

## Final report

### FREP Project 07-0174

#### Evaluation of humic substances used in commercial fertilizer formulations

T.K. Hartz  
Extension Specialist  
Department of Plant Sciences  
University of California  
1 Shields Ave  
Davis, CA 95616  
(530) 752-1738  
[tkhartz@ucdavis.edu](mailto:tkhartz@ucdavis.edu)

#### Executive summary:

This project examined the effects of five commercial humic acid formulations on soil microbial activity, seed germination, early growth, nutrient uptake and crop productivity of lettuce and processing tomato. Humic acid solutions ranging from 250-750 PPM a.i. were used to imbibe coated lettuce seed; those seeds germinated at the same rate and frequency as seed imbibed with deionized water. In a greenhouse trial lettuce plants were grown from seed in pots of four field soils differing in phosphorus availability. The pots received pre-seeding banded application of humic acid alone, P fertilization (liquid 10-34-0) alone, both humic acid and P fertilization, or neither treatment. In one of the four soils humic acid plus P increased lettuce growth above that of P fertilization alone. In the absence of P fertilization, no humic acid formulation increased lettuce growth in any soil. P fertilization increased plant P uptake in all soils, but humic acid did not increase P uptake in any soil.

The effect of humic acid on soil microbial activity was evaluated in a laboratory assay using a low organic matter soil and a high organic matter soil (0.8 and 2.5% organic matter, respectively). The soils were wetted with tap water alone, P fertilizer solution, humic acid solution, or a solution containing both humic acid and P fertilizer. The wetted soils were incubated at a constant 77 °F (25 °C) for 7 days in sealed containers, and CO<sub>2</sub> evolution was determined as a measure of overall microbial activity. After 7 days, moist soil samples were subjected to phospholipid fatty acid (PLFA) analysis by gas chromatography; this technique quantifies the type and amount of phospholipid fatty acids present, which provides a 'fingerprint' of the active microbial communities in the soil (fungi, bacteria, actinomycetes, etc.). In the absence of P fertilization, humic acids had no effect on soil microbial activity; with P fertilization, humic acids caused a small but statistically significant increase in microbial activity in the low organic matter soil only. In the low organic matter soil humic acids significantly increased the PLFA's associated with fungi, bacteria and actinomycetes. In the

high organic matter soil the P-fertilized treatment had higher PLFA levels than the humic plus P treatments.

Processing tomato trials were conducted in both 2008 and 2009 to determine the effects of humic acids applied with preplant P fertilizer (liquid 10-34-0) on crop nutrient uptake, early growth, nutrient uptake and fruit yield and quality. Two rates (1 and 3 lb a.i. per acre) of the five humic acid formulations were evaluated. Early season crop growth was evaluated by the harvest of whole plants 6 weeks (2008) or 4 weeks (2009) after transplanting; tissue macro- and micronutrient analysis was performed at that time. The trials were mechanically harvested at commercial maturity. In both years P fertilization significantly increased early plant growth, but no humic acid treatment increased growth above that in the P-fertilized control. Petiole  $\text{PO}_4\text{-P}$  and leaf P concentration were significantly enhanced by P fertilization, but humic acids did not increase tissue P compared to the P-fertilized control. Compared to the P-fertilized control, humic acids had no consistent effect on tissue concentration of other nutrients. In neither year did humic acid application affect fruit yield, soluble solids concentration or color.

In summary, when used at commercial application rates, no consistent agronomic advantage was observed with the application of commercial humic acid formulations to typical agricultural soils.

## **Introduction:**

The use of humic substances to improve crop growth has been the subject of a substantial body of research over decades. The term 'humic substances' refers to a complex, heterogeneous mixture of organic materials arising from the decay of plant and animal residues (McCarthy et al., 1990). Humic substances can be characterized as humic acid, fulvic acid and humin on the basis of solubility in water as a function of pH. Both humic acid and fulvic acid, the soluble fractions, have been widely studied. The reported effects of humic substances include:

a) modified soil physical properties of soil. Humic substances can stabilize soil structure (Piccolo and Mbagwu, 1990) and increase cation exchange (Allison, 1973).

b) increased root growth. Root growth enhancement has been attributed to improved soil structure, stimulation of soil microflora, and plant growth regulator effects (Chen and Aviad, 1990).

c) enhanced nutrient availability. This can result from direct availability of nutrients from the humic substances (Stevenson and He, 1990; Tarafdar and Jungk, 1987), chelation of nutrients by the humates (Stevenson, 1991), or through more complex physiological interactions (Vaughan et al., 1985). For comprehensive reviews of humic acid effects on plants see Chen and Aviad (1990) and Varanini and Pinton (1995).

It is the potential to enhance nutrient availability (particularly for phosphorus and micronutrients) that is the main rationale for the use of humic substances by the commercial fertilizer industry. Many companies incorporate these materials into standard fertilizer formulations; such products are sold both for soil- and foliar application. The source of most of the humic substances incorporated into commercial fertilizers is leonardite, a carbonaceous mineral found in geological deposits around the world.

While the potential bioactivity of humic substances has been well documented, there are serious limitations in the existing scientific literature. The vast majority of positive reports of humic acid effects have come from solution culture or hydroponic experiments; very few studies showing positive crop response to humic acid have been conducted in representative agricultural soils, and even fewer under normal agricultural field conditions. The handful of studies showing positive responses in field conditions tend to have been conducted in low organic matter soils (Fagbenro and Agboola, 1993; Kunkel and Holstad, 1968; Lee and Bartlett, 1976). Recent U.S. research suggests that, under representative field conditions, commercial humic acid formulations do not reliably provide agronomic benefits for vegetable production. Boyhan et al. (2001) found no humic acid effects on onion yield in three years of field trials, but reported enhanced storage life in one year. Feibert et al. (2003) and Duval et al. (1998) reported no benefit from humic acid application in field production of onion and mustard greens, respectively.

This project examined the effects of five commercial humic acid formulations when applied to representative agricultural soils. Using laboratory, greenhouse and field experiments, humic acid effects on soil microbial activity,

seed germination, early growth, nutrient uptake, and crop yield were determined on lettuce and processing tomato.

### **Objectives**

- a) Quantify the effects of humic acid materials used in commercial fertilizer formulations on soil microbial activity, early growth, nutrient uptake, and crop yield
- b) Determine whether crop response to humic acid materials is soil-specific

### **Methods:**

Five commercial humic acid formulations were evaluated in greenhouse, laboratory and field experiments. The chemical composition of these products was characterized by various means. The humic acid content was analyzed by A&L Western Agricultural Laboratory, Modesto, California, using a protocol developed by the California Department of Food and Agriculture. In short, this protocol determined the percentage of product weight soluble in NaOH but insoluble in HCl. Samples of all formulations were oven-dried at 75° C (167 °F) and ground to pass a 40 mesh screen. Analysis of hydrogen and oxygen content was performed using an Isotope Ratio Mass Spectrometer (IRMS). Carbon and N content was determined by a combustion technique, while P, K and S content were determined by atomic absorption spectrometry (AAS) or Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) following microwave-acid digestion.

For the greenhouse experiment four field soils were collected, two from the San Joaquin Valley and two from the Sacramento Valley. The soils chosen had been in typical row crop / vegetable crop rotations, and all had low P availability [ $< 15$  PPM bicarbonate extractable (Olsen) P]; physiochemical properties are given in Table 1. The soils were air-dried, screened through 5 mm mesh and blended for uniformity. Plastic pots of one liter volume were partially filled with 750 g of dry soil. To simulate a banded preplant fertilizer application a band of liquid was applied to the soil surface, then covered by an additional 250 g of soil. The liquid band contained one of the humic acids alone, 10-34-0 fertilizer alone, or a combination of humic acid and 10-34-0. Comparison pots receiving neither humic acid nor 10-34-0 were also included. Application rates for the humic acids were equivalent to a field rate of 2 lb a.i. per acre, based on a banded application in a field of 40 inch-wide beds with two rows of lettuce per bed. The  $P_2O_5$  rate for all fertilized treatments was 50 lb  $P_2O_5$  / acre.

Ten pelleted seeds of 'Green Towers' romaine lettuce were sown in each pot, in a line 1 inch above and 1 inch to the side of the humic acid / fertilizer band. The seeds were covered with a thin layer of sand and the pots were placed in a greenhouse. A randomized complete block experimental design was used, with 5 replicate pots of each soil x treatment combination. The pots were wetted on 2 November, 2007. The number of emerged seedlings in each pot was recorded daily from 8-16 November, after which the seedlings were thinned to one representative plant per pot. The greenhouse was maintained at 75 / 70

°F (24 / 20 °C) day/night. Watering was done daily with a calcium nitrate solution containing 100 PPM N. Whole plants were harvested on 19 December (47 days after sowing). The plants were oven-dried, weighed, ground and analyzed for P content.

Additionally, a lettuce germination assay was conducted in petri dishes. In 10 cm-wide petri dishes blotter papers were wetted with either deionized water, or water containing 250, 500 or 750 PPM a.i. humic acid (based on the commercial label analysis). Twenty five pelleted seeds of 'Green Towers' romaine lettuce were placed in each dish. The dishes were covered and incubated at 59 °F (15 °C); there were four replicate dishes per humic acid formulation per humic acid concentration, for a total of 64 dishes. Germination counts were taken daily, with germination scored when the radicle had extended at least 3 mm from the seed.

A laboratory incubation experiment was conducted to evaluate the effects of the humic acids on soil microbial activity and microbial community structure. Two agricultural soils were selected, one a low organic matter soil from the San Joaquin Valley, one a higher organic matter soil from the Salinas Valley; physiochemical characteristics are given in Table 2. The soils were air-dried, passed through a 5 mm screen, and blended for uniformity. One hundred grams of dry soil was placed in glass jars of 1 liter volume. The soil was wetted to field capacity moisture content by adding tap water alone, P fertilizer solution, humic acid solution, or a solution containing both humic acid and P fertilizer. The concentrations of P and humic acids were calculated to represent the concentration of these materials in a banded application of 20 lb P<sub>2</sub>O<sub>5</sub> and 2 lb a.i. humic acid per acre. Four replicate jars of each humic acid x P fertilizer combination per soil were prepared along with unfertilized and P-fertilized controls. Once wetted, the jars were sealed and placed in a 77 °F (25 °C) chamber. After 3 and 7 days, samples of the headspace air were removed from the jars and analyzed for CO<sub>2</sub> concentration by infrared gas analyzer; from these data the amount of carbon mineralized by microbial activity was calculated. At the end of 7 days the jars were removed from the chamber, and 50 g of moist soil was removed from each jar. These soil samples were subjected to phospholipid fatty acid (PLFA) analysis by gas chromatography; this technique quantifies the type and amount of phospholipid fatty acids present. The various PLFAs detected can be classified according to the microbial group (fungi, bacteria, actinomycetes, etc.) with which they are most closely associated; while not all PLFAs are exclusive to a particular group of microorganisms, this classification is widely recognized as providing a 'fingerprint' of the active microbial communities in the soil (Drenovsky et al., 2004). This technique provided a method to determine which microbial communities were significantly affected by humic acid application.

The effect of the humic acid formulations on the production of processing tomatoes was evaluated in field trials conducted at UC Davis. In 2008 a field of silt loam soil with an Olsen P value of 12 PPM was tilled into 60 inch-wide raised beds. On 18 April, a pre-transplanting banded application of fertilizer was applied 4-5 inches deep, offset approximately 1 inch from the bed center. The

treatments applied included each of the humic acid formulations at both a 1 and 3 lb a.i./acre rate applied with 10-34-0 fertilizer, a P-fertilized control and a no P control. In all treatments receiving P fertilization 70 lb P<sub>2</sub>O<sub>5</sub>/acre was applied. The humic acids were thoroughly blended with the 10-34-0 before application to simulate commercial humic/fertilizer solutions. The no P control treatment received preplant N equivalent to that contained in the treatments receiving 10-34-0. The field was transplanted with Heinz 9780 processing tomato plants on 24 April; planting density was approximately 7,000 plants / acre. The experimental design was randomized complete block with 5 replications; individual single row plots were 100 feet long. On 10 June four whole plants per plot were harvested, dried and analyzed for macro- and micronutrient concentrations. A seasonal total of 180 lb N/acre was applied in 8 weekly fertigation; no K fertilization was required. The plots were mechanically harvested on 28 August; total and marketable yield were determined, and fruit samples were evaluated by the Processing Tomato Advisory Board grading station in Dixon for soluble solids, blended color and pH.

The processing tomato field experiment was repeated at UC Davis in the 2009 production season in a field of loam soil with Olsen P of 13 PPM. The trial structure was similar to the 2008 trial, with minor modifications. All humic acid treatments and the fertilized control received only 40 lb P<sub>2</sub>O<sub>5</sub>/acre. Also, the manufacturer of the ESP-50 product expressed a desire to eliminate the high rate of that product, as it was not economically feasible at that rate; an additional fertilized control treatment receiving 80 lb P<sub>2</sub>O<sub>5</sub>/acre was substituted. Heinz 9780 plants were transplanted on 29 April. A seasonal total of 170 lb N / acre was applied in eight weekly fertigation. On 22 May four whole plants per plot were harvested for dry weight determination, and whole leaf and petiole samples were collected and dried for tissue macro- and micronutrient determination. Leaf and petiole sampling was repeated on 12 June. The plots were mechanically harvested on 2 September; total and marketable yield were determined, and fruit samples were evaluated by the Processing Tomato Advisory Board grading station in Dixon for soluble solids and blended color.

### **Results:**

The humic acid formulations differed considerably in their chemical composition. Humic acid content by the label analysis varied from 6-50%, while laboratory analysis was generally somewhat lower (Table 3). Humic acid is a general term for a very heterogeneous material, and there is some disagreement regarding the most appropriate analytical technique for its determination. In all experiments humic acid application rates were calculated based on the label analysis.

The elemental composition also varied widely among the commercial formulations tested, undoubtedly reflecting the different Leonardite deposits from which they originated (Table 4). While the large differences in macronutrient content, particularly P, suggest that these products have substantial differences in chemical structure, the implications for crop fertility are limited; at the normal

application rate to field soils (typically < 3 lb of humic acid per acre) the amount of nutrient supplied to a crop directly from humic acid is insignificant.

In the laboratory germination experiment humic acid had no statistically significant effect on germination percentage or the speed of germination. All treatments had > 98% germination, and the mean days to germination ranged only from 2.6 to 3.0 days among treatments. Similarly, neither humic acid nor P fertilization significantly affected lettuce seedling emergence percentage or speed of emergence in the greenhouse study (Table 5). The soils varied significantly in final emergence percentage, but not in speed of emergence.

There was significant treatment x soil interaction in lettuce growth and P uptake in the greenhouse study, so treatment effects were evaluated separately for each soil. P fertilization had a profound influence on lettuce growth in all soils (Table 6); unfertilized treatments in soils 1 and 2 were severely P-limited. However, only in soil 3 did the addition of humic acid increase lettuce growth above that of P fertilization alone. In the absence of P fertilization, no humic acid formulation increased lettuce growth in any soil. Table 7 shows lettuce P uptake. The effect of P fertilization was profound in all soils; humic acids did not increase lettuce P uptake in any soil.

In the incubation experiment P fertilization stimulated soil microbial activity (increased CO<sub>2</sub> evolution) in both soils (Table 8). In the absence of P fertilization, humic acid had no effect on microbial activity; with P fertilization, humic acids caused a small but statistically significant increase in microbial activity after 7 days in the low organic matter soil (soil 1). It is generally recognized that the carbon in humic acid is fairly resistant to microbial metabolism, so most of the increase in carbon mineralization resulting from humic acid application was presumably due to a stimulatory effect on the microbial community rather than an increase in the supply of labile carbon. Microbial activity was not enhanced by humic acid application in the soil with higher organic matter (soil 2).

In the PLFA analysis of the soils from the incubation experiment there was a statistical interaction between humic treatment and soil, so the humic acid effects were analyzed separately for each soil (Table 9). In the low organic matter soil (soil 1) humic acid significantly increased the PLFA's associated with fungi, bacteria and actinomycetes compared to the control treatment receiving neither humic acids nor P fertilization, or the treatment receiving only P fertilization. P fertilization alone was also stimulatory for PLFA's associated with fungi and bacteria. In the higher organic matter soil (soil 2) P application did not significantly influence PLFA type or amount; this was not surprising considering the high initial P level of that soil (59 PPM Olsen P, compared to 7 PPM in soil 1). In soil 2 humic acids were not stimulatory; in fact, the P-fertilized control had slightly higher PLFA levels than the humic plus P treatments. The explanation for this phenomenon was unclear.

In the 2008 field trial P fertilization significantly increased early plant growth, but no humic acid treatment increased growth compared to the P-fertilized control (Table 10). Both petiole PO<sub>4</sub>-P and leaf P concentration were significantly enhanced by P fertilization, but humic acids did not increase tissue P

compared to the P-fertilized control. Compared to the P-fertilized control, humic acids had little effect on the tissue concentration of other nutrients, as a group increasing leaf Fe but decreasing leaf Zn. The lower Zn and Cu concentrations in all fertilized treatments compared to the no P control may have reflected a 'dilution' effect, in that the micronutrients were spread over a larger amount of plant dry matter in the P-fertilized treatments.

While no individual treatment was significantly higher-yielding than the no P control, in the aggregate P fertilization increased both total and marketable fruit yield (as determined by orthogonal contrast, Table 11). Humic acid did not increase fruit yield above the P-fertilized control. Neither P fertilization nor humic acid treatment affected fruit soluble solids concentration or fruit color.

P fertilization significantly increased early plant growth and tissue P concentration in the 2009 trial (Table 12). However, the addition of humic acids to the P fertilizer again had no significant effect; increasing P fertilization from 40 to 80 lb P<sub>2</sub>O<sub>5</sub>/acre did not significantly increase plant growth. Similarly, P fertilization increased petiole NO<sub>3</sub>-N, PO<sub>4</sub>-P and K concentration, and leaf N and P concentration, above that of the no P control. However, the addition of humic acid did not increase tissue nutrient concentrations above P fertilization alone. On 12 June petiole PO<sub>4</sub>-P was still elevated in the P-fertilized treatments, with petiole NO<sub>3</sub>-N higher in the no-P control (Table 13). However, the only significant effect of humic acid was an increase in leaf N compared to the 40 lb P<sub>2</sub>O<sub>5</sub>/acre treatment; this appeared to be an anomaly, as the higher rate of P fertilizer and the no-P control both had leaf N similar to the humic treatments. No significant treatment effects were observed on leaf micronutrient concentrations at early growth (Table 14).

Although early growth was clearly P-limited (Table 12), by the end of the season there were no significant treatment differences in tomato yield or fruit quality (Table 15). In the aggregate, the humic acid treatments showed no advantage in any parameter over the treatment receiving 40 lb P<sub>2</sub>O<sub>5</sub>/acre alone.

### **Discussion:**

Commercial humic acid formulations can be biologically active in representative field soils, as evidenced in the soil incubation experiment, and in soil 3 of the greenhouse experiment. However, no consistent effect of humic acid application was observed in seed germination, crop growth, nutrient uptake or commercial yield. These results were consistent with the conclusions drawn by Chen et al. (2004) in their recent review of the use of humic substances in agriculture. They concluded that, although humic substances can affect plant productivity through a variety of mechanisms, soil application of commercial humic products at typical use rates is unlikely to elicit a significant agronomic response. They based this conclusion on the observation that, across numerous nutrient solution studies, the concentration of HS required to stimulate plant growth was typically in the range of 75 PPM. Applying that analogy to field soils, it would take in excess of 50 lb/acre of humic substances to reach that concentration in the root zone soil solution. Humic acid use rates in these

experiments were 1-3 lb active ingredient/acre, in keeping with the manufacturers' recommendations.

Even at such low use rates, banded application of humic acid creates zones of higher concentration. In the incubation study we mimicked a banded application of 2 lb/acre, with a resultant soil solution concentration of 80 PPM humic acid. In the low organic matter soil that was sufficient to enhance microbial activity, at least in the short term (the study terminated after 7 days). The humic acid applications in the greenhouse and field studies were also banded, but there were important differences. A considerable period of time elapsed between soil application and the establishment of a substantial root system, time in which irrigation could have diluted HA concentration, and soil microbial activity may have altered it.

Beyond the issue of humic acid application rate, there are at least two other important factors that may limit agronomic benefit from humic acid application to agricultural soils. In nutrient solution studies plant growth response to humic substances tended to peak around 100 PPM (Chen and Aviad, 1990). Native soil dissolved organic matter (DOM), which can perform some of the same functions as applied HA (Chen et al., 2004), may be present at sufficient concentration to negate any benefit of applied humic acid. While DOM may be less than 30 PPM in very low organic matter soils (Chen and Katan, 1980), in higher organic matter soils DOM may reach 400 PPM (Chen and Schnitzer, 1978). This may explain the lack of positive benefits of humic acid in the higher organic matter soil in the incubation study, and why reports of beneficial effects of humic acid in field trials have been limited to low organic matter soils (Fagbenro and Agboola, 1993; Kunkel and Holstad, 1968; Lee and Bartlett, 1976).

Finally, some potential benefits of humic acid are of practical significance only in a minority of fields. Consider enhanced micronutrient uptake, a commonly reported benefit of HA in nutrient solution studies (Chen and Aviad, 1990; Varanini and Pinton, 1995). Growth-limiting micronutrient deficiencies are rare in California vegetable fields; for example, in a survey of 78 coastal lettuce fields (Hartz et al., 2007), only one field had tissue Zn concentration below the established sufficiency level, and no fields had deficient tissue levels of Mn or Fe.

In summary, under certain circumstances commercial humic acid formulations can be biologically active when applied to representative field soils. However, at typical application rates, significant improvements in vegetable crop nutrient uptake or productivity appear unlikely in most soils.

#### **Outreach activities:**

Presentations summarizing these results were made at processing tomato grower meetings in Woodland on 14 January and Five Points on 18 February, 2010, and at the annual California Tomato Growers Association meeting in Modesto on 27 January, 2010. A presentation will be made at the American Society for Horticultural Science at the 2010 Annual Meeting in Palm Desert, California. Additionally, a summary has been accepted for publication in the ASHS journal *HortScience*, and will appear later in 2010.

## Literature cited:

- Allison, F.E. 1973. Soil organic matter and its role in crop production. Elsevier Company, Amsterdam, 637 pp.
- Boyhan, G.E., W.M. Randle, A.C. Purvis, P.M. Lewis, R.L. Torrance, D.E. Curry and D.O. Linton. 2001. Evaluation of growth stimulants on short-day onions. HortTechnology 11:38-42.
- Chen, Y., M. De Nobili and T. Aviad. 2004. Stimulatory effects of humic substances on plant growth. *In*: F. Magdoff and R. R. Weil (eds.). Soil organic matter in sustainable agriculture. CRC Press, Boca Raton, FL.
- Chen, Y. and T. Aviad. 1990. Effects of humic substances on plant growth. *In*: P. MacCarthy et al. (eds.). Humic substances in soil and crop sciences: selected readings. Amer. Soc. Agronomy, Madison, WI., pp. 161-186.
- Chen, Y. and J. Katan. 1980. Effects of solar heating of soils by transparent polyethylene mulching on their chemical properties. Soil Sci. 130:271-277.
- Chen, Y. and M. Schnitzer. 1978. The surface tension of aqueous solutions of soil humic substances. Soil Sci. 125:7-15.
- Drenovsky, R.E., G.N. Elliott, K.J. Graham and K.M. Scow. 2004. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. Soil Biol. Biochem. 36:1793-1800.
- Duval, J.R., F.J. Dainello, V.A. Haby and D.R. Earhart. 1998. Evaluating leonardite as a crop growth enhancer for turnip and mustard greens. HortTechnology 8:564-567.
- Fagbenro, J.A. and A.A. Agboola. 1993. Effect of different levels of humic acids on growth and nutrient uptake of teak seedlings. J. Plant Nutrition 16:1465-1483.
- Feibert, E.B.G., C.C. Shock and L.D. Saunders. 2003. Nonconventional additives leave onion yield unchanged. HortScience 38:381-386.
- Hartz, T. K., P. R. Johnstone, E. Williams and R. F. Smith. 2007. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. HortScience 42:143-146.
- Kunkel, R. and N. Holstad. 1968. Effect of adding humates to the fertilizer on the yield and quality of russet burbank potatoes. Potato J. 45:449-457.
- Lee, Y.S. and R.J. Bartlett. 1976. Stimulation of plant growth by humic substances. Soil Sci. Soc. Amer. J. 40:876-879.

MacCarthy, P., C.E. Clapp, R.L. Malcolm and P.R. Bloom. 1990. An introduction to soil humic substances. In: P. MacCarthy et al. (eds.). Humic substances in soil and crop sciences: selected readings. Amer. Soc. Agronomy, Madison, WI., pp. 1-12.

Piccolo, A. and J.S.C. Mbagwu. 1990. Effects of different organic waste amendments on soil microaggregates stability and molecular sizes of humic substances. *Plant Soil* 123:27-37.

Stevenson, F.J. 1991. Organic matter – micronutrient reactions in soil. In: J.J. Mortvedt et al (eds.). Micronutrients in agriculture. Soil Sci. Soc. Amer., Madison, WI., pp. 145-186.

Stevenson, F.J. and X. He. 1990. Nitrogen in humic substances as related to soil fertility. In: P. MacCarthy et al. (eds.). Humic substances in soil and crop sciences: selected readings. Amer. Soc. Agronomy, Madison, WI., pp. 91-109.

Tarafdar, J.C. and A. Jungk. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. *Biol. Fertil. Soils* 3:199-204.

Varanini, Z. and R. Pinton. 1995. Humic substances and plant nutrition. *Progress in Botany* 56:97-117.

Vaughan, D., R.E. Malcolm and B.G. Ord. 1985. Influence of humic substances on biochemical processes in plants. In: D. Vaughan and R.E. Malcom (eds.). Soil organic matter and biological activity. Martin Nijhoff / Dr. W. Junk Publishers, Dordrecht, Netherlands, pp. 77-108.

Table 1. Physiochemical characteristics of the soils used in the greenhouse lettuce experiment.

Soil attribute	Soil 1	Soil 2	Soil 3	Soil 4
location	Fresno County	Fresno County	Yolo County	Yolo County
texture	sandy clay loam	clay loam	loam	loam
pH	7.7	7.8	7.4	7.3
organic matter	0.8	0.9	0.9	1.1
CEC (meq/100 g)	18.9	23.6	19.3	21.7
exchangeable K (PPM)	291	352	208	267
Olsen P (PPM)	3	5	12	10

Table 2. Physiochemical characteristics of the soils used in the laboratory incubation experiment.

Soil attribute	Soil 1	Soil 2
location	Fresno County	Monterey County
texture	sandy clay loam	loam
pH	7.8	7.9
organic matter (%)	0.80	2.5
NO <sub>3</sub> -N (PPM)	23	8
Olsen P (PPM)	7	59
exchangeable K (PPM)	295	155

Table 3. Description of the humic acid products tested.

Year	Humic formulation	Manufacturer	Form	% humic acid by label analysis	
2008	Actagro Humic Acid	Actagro, LLC	liquid	10	10
	Actagro Liquid Humus	Actagro, LLC	liquid	11	8
	Organo Liquid Hume	Black Earth Humates, Ltd.	liquid	6	4
	Quantum-H	Horizon Ag Products	liquid	6	5
	ESP-50	Earthgreen Products, Inc.	powder	50	39
2009	Actagro Humic Acid	Actagro, LLC	liquid	10	11
	Actagro Liquid Humus	Actagro, LLC	liquid	22	10
	Organo Liquid Hume	Black Earth Humates, Ltd.	liquid	6	6
	Quantum-H	Horizon Ag Products	liquid	6	3

Table 4. Elemental composition of the humic acid products tested.

Year	Humic formulation	% of dry weight						
		C	H	O	N	P	K	S
2008	Actagro Humic Acid	32	3	33	0.66	< 0.01	17.7	2.35
	Actagro Liquid Humus	29	4	43	0.41	< 0.01	10.2	0.67
	Organo Liquid Hume	41	4	34	1.24	0.02	9.2	0.52
	Quantum-H	36	3	32	1.21	3.10	12.7	0.60
	ESP-50	24	3	35	2.08	7.72	16.3	0.38
2009	Actagro Humic Acid	31	3	35	0.71	< 0.01	18.8	2.85
	Actagro Liquid Humus	29	4	43	0.71	0.21	10.5	0.90
	Organo Liquid Hume	41	3	34	1.30	0.20	8.9	0.52
	Quantum-H	39	3	31	1.25	0.61	12.3	1.05

Table 5. Effect of humic acid and P fertilizer on lettuce seed emergence percentage and speed, greenhouse trial.

Treatment	Emergence (%)	Mean days to emergence
Actagro Humic Acid	78	9.7
Actagro Liquid Humus	82	9.5
Organo Liquid Hume	78	10.1
Quantum-H	81	9.8
ESP-50	76	9.5
Actagro Humic Acid + P	78	9.6
Actagro liquid Humus + P	76	9.3
Organo Liquid Hume + P	78	9.5
Quantum-H + P	85	9.9
ESP-50 + P	78	9.6
P alone	78	9.6
No humic acid or P	79	9.8
	ns	ns
<i>Soils</i>		
1	86 a <sup>z</sup>	9.5
2	83 a	9.8
3	73 b	9.8
4	72 b	9.6
		ns

<sup>z</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$

<sup>ns</sup> not significant at  $p < 0.05$

Table 6. Effect of humic acid and P fertilizer on lettuce plant dry weight, greenhouse experiment.

Treatment	Lettuce dry wt (g/plant)			
	Soil 1	Soil 2	Soil 3	Soil 4
Actagro Humic Acid	0.19 b <sup>z</sup>	0.43 b	0.86 d	1.37 b
Actagro Liquid Humus	0.19 b	0.44 b	0.96 d	1.24 b
Organo Liquid Hume	0.28 b	0.52 b	0.92 d	1.03 b
Quantum-H	0.26 b	0.61 b	0.81 d	1.10 b
ESP-50	0.36 b	0.65 b	0.91 d	1.29 b
Actagro Humic Acid + P	1.64 a	1.72 a	3.44 a	2.96 a
Actagro liquid Humus + P	1.73 a	1.87 a	3.28 ab	2.78 a
Organo Liquid Hume + P	1.91 a	1.52 a	3.44 a	2.99 a
Quantum-H + P	1.67 a	1.91 a	3.02 abc	2.49 a
ESP-50 + P	1.91 a	1.48 a	2.63 c	3.20 a
P alone	2.08 a	1.89 a	2.69 bc	2.74 a
No humic acid or P	0.21 b	0.50 b	0.79 d	1.06 b
<i>contrasts</i>				
humics alone vs. humics + P	**	**	**	**
humics + P vs. P alone	ns	ns	*	ns
humics alone vs. no humics or P	ns	ns	ns	ns

<sup>z</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$   
 ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 7. Effect of humic acid and P fertilizer on lettuce P uptake, greenhouse experiment.

Treatment	Lettuce P uptake (mg/plant)			
	Soil 1	Soil 2	Soil 3	Soil 4
Actagro Humic Acid	0.36 b <sup>z</sup>	0.82 c	1.91 c	4.28 c
Actagro Liquid Humus	0.42 b	0.93 c	2.06 c	3.81 c
Organo Liquid Hume	0.51 b	1.11 c	1.90 c	3.10 c
Quantum-H	0.55 b	1.18 c	1.83 c	3.20 c
ESP-50	0.80 b	1.43 c	2.05 c	3.55 c
Actagro Humic Acid + P	6.72 a	6.40 ab	19.85 a	14.60 b
Actagro liquid Humus + P	6.52 a	6.74 ab	19.72 a	16.95 ab
Organo Liquid Hume + P	7.35 a	6.08 ab	17.68 a	16.63 ab
Quantum-H + P	6.59 a	7.04 a	18.80 a	14.96 b
ESP-50 + P	7.38 a	5.48 b	12.76 b	20.57 a
P alone	7.52 a	6.56 ab	15.66 ab	15.39 b
No humic acid or P	0.48 b	1.03 c	1.68 c	2.80 c
<i>contrasts</i>				
humics alone vs. humics + P	**	**	**	**
humics + P vs. P alone	ns	ns	ns	ns
humics alone vs. no humics or P	ns	ns	ns	ns

<sup>z</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$

ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 8. Effects of humic acid and P fertilization on soil microbial activity (mg carbon mineralized / jar), incubation experiment.

Treatment	Soil 1		Soil 2	
	3 days	7 days	3 days	7 days
Actagro Humic Acid	2.22 b <sup>z</sup>	4.27 c	6.27 e	8.86 e
Actagro Liquid Humus	2.34 b	4.06 c	6.50 de	9.36 d
Organo Liquid Hume	2.23 b	3.90 c	6.58 d	9.27 de
Quantum-H	2.24 b	3.93 c	6.25 e	8.84 e
ESP-50	2.29 b	4.19 c	6.29 de	8.91 de
Actagro Humic Acid + P	2.84 a	5.69 b	7.35 c	10.77 bc
Actagro liquid Humus + P	2.48 a	5.84 b	7.52 bc	11.06 ab
Organo Liquid Hume + P	2.85 a	5.83 b	7.90 a	11.26 a
Quantum-H + P	3.04 a	6.30 a	7.26 c	10.56 c
ESP-50 + P	3.04 a	5.89 ab	7.84 a	11.24 a
P alone	2.86 a	5.45 b	7.71 ab	11.22 a
No humic acid or P	2.32 b	3.99 c	6.40 de	9.12 de
<i>contrasts</i>				
humics alone vs. humics + P	**	**	**	**
humics + P vs. P alone	ns	**	ns	ns
humics alone vs. no humics or P	ns	ns	ns	ns

<sup>z</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$   
 ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 9. Effects of humic acid and P fertilization on the amount of phospholipid fatty acids detected in soil, incubation experiment.

Soil	Treatment	Phospholipid fatty acids detected (nmol/g dry soil)			
		Total	Fungi	Bacteria	Actinomycetes
1	Actagro Humic Acid	26.1 ab <sup>z</sup>	5.7 ab	13.5 ab	1.44 ab
	Actagro Liquid Humus	27.4 ab	6.0 ab	14.2 ab	1.48 ab
	Organo Liquid Hume	25.4 ab	5.6 ab	13.2 ab	1.44 ab
	Quantum-H	29.8 a	6.3 a	15.2 a	1.59 ab
	ESP-50	26.2 ab	5.7 ab	13.9 ab	1.48 ab
	Actagro Humic Acid + P	30.2 a	6.6 a	16.0 a	1.61 ab
	Actagro liquid Humus + P	28.8 a	6.2 a	15.0 a	1.53 ab
	Organo Liquid Hume + P	28.5 a	6.1 a	15.0 a	1.53 ab
	Quantum-H + P	25.3 ab	5.4 ab	13.4 ab	1.40 ab
	ESP-50 + P	29.9 a	6.4 a	15.6 a	1.63 a
	P alone	22.0 b	4.4 b	11.6 b	1.28 bc
	No humic acid or P	14.9 c	2.6 c	8.0 c	1.09 c
	<i>contrasts</i>				
humics alone vs. humics + P		*	ns	*	ns
humics + P vs. P alone		**	*	**	*
humics alone vs. no humics or P		**	**	**	**
P alone vs. no humics or P		**	**	**	ns
2	Actagro Humic Acid	52.3 abc	11.9 abc	29.2 abc	3.02 abc
	Actagro Liquid Humus	58.5 a	13.4 a	32.8 a	3.34 a
	Organo Liquid Hume	49.4 abc	11.6 abc	27.7 abc	2.71 bcd
	Quantum-H	57.7 a	13.4 a	32.3 a	3.24 ab
	ESP-50	59.4 a	13.7 a	33.0 a	3.37 a
	Actagro Humic Acid + P	43.0 c	10.1 c	24.1 c	2.45 d
	Actagro liquid Humus + P	55.5 ab	12.9 ab	31.0 ab	3.11 abc
	Organo Liquid Hume + P	46.3 abc	10.7 bc	25.8 bc	2.60 cd
	Quantum-H + P	56.7 ab	13.1 ab	31.8 a	3.06 abc
	ESP-50 + P	51.5 abc	12.0 abc	29.7 abc	2.83 abcd
	P alone	59.3 a	13.6 a	33.0 a	3.32 a
	No humic acid or P	54.3 ab	12.4 abc	30.3 ab	3.10 abc
	<i>contrasts</i>				
humics alone vs. humics + P		ns	ns	ns	ns
humics + P vs. P alone		**	**	**	**
humics alone vs. no humics or P		ns	ns	ns	ns
P alone vs. no humics or P		ns	ns	ns	ns

<sup>z</sup> mean separation by Duncan's multiple range test at  $p < 0.05$

ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 10. Effect of humic acid and P fertilization on processing tomato early growth and tissue nutrient concentration, 2008 trial.

Treatment	Humic rate (lb/acre)	Plant dry wt (g) <sup>z</sup>	Petiole				Whole plant					
			NO <sub>3</sub> -N (PPM)	PO <sub>4</sub> -P (PPM)	K (%)	N (%)	P (%)	K (%)	Zn (PPM)	Mn (PPM)	Fe (PPM)	Cu (PPM)
Actagro Humic Acid	1	84.0 ab <sup>y</sup>	11,540	4,060 a	5.64	4.74	0.46 a	3.48	22.4	143 a	730	15.2
Actagro Liquid Humus		92.8 a	11,480	4,110 a	5.40	4.59	0.40 b	3.37	21.8	148 a	728	14.7
Organo Liquid Hume		82.0 ab	10,610	3,550 ab	5.71	4.58	0.39 b	3.49	22.4	122 a	752	15.5
Quantum-H		92.4 a	10,530	4,010 a	5.35	4.64	0.44 ab	3.38	23.2	144 a	703	15.0
ESP-50		91.2 a	10,300	3,880 ab	5.45	4.68	0.40 b	3.37	24.0	145 a	730	15.6
Actagro Humic Acid	3	83.6 ab	9,660	3,930	5.27	4.67	0.43 ab	3.45	23.2	136 a	702	15.7
Actagro liquid Humus		85.6 ab	10,420	3,830 ab	5.20	4.77	0.44 ab	3.59	22.8	144 a	742	15.3
Organo Liquid Hume		94.4 a	8,950	3,780 ab	5.34	4.57	0.40 b	3.43	22.2	134 a	749	15.0
Quantum-H		83.2 ab	10,270	3,710 ab	5.28	4.74	0.45 a	3.57	23.0	150 a	777	15.5
ESP-50		86.4 ab	9,890	3,700 ab	5.05	4.61	0.40 b	3.50	23.2	142 a	751	15.8
P alone		86.8 ab	9,940	3,340 b	5.25	4.63	0.39 b	3.43	25.0	140 a	652	16.0
No humic acid or P		69.6 b	9,300	2,730 c	5.65	4.60	0.34 c	3.47	26.8 a	96 b	773	18.1
<i>contrasts</i>			ns		ns	ns		ns			ns	
Humic @ 1 lb vs. 3 lb			ns	ns	ns	*	ns	ns	ns	ns	ns	ns
all humic treatments vs. P alone			ns	ns	ns	ns	ns	ns	*	ns	*	ns
all P treatments vs. no P control			**	ns	**	ns	ns	**	ns	**	**	ns

<sup>z</sup> plants collected 47 days after transplanting

<sup>y</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$

ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 11. Effect of humic acid and P fertilization on processing tomato fruit yield and quality, 2008 trial.

Treatment	Humic rate (lb/acre)	Total fruit yield (tons/acre)	Mkt. fruit yield (tons/acre)	Fruit soluble solids (°brix)	Fruit color <sup>z</sup>
Actagro Humic Acid	1	54.2	51.9	5.58	25.2
Actagro Liquid Humus		52.9	50.2	5.54	24.2
Organo Liquid Hume		53.9	52.7	5.42	24.2
Quantum-H		52.5	49.4	5.58	24.8
ESP-50		52.2	50.3	5.50	24.8
Actagro Humic Acid	3	50.4	48.6	5.46	25.0
Actagro liquid Humus		54.9	52.2	5.44	24.2
Organo Liquid Hume		54.9	53.6	5.52	24.0
Quantum-H		53.5	51.4	5.54	24.0
ESP-50		55.7	53.2	5.54	24.2
P alone		55.2	52.7	5.62	25.0
No humic acid or P		49.8	47.7	5.42	24.0
		ns	ns	ns	ns
<i>contrasts</i>					
Humic @ 1 lb vs. 3 lb		ns	ns	ns	ns
all humic treatments vs. P alone		ns	ns	ns	ns
all P treatments vs. no P		*	*	ns	ns
control					

<sup>z</sup> 'Agtron' value, a dimensionless unit; lower value indicates more red

ns, \* not significant at  $p < 0.05$ , or significant at  $p < 0.05$

Table 12. Effect of humic acid and P fertilization on processing tomato early growth and tissue nutrient concentration, 22 May, 2009.

Treatment	Humic rate (lb/acre)	Plant dry wt (g) <sup>z</sup>	Petiole			Whole leaf		
			NO <sub>3</sub> -N (PPM)	PO <sub>4</sub> -P (PPM)	K (%)	N (%)	P (%)	K (%)
Actagro Humic Acid	1	19.3 a <sup>y</sup>	10,920 a	4830 b	6.02	5.64	0.62 b	2.39
Actagro Liquid Humus		22.1 a	11,040 a	4882 b	5.92	5.58	0.61 b	2.38
Organo Liquid Hume		20.5 a	10,730 a	4906 b	6.03	5.62	0.64 b	2.42
Quantum-H		19.8 a	11,050 a	4902 b	6.08	5.68	0.63 b	2.39
ESP-50		22.2 a	10,890 a	4804 b	6.12	5.65	0.62 b	2.38
Actagro Humic Acid	3	20.5 a	11,080 a	5102 b	6.05	5.60	0.63 b	2.40
Actagro liquid Humus		22.7 a	11,000 a	4754 b	6.17	5.65	0.67 b	2.45
Organo Liquid Hume		22.5 a	10,730 a	4670 b	6.00	5.66	0.62 b	2.39
Quantum-H		21.7 a	11,200 a	5198 b	5.91	5.62	0.64 b	2.42
P control @ 40 lb P <sub>2</sub> O <sub>5</sub> /acre		21.8 a	11,060 a	5032 b	5.97	5.72	0.68 ab	2.39
P control @80 lb P <sub>2</sub> O <sub>5</sub> /acre		23.0 a	10,980 a	5694 a	6.05	5.73	0.76 a	2.39
Control (no humic acid or P)		15.5 b	9,810 b	2290 c	5.81	5.45	0.43 c	2.37
<i>contrasts</i>					ns	ns		ns
all humic treatments vs. 40 lb P <sub>2</sub> O <sub>5</sub> /acre alone		ns	ns	ns	ns	ns	ns	ns
all P treatments vs. no P control		**	**	**	*	**	**	ns

<sup>z</sup> plants collected 23 days after transplanting

<sup>y</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$

ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 13. Effect of humic acid and P fertilization on processing tomato tissue nutrient concentration on 12 June, 2009.

Treatment	Humic rate (lb/acre)	Petiole			Whole leaf		
		NO <sub>3</sub> -N (PPM)	PO <sub>4</sub> -P (PPM)	K (%)	N (%)	P (%)	K (%)
Actagro Humic Acid	1	7,500 b	2,400 bc	4.49	4.22	0.32 b	2.88
Actagro Liquid Humus		7,260 b	2,380 bc	4.42	4.12	0.32 b	2.91
Organo Liquid Hume		7,490 b	2,650 ab	4.77	4.15	0.31 b	2.80
Quantum-H		7,250 b	2,220 cd	4.25	4.17	0.30 b	2.87
ESP-50		7,140 b	2,260 cd	4.34	4.17	0.32 b	2.87
Actagro Humic Acid	3	7,510 b	2,480 bc	4.52	4.08	0.30 b	2.82
Actagro liquid Humus		6,840 b	2,460 bc	4.51	4.17	0.32 b	2.93
Organo Liquid Hume		6,880 b	2,260 cd	4.66	4.21	0.32 b	2.84
Quantum-H		8,040 ab	2,580 b	4.35	4.04	0.32 b	3.00
P control @ 40 lb P <sub>2</sub> O <sub>5</sub> /acre		7,220 b	2,580 bc	4.44	3.93	0.30 b	2.86
P control @ 80 lb P <sub>2</sub> O <sub>5</sub> /acre		7,830 b	2,910 a	4.62	4.27	0.36 a	2.85
Control (no humic acid or P)		9,100 a	2,090 cd	4.42	4.24	0.31 b	2.95
<i>contrasts</i>							
all humic treatments vs. 40 lb P <sub>2</sub> O <sub>5</sub> /acre alone		ns	ns	ns	*	ns	ns
all P treatments vs. no P control		**	**	ns	ns	ns	ns

<sup>z</sup> mean separation within columns by Duncan's multiple range test,  $p < 0.05$   
 ns, \*, \*\* not significant at  $p < 0.05$ , or significant at  $p < 0.05$  or  $0.01$ , respectively

Table 14. Effect of humic acid and P fertilization on processing tomato leaf micronutrient concentration, 22 May, 2009.

Treatment	Humic rate (lb/acre)	Ca (%)	Mg (%)	S (%)	B (PPM)	Zn (PPM)	Cu (PPM)	Mn (PPM)	Fe (PPM)
Actagro Humic Acid	1	1.63	1.13	0.58	47	28	18.0	102	772
Actagro Liquid Humus		1.57	1.20	0.57	53	27	17.8	100	687
Organo Liquid Hume		1.58	1.13	0.56	50	27	17.5	105	724
Quantum-H		1.60	1.16	0.57	53	27	17.7	107	758
ESP-50		1.67	1.20	0.57	53	27	19.1	95	787
Actagro Humic Acid	3	1.66	1.20	0.57	52	28	18.8	105	881
Actagro liquid Humus		1.56	1.14	0.56	49	27	17.9	106	769
Organo Liquid Hume		1.62	1.16	0.55	50	28	21.0	98	737
Quantum-H		1.58	1.13	0.58	50	27	17.7	108	740
P control @ 40 lb P <sub>2</sub> O <sub>5</sub> /acre		1.65	1.19	0.57	49	26	17.5	106	753
P control @80 lb P <sub>2</sub> O <sub>5</sub> /acre		1.56	1.12	0.57	50	27	17.7	110	743
Control (no humic acid or P)		1.60	1.14	0.56	48	27	18.6	102	757
		ns	ns	ns	ns	ns	ns	ns	ns

<sup>ns</sup> not significant at  $p < 0.05$

Table 15. Effect of humic acid and P fertilization on processing tomato fruit yield and quality, 2009 trial.

Treatment	Humic rate (lb/acre)	Total fruit yield (tons/acre)	Mkt. fruit yield (tons/acre)	Fruit soluble solids (%brix)	Fruit color <sup>z</sup>
Actagro Humic Acid	1	42.8	41.3	5.58	27.2
Actagro Liquid Humus		45.2	43.5	5.44	26.6
Organo Liquid Hume		43.1	41.6	5.52	26.2
Quantum-H		43.6	42.4	5.48	26.0
ESP-50		42.6	41.5	5.54	26.4
Actagro Humic Acid	3	47.1	45.3	5.30	26.4
Actagro liquid Humus		46.8	44.5	5.60	25.4
Organo Liquid Hume		49.3	47.9	5.44	27.4
Quantum-H		46.0	44.5	5.64	26.6
P control @ 40 lb P <sub>2</sub> O <sub>5</sub> /acre		46.0	44.2	5.62	25.4
P control @80 lb P <sub>2</sub> O <sub>5</sub> /acre		45.6	44.3	5.92	24.8
Control (no humic acid or P)		45.1	43.3	5.44	26.8
<i>contrasts</i>					
all humic treatments vs. 40 lb P <sub>2</sub> O <sub>5</sub> /acre alone	ns	ns	ns	ns	ns
all P treatments vs. no P control	ns	ns	ns	ns	ns

<sup>z</sup> 'Agtron' value, a dimensionless unit; lower value indicates more red

<sup>ns</sup> not significant at  $p < 0.05$