuse of fertilizers and pesticides are a result of the trend in crop production toward homogeneous crop genotypes for certain agricultural areas (Browning and Frey, 1969). In fact, monoculture is presently the dominant form of crop management worldwide (Zhu *et al.*, 2000). Monoculture is convenient because one variety is easier to plant, harvest, and market than mixtures of several. Most field crops and vegetables are grown as isoline varieties meaning individuals are almost identical genetically (Leonard and Fry, 1989). Such genetic uniformity increases the potential for devastation of an entire field by a pathogen.

Mixed varietal plantings may limit diseases by inhibiting the spread of pathogen propagules to susceptible plants, increasing the distance between susceptible plants, or activating non-specific host defenses (Ngugi *et al.*, 2001; Wolfe, 2000). Disease reductions by interplanting resistant cultivars with susceptible ones have been documented in China with rice blast (caused by *Magnaporthe grisea*) (Zhu *et al.*, 2000); in bell pepper, with bacterial spot (caused by *Xanthomonas campestris*) (Kousik and Ritchie, 1996); in sorghum with anthracnose (caused by *Colletotrichum sublineolum*) and leaf blight (caused by *Exserohilum turcicum*) (Ngugi *et al.*, 2001); and in potato with late blight (caused by *Phytophthora infestans*) (Garrett and Mundt, 2000).

The rate of disease increase in fields and the cumulative amount of disease over a season (expressed as Area Under the Disease Progress Curve) provide useful overall measures of disease progress. More importantly, the mechanism of disease reduction due to intercropping with resistant varieties can be determined in a number of ways when disease is initiated from a single point of infection in the field. If disease is reduced primarily because of a lower volume of

susceptible plant material in the field, then the onset of symptoms on the susceptible variety should be delayed due to blocking of spores by the resistant variety. Also, the number of lesions will be lower due to reduced inoculum deposition on susceptible tissue. But ultimately, the size of individual lesions that develop on susceptible plants will be identical to those in monoculture. However, if resistance is being induced in susceptible varieties when they are intercropped with resistant varieties, then the rate of lesion expansion on the susceptible varieties may be slower in intercropped compared to monoculture plants. There also could be a higher frequency of failed infections on intercropped susceptible varieties that can be observed under the microscope (Hammerschmidt and Nicholson, 2000).

The planting of susceptible varieties when resistant varieties are available may not appear, at first, to be a good management practice. However, there are several reasons for this practice. Often, organic growers and their customers prefer the flavor and horticultural characteristics of heirloom varieties and grow them in spite of their potential for disease problems. Where resistance is linked to undesirable traits, low yields or low quality, growers may plant susceptible varieties despite the risk, especially if disease is weather-dependant and epidemics do not occur every year. From an evolutionary perspective, planting crop mixtures increases the genetic diversity of the host within the field, thereby reducing the likelihood of an epidemic while also reducing the pressure on the pathogen to overcome host resistance genes. Thus, crop mixtures would allow growers to plant varieties that are otherwise avoided because they are highly susceptible to disease. In addition, the ability to grow 'heirloom' varieties economically would preserve rare plants whose germ lines may become useful in the future for developing new varieties. Finally, crop mixtures allow various genes to be expressed within the same field, without the high costs that result from genetically engineered crops.

3. Objectives of the Project

The objectives of this research were to:

- 1) Evaluate susceptibility to early blight in 16 heirloom and modern hybrid cultivars.
- 2) Evaluate whether disease incidence and severity are reduced on a susceptible tomato variety intercropped with a resistant variety, compared to a monoculture of the susceptible variety.

4. Materials and Methods

Variety Evaluations:

Sixteen tomato varieties were tested for resistance to *A. solani* in the field (Table 1). Five replicate blocks were planted with four tomato plants of the each variety in a complete randomized block design. Six-week-old seedlings of the 16 tomato varieties were transplanted on May 27, 2001 to the WVU Organic Research Farm. Plants were spaced 90 cm apart, both within and between rows. Composted cow manure (10 tons/acre) was used to meet fertility needs of the crop. Plants were staked with 4' bamboo poles and mulched with newspaper and hay. Inoculum consisted of naturally occurring spores of *A. solani*. Conditions for early blight were favorable throughout the season. Disease was measured at 7-day intervals from July 13 to Oct. 5. Percent defoliation due to early blight was rated and area under the disease progress curve (AUDPC) was determined.

The experiment was repeated in 2002 in a new field with tomato transplants planted on May 31. Cultural practices were identical, except that drip irrigation was applied as needed, and

plants were not staked. Epidemics again relied on naturally occurring field inoculum. Disease was assessed on 5 dates: Aug. 11, 16, 30, and Sept. 14 and 29. The experiment ended at the first frost, Oct. 18.

Intercropping Field Study.

Twenty plots, 7.3 by 7.3 m, were established at the WVU Organic Research Farm in Dormont (fine-loamy, superactive, mesic, mixed, hapludalf) soil. Four treatments were established based on planting pattern and pathogen inoculations: 1) 'Brandywine' in monoculture inoculated with *A. solani*; 2) Brandywine monoculture inoculated with *M. incognita*; 3) 'Brandywine' intercropped with early blight resistant cultivar 'Juliet' in a design alternating resistant and susceptible plants (Figure 1) and inoculated with *A. solani*; and, 4) 'Brandywine' intercropped with root knot disease resistant cultivar 'Celebrity' and inoculated with *M. incognita*. Treatments were arranged in a randomized complete block design with five replicates. Disease progression in the monoculture was compared with the resistant intercrop.

Seven-week-old tomato seedlings were transplanted May 27. Plants were spaced 90 cm apart, staked, and mulched with newspaper and straw hay. Plots were alternated according to the pathogen with which they were inoculated, but the monoculture versus intercropping treatments were randomized within blocks. Only one 'Brandywine' plant in the Northwest corner of each plot was inoculated. The plant in each early blight plot was inoculated July 13 by spraying leaves with a 20,000-spore/ml suspension of *A. solani*. Cultures of *A. solani* were originally collected from the WVU Organic Research Farm and maintained in the lab on V8 agar plates under fluorescent lights with 8 hours of darkness each day. Two-week-old fungal colony surfaces were gently scraped with a sterile blade to disrupt hyphae and, 24 hours later, distilled water was poured on the plates and a paintbrush was used to suspend the spores (Yan and Reddy, 1999).

Nematode plots were inoculated in the NW corner at transplanting by planting a tomato plant heavily infected with *M. incognita*. Cultures of *Meloidogyne incognita* were isolated from tomato plants from a garden in Kanawha County, West Virginia. Single egg mass cultures were established in sterile soil in the greenhouse on 'Rutgers' tomato and identity of *M. incognita* was confirmed based on perineal patterns. To assure a high level of inoculum, tomato seedlings were transplanted into heavily infested soil from these single egg mass cultures approximately 3 months prior to the field experiment. These inoculum plants were heavily galled when transplanted to the field plots, however no other attempt to quantify inoculum was made. Root knot severity was measured at the end of the study by counting the number of galls observed on 5 grams of plant roots from selected 'Brandywine' plants.

Early blight disease progress was measured on the inoculated 'Brandywine' plant and the first, third, fifth, and seventh plant in each of three transects (designated A, B, and C, Figure 1) in each plot every 7 days. Total percent symptomatic leaf area on the selected 'Brandywine' plants was recorded, and rate of disease increase (r-value) was calculated by plotting percent symptomatic leaf area over time and determining the slope of the best-fit line with JMP statistical software. Disease gradients were assessed based on the distance from the inoculated plant (Table 3).

Lesion diameter on randomly selected old and middle-aged leaves on pre-selected monitored 'Brandywine' plants was measured at intervals to determine the rate of lesion expansion. Old leaves were near the bottom of the main stem. Middle-aged leaves were at least half way up the main stem. The leaves were tagged and a diagram of the leaf was made with

lesion location. Monitored lesions were marked with a permanent marker so that the same lesion could be found each week.

Fruits on the 13 pre-selected 'Brandywine' plants were harvested when ripe and yield at each picking was composited within each plot.

Statistical Analyses.

For all field experiments, AUDPC was calculated by summing the products of the number of days in the sampling interval and the percent defoliation due to early blight at each monitoring date over the season.

In the intercropping experiment, rate of early blight increase, area under the disease progress curve (AUDPC), average lesion expansion rate, and yield in the 'Brandywine' monoculture was compared with 'Brandywine' intercropped with the resistant cultivars. Rate of early blight increase (r-value) was determined by plotting percent symptomatic leaf area over time and calculating the slope of the best-fit line. A logistic model was compared with a linear regression, and the linear regression gave better fits and randomly distributed residuals. Rate of lesion expansion was calculated by plotting lesion diameters over time and calculating the slope of the best-fit line. Data for the AUDPC, r-values, and rate of lesion expansion were collected and analyzed on a per-plant basis using two-way analysis of variance. Transects within plots did not differ and were treated as replicates. One-way ANOVA and Tukey's HSD were used to compare treatment-by-distance combinations for AUDPC, lesion expansion rates, and r-values.

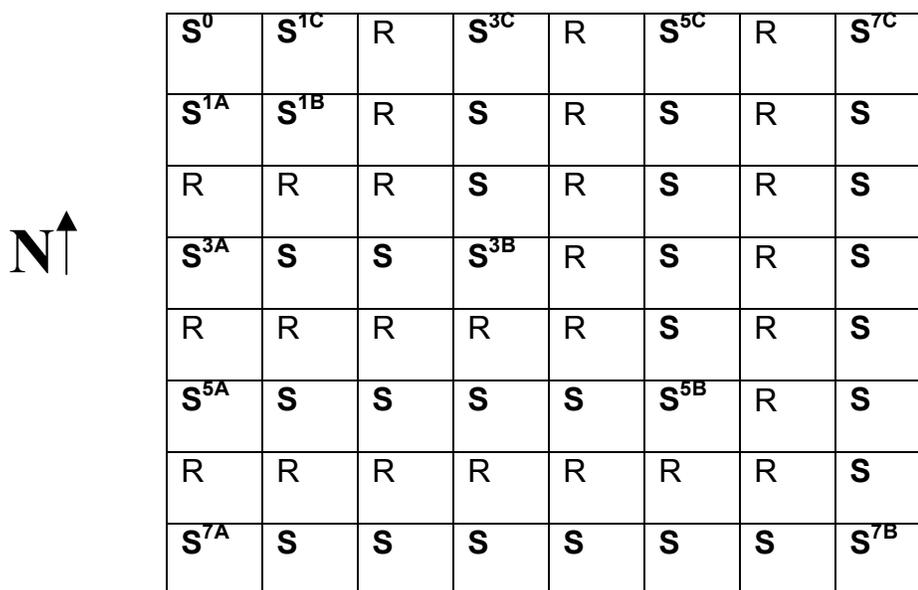


Figure 1. Field plot layout and numbering scheme for intercropping plots. Disease gradients were monitored in susceptible ‘Brandywine’ plants (S) numbered (0, 1, 3, 5, 7) according to distance from the inoculated plant (S^0) in three transects (A, B, C). Resistant plants (R) were cultivar ‘Celebrity’ in nematode plots, and cultivar ‘Juliet’ in early blight plots.

5. Project Results

Variety Trials

Early blight progressed linearly throughout the 2001 season. An outbreak of late blight was observed in plots, beginning on August 24. Disease measurements after September 14 were not included in data analyses due to the inability to distinguish between the two foliar diseases. Early blight disease severity was highest on ‘Daybreak’ and lowest on ‘Matt’s Wild Cherry’, ‘99199’, ‘Red Currant’, ‘99197’, ‘99203’, and ‘Juliet’. ‘Sungold’ and ‘Pruden’s Purple’, an heirloom variety, had intermediate levels of disease (Table 1). By the first frost on October 7, the only variety with remaining green foliage was ‘Matt’s Wild Cherry’ which appeared to be resistant to both late blight and early blight.

Early blight was much less severe in 2002 and the first lesions were not observed until early August. AUDPC was determined using data through Sept. 14 only, so that disease severity values from the two years could be compared (Table 1). Cultivar rankings using the entire season’s data did not differ from those on Sept. 14 (data not shown). Cultivar rankings were similar to the previous year, except that ‘Pruden’s Purple’ and ‘99197’ ranked higher in terms of disease levels, compared to other cultivars, than in 2001, while ‘Green Zebra’, ‘Striped German’, ‘Celebrity’ and ‘Arkansas Traveler’ had relatively lower levels of disease compared to other cultivars, than in 2001.

Intercropping Experiment

No spread of the Southern Root Knot nematode, *Meloidogyne incogita*, from the inoculated corner plant was observed in any plot, thus, no evaluation of the effect of intercropping treatments on nematode infection was possible.

Early blight lesions were observed in early July on corner plants and disease increased linearly throughout the summer until frost on October 7. An outbreak of late blight began in late August and percent defoliation due to late blight was measured separately from early blight between Aug. 24 and Sept. 14. By late September it became difficult to differentiate between defoliation due to the two diseases, therefore, observations after Sept. 14 were not included in the analysis.

An early blight disease gradient from the point of inoculation was established across the plots. Early blight AUDPC ($P = 0.038$) and r -values ($P = 0.006$) were significantly lower in the intercrop compared with the monoculture when averaged across all the measured plants (Table 2).

The average lesion expansion rate was significantly greater on older leaves than younger leaves. The rates on both old and middle aged leaves was lower on the intercropped 'Brandywines' than in the monoculture, but differences were not significant (Table 2).

Yield was significantly increased on 'Brandywine' when it was intercropped with 'Juliet' (Table 2). Yield was measured on the same 13 plants on a per plot basis. 'Brandywine' yield was 17.3% greater when intercropped with 'Juliet' than with monoculture.

Disease severity was compared among plants at increasing distances from the inoculated plant to assess the effect of interplanting with a resistant cultivar on disease (Table 3). Plants at the position 0 were the inoculated 'Brandywine' plants, and those at position 1 were 'Brandywine' plants directly adjacent to the inoculated plants (Fig. 1). As expected, the rate of disease increase (r -values) in these plants did not differ among treatments. However, r -values are significantly lower in the intercropped plants at positions 3, 5 and 7 than in monoculture plants. Comparisons of AUDPC values by position showed a similar pattern, but differences between monoculture versus intercropped plants were not significant. The rate of lesion expansion at different distances from the inoculated plant tended to be greater in monoculture than intercrop plots, but differences were not statistically significant.

6. Conclusions and Discussion

Varietal resistance is an important factor to be considered in organic crop production where synthetic fungicides are not allowed. However, varietal resistance to early blight is not well characterized. Varieties that are listed as 'Resistant' in seed catalogs may still show symptoms of early blight, but lesion expansion is slower, or spore production is inhibited. Resistance to *A. solani* in commercially acceptable tomato cultivars has been difficult to achieve due to the complex patterns of inheritance and the lack of single-gene resistance (Spletzer and Enyedi, 1999). In general, early-maturing cultivars of tomato are more susceptible to early blight than late maturing cultivars (Rands, 1917).

For these field experiments, weather throughout 2001 was ideal for the spread of early blight. Lesions developed in early July and disease progressed linearly. Late blight appeared at the end of August and made early blight disease assessment difficult. Climate was much less

favorable for early blight in 2002, thus conclusions regarding differences in disease resistance among varieties in 2002 are less reliable.

'Juliet' was the most resistant variety to early blight in 2001 and was among the most resistant in 2002. Greenhouse studies verified that early blight lesion expansion rates are reduced and spore production does not occur in 'Juliet' (Smith, 2002). '99203', '99197', and '99199' are resistant varieties produced by North Carolina State University and were not significantly different from 'Juliet' in either year. 'Matt's Wild Cherry' and 'Red Currant' are cherry varieties and both had levels of resistance statistically similar to 'Juliet'. Cherry varieties tend to have higher levels of resistance to early blight. The only other cherry variety was 'Sungold' and though it had moderate susceptibility to early blight, it was statistically different from the most susceptible variety in both years.

'Pruden's Purple' is an heirloom variety that many organic growers claim has tolerance to early blight. This was observed in 2001, but not 2002. Green Zebra was very susceptible in 2001, but had the least disease of any variety in 2002. 'Striped German', 'Arkansas Traveler' and 'Celebrity' were also very susceptible in 2001, but showed statistically less disease in 2002 than 'Daybreak', 'Brandywine', 'Johnny's 361', and 'Valley Girl', which were very susceptible to early blight in both years.

Interplanting with resistant cultivars may be useful for disease management, even in susceptible varieties. Early blight AUDPC was reduced on 'Brandywine' intercropped with resistant variety 'Juliet' in the field experiment. It is likely that the reduction in disease occurred due to the decreased proportion of susceptible plant material and because the early blight lesions on resistant variety 'Juliet' do not produce spores. Despite the large size of the tomato plants, autoinfection must have been limited in order to observe the effect of disease reduction in the resistant intercrop (Garrett and Mundt, 2000), indicating that inoculum was dispersed far from individual plants.

It is also possible that intercropped 'Brandywine' may have reduced disease because of induced resistance in addition to the reduction in susceptible plant material. Neighboring 'Juliet' tomato plants produce salicylic acid and develop systemic acquired resistance (SAR) in response to *A. solani* infection (Spletzer and Enyedi, 1999; Smith, 2002). Salicylic acid is converted to methyl salicylate, a volatile compound that can activate disease resistance in neighboring plants (Shulaev et al., 1997). We used lesion diameter and expansion as an indication of the level of resistance in the neighboring plant (Berger et al., 1997). Lesions on middle-aged leaves expanded 0.00529 cm/day faster on 'Brandywine' in monoculture ($p=0.0936$) than on intercropped 'Brandywine'. Lesions on older leaves expanded 0.00785 cm/day faster on 'Brandywine' in monoculture ($p=0.1602$). This is an 11% and 12% reduction in lesion expansion on middle and old-aged leaves, respectively. More experiments are needed to identify the mechanism of disease reduction on susceptible plants in resistant intercrops.

The mechanism of disease reduction by intercropping with 'Juliet' can be better understood by comparing rates of disease increase (r-values) on 'Brandywine' at different distances from the inoculated plant for both treatments (Table 3). The tomato plants that were measured for disease in each plot included some that were directly adjacent to the inoculated plant (position 1) and others that had resistant variety 'Juliet' between them and the inoculated plant (positions 3, 5 and 7). Rate of disease increase and lesion expansion rates on plants at position 1 can be compared with those at positions 3, 5, and 7 to determine whether induced resistance played a part in disease reduction in 'Brandywine' plants. Rate of disease increase was statistically the same on the inoculated plant and 'Brandywine' at position 1 in both the

monoculture and the intercrop. However, rate of disease increase on 'Brandywine' was progressively lower on intercropped plants at positions 3, 5, and 7. This is an indication that the resistant variety 'Juliet' not only acts as a buffer between the inoculum source and the susceptible plant, but may be inducing resistance in neighboring plants after it is infected with *A. solani*. The reduction in disease on plants with a distance of 5 and 7 in the monoculture indicates that autoinfection is somewhat limited, and spore dissemination from source plants is important to initiate and maintain an epidemic.

Lesion expansion was 21% lower on 'Brandywine' in monoculture, but this difference was not statistically significant ($P=0.125$). If salicylic acid and induced resistance are involved in disease reduction in mixtures, it is possible that plants immediately adjacent to the inoculated plant could be affected by volatile methyl salicylate as well. Experiments which monitor salicylic acid levels in the field might help to further understand the role of induced resistance in varietal mixtures.

Yield increases in intercropped 'Brandywine' compared to monoculture 'Brandywine' are particularly interesting and further evaluation is needed. In many cases, yield differences in intercrop trials are confounded by differences in plant size and spacing and the corresponding competition for resources. Since both 'Brandywine' and 'Juliet' are large indeterminate plants, differences in plant-to-plant spacing seem unlikely to account for the yield response of 'Brandywine' to intercropping.

The use of resistant intercropping for the reduction in the rate of spread of *A. solani* to susceptible varieties has not been tested for its effectiveness under natural inoculation, and it may require the use of other cultural practices for effective control of early blight. However, the 21 % increase in crop yield on 'Brandywine' intercropped with 'Juliet' indicates that intercropping susceptible varieties with resistant ones, where practical, has significant benefits for disease control and productivity.

7. Outreach

Results from this project were disseminated to the public via tours and field days at the WVU Organic Research Farm. In addition, the results contributed to a Master's thesis (Smith, 2002) and a preliminary report was presented at the American Phytopathological Society meetings. Research articles are being prepared for publication in peer-reviewed scientific journals.

Table 1. Early blight disease severity (Area Under the Disease Progress Curve, AUDPC) on 16 tomato varieties in field trials in 2001 and 2002.

Variety	AUDPC	
	2001	2002
Daybreak	358 a	145 abcd
Brandywine	338 ab	224 ab
Green Zebra	306 ab	9 d
Johnnys 361	305 ab	190 abc
Striped German	297 ab	81 cd
Valley Girl	295 ab	103 abcd
Celebrity	294 ab	66 cd
Arkansas Traveler	287 abc	57 cd
Sungold	265 bcd	60 cd
Pruden's Purple	257 bcde	242 a
Matts Wild Cherry	207 cde	54 cd
Red Currant	199 de	99 bcd
99199	190 de	66 cd
99197	188 de	101 abcd
99203	177 e	51 cd
Juliet	174 e	68 cd

Means (of five replicates) followed by the same letter due not differ significantly (P=0.05) according to Tukey's HSD test.

Table 2. Average rate of early blight increase (r-value), Area Under the Disease Progress Curve (AUDPC), lesion expansion rate, and total yield, in intercropped and monoculture plots of 'Brandywine' tomato.

Treatment	r-value	AUDPC	Lesion expansion rate (mm/day)		Yield kg/plot
			Old leaves	Middle leaves	
Monoculture	1.13 b	442 b	0.65 a	0.53 a	48.3 a
Intercrop	0.91 a	397 a	0.57 a	0.35 a	58.4 b

Means followed by the same letter in the same column do not differ significantly (p<0.05) according to Tukey's HSD.

Table 3. Rate of early blight disease progress (r) and Area Under the Disease Progress Curve (AUDPC) on ‘Brandywine’ in monoculture or intercropped with resistant cultivar ‘Juliet’ at different positions from the inoculated plant (Figure 2.3).

Treatment	Position ¹	r-value ²	AUDPC
Monoculture	0	1.24 a ³	695 A
	1	1.21 a	450 B
	3	1.15 ab	383 B
	5	1.05 b	373 B
	7	0.99 b	308 BC
Intercrop	0	1.23 a	744 A
	1	1.23 a	384 B
	3	0.74 c	318 BC
	5	0.71 c	313 BC
	7	0.61 d	226 C

¹ Position = Number of plants from the inoculated plant (Plant Distance = 0). Plants were spaced 90 cm apart both between and within rows.

² r-values were determined using linear regression of disease severity versus time.

³ Means of fifteen replicates. Means followed by the same letter do not differ significantly ($P < 0.05$) according to Tukey’s HSD.

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