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MATURITY SPRAY RESIDUE DETERMINATION AND EARLY SEASON ACID ACCUMULATION IN GRAPEFRUIT

J. D. RICE[1], S. NIKDEL[2], AND A. C. PURVIS[3]
University of Florida, IFAS
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

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Abstract. **Leaves and juice vesicles of 'Marsh' and 'Duncan' grapefruit (*Citrus paradisi* Macf.) were examined for lead (Pb) and arsenic (As) accumulation following postbloom maturity spray application. A procedure for rapid element extraction by microwave dissolution and detection by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) is described. Spray concentrations applied were 700 ppm lead arsenate (LA) (pH 4.5) for 'Duncan' and 1400 ppm LA (pH 7.0) for 'Duncan' and 'Marsh'. Based on Pb:As ratios determined from the spray solutions and initial total leaf residue levels, Pb accumulation by leaves was initially retarded compared to As. The effect was more pronounced with the low pH, 700 ppm LA treatment, but was at least partially overcome with time with all treatments. Regardless of the initial delay, total Pb content was greater than As in all treatments. Leaves from 'Marsh' and 'Duncan' unsprayed controls also exhibited detectable levels of Pb and As. Neither element was detectable in the juice sac preparation of any treatment, while all LA treatments resulted in less titratable acid accumulation in the juice of developing fruit. The low pH, 700 ppm LA spray afforded similar acidity reduction as the neutral pH, 1400 ppm LA spray. The effectiveness of the LA sprays in reducing juice acid accumulation, lack of element residues in the juice, and high levels of Pb and As associated with the leaves indicate that the effects of the maturity spray may be elicited in some manner in the leaves.**

The postbloom foliar application of lead arsenate (LA) to grapefruit trees results in earlier legal maturity of the fruit, primarily due to reduced acid levels in the juice (5). The site at which the compound elicits its effect has not been demonstrated, although results of early investigations indicated that arsenic (As) is present in significant levels only on the leaves of sprayed trees (9, 11). However, these experiments were designed to measure weathering of As from the leaves in the period following spray application. As a result, there are no reports documenting deposition characteristics and thus, possible levels of As within the leaves of citrus following spray applications.

The characterization of the lead (Pb) component, with respect to citrus maturity sprays, has received almost no attention, since As has been generally regarded as the active component of the spray compound. This is based, in part, on an early report by Gray and Ryan (7) that acidification of LA sprays resulted in greater reduction of acid levels as compared to the juice of fruit from trees to which more basic sprays were applied. It was speculated that lowering the pH preferentially increased the solubility of As, although residue analyses were not reported for either element in their experiments. Secondly, the sprays compared by Gray and Ryan were not altered by pH alone. The compound applied as basic LA also contained a lime-sulfur additive. Since liming has been shown to decrease the uptake of Pb, at least in several plant species (4), it is possible that liming of one solution and acidification of the other may have interfered with the interpretations presented by Gray and Ryan. Lead contamination has been shown to affect physiological processes such as net photosynthesis (2) and transpiration (1, 2) of leaves of several plant species. In this case, the complete exclusion of Pb as an active component in the spray compound may be premature. Regardless, Pb content of the leaves and developing fruit of citrus following maturity spray application has not been examined.

In order to examine the physiological nature of acidity reduction by postbloom sprays in citrus, the location(s) and levels of the spray constituents first must be considered. Towards this end, the characteristics of Pb and As deposition were observed in the weeks following spray application to grapefruit trees. Over the same period, developing fruit from sprayed and control trees were harvested to determine if detectable levels of either element entered the juice and the stage of maturity after spray application lowered acidity could be observed. Finally, a method which allows for rapid and simultaneous extraction and detection of Pb and As is described.

Materials and Methods

Lead arsenate was applied at spray concentrations of 700 ppm (pH 4.5) and 1400 ppm (pH 7.0) to mature grapefruit (*Citrus paradisi*) trees of the 'Duncan' variety on 29 Apr. 1983, and 1400 ppm (pH 7.0) to the 'Marsh' variety on 31 May 1984. Fruit and mature leaves were selected from each quadrant of individual trees (each tree serving as a replicate) to examine residue uptake and, in the case of fruit, acid levels. Prior to each harvest, fruit were measured with hand calipers to obtain samples of near equal size among the replicates. In the laboratory, leaves were rinsed for approximately 1 min under running deionized water to remove the free surface residues, blotted dry and diced. Whole fruit were rinsed with deionized water and weighed prior to the removal of intact juice sacs. Leaf and juice sac subsamples were weighed, dried at 65° C for 1 week and stored in glass vials at room temperature until Pb and As residues were determined. Prior to element analysis, the samples were charred at 150° C for 2 hr and ground to a fine powder with a mortar and pestle. The elements were extracted by modification of the procedure of Nadkarni (10). One half gram samples were placed in open beakers, 10-ml aqua regia was added and the sample

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[1]Fruit Crops Department, University of Florida, Gainesville, FL 32611.
[2]Florida Department of Citrus, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850.
[3]Department of Horticulture, Louisiana State University, Baton Rouge, LA 70802.

was placed in a covered desiccator (without desiccant) in a microwave oven (Jet 210, GE). Extraction was accomplished with three 1-min cycles at full power (625 Watts rating) with 2-min cooling intervals. The samples were allowed to come to room temperature, gravity filtered through #41 Whatman ashless filter paper, and taken to 25 ml with glass distilled deionized water. Lead and As recovery by this procedure was determined to be approximately 99%. Arsenic and Pb concentrations were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using a Jarrel-Ash AtomScan 2000 sequential plasma spectrometer interfaced with an Apple II data acquisition station under standard operating conditions. The spectral lines at 228.812 nm and 220.353 nm were used to determine As and Pb, respectively (13). Aqua regia:distilled water (1:1.5) was used as a blank between each sample determination. Plasma-grade materials of $NH_4H_2AsO_4$ and Pb $(NO_3)_2$ (HiPure Materials, from SPEX Industries, NJ) were used as reference standards for concentration determinations. Counts registered in the computer as the average of four 5-second integrations per element per sample were compared to the reference elements and final concentrations are given in terms of tissue fresh weight.

Subsamples of juice sacs were used for acidity determinations. For small fruit, juice sacs were ruptured with a Ten Broeck homogenizer, taken to volume with glass distilled water, poured through cheese cloth and acidity determined by titration. It was determined that diluting with water did not interfere with linear conversion of percent acid in the diluted samples to a full strength basis (r = 0.9999); and the results are reported as such. Fruit larger than 100 g were juiced with a hand reamer and titrated directly. Samples were titrated to pH 8.3 using standard NaOH with either a "direct reading" or a "standard" burette. Values obtained with the "standard" burette were adjusted as described by Wardowski et al. (12).

Results and Discussion

Neither Pb nor As could be detected in the juice sac preparations in our experiments. Since the theoretical detection limits using ICP-AES are 0.08 ppm and 0.04 ppm for As and Pb, respectively (13), these elements do not appear to accumulate in the juice.

Leaves exhibited appreciable levels of both As and Pb throughout the experiment. 'Marsh' leaves showed As levels averaging approximately 9 ppm within the first 2 weeks after spray application and levels remained nearly constant for the remainder of the experiment. (Fig. 1A). Lead residue accumulated to a greater extent than As, and levels averaged approximately 16 ppm over the experi-

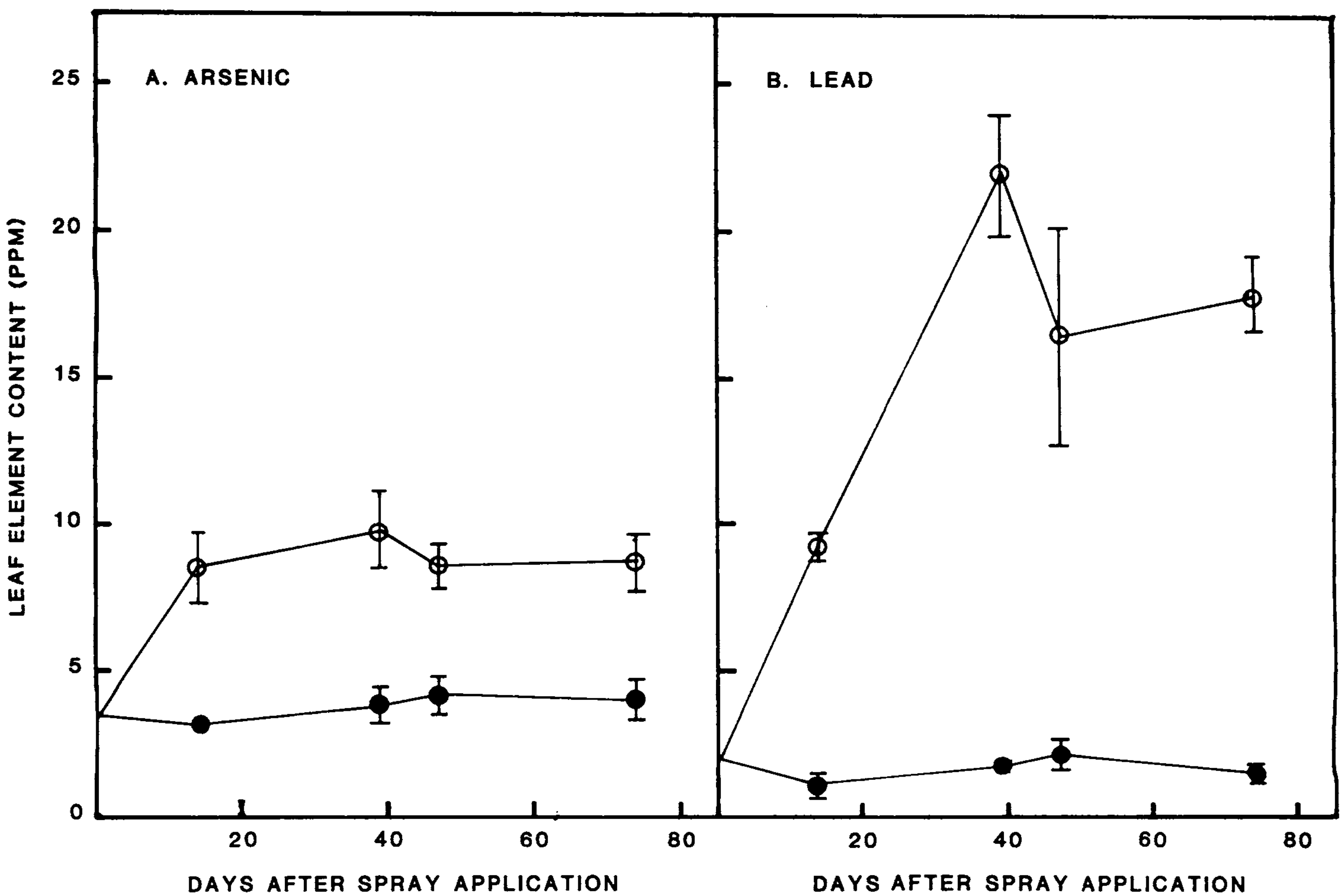

Fig. 1. A. Arsenic content in leaves of 'Marsh' grapefruit trees as a result of lead arsenate (LA) spray application. (●—●), control; (o—o), 1400 ppm LA spray. B. Lead content. (●—●), control: (o—o), 1400 ppm LA spray. Each point represents the +/– SD of 3 samples.

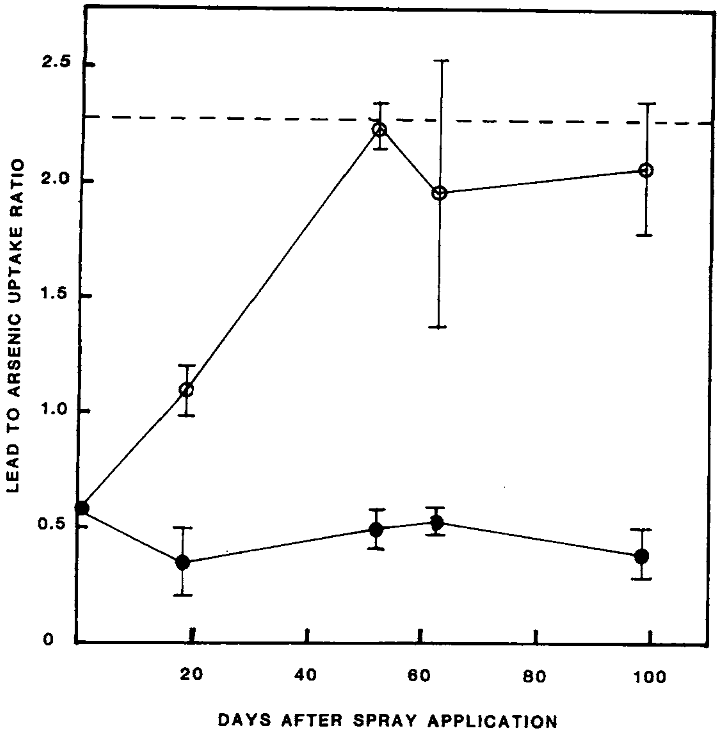

Fig. 2. Ratio of lead to arsenic content of leaves of 'Marsh' grapefruit trees following lead arsenate (LA) spray application. (●—●), control; (o—o), 1400 ppm LA spray. Each point represents the mean +/– SD of 3 samples. Dashed line represents the lead to arsenic ratio in the initial spray solution.

ment (Fig. 1B). However, in terms of the lead to arsenic (Pb:As) ratio (2.26 in the spray solution and leaf samples obtained immediately after spray application), the results indicate that Pb deposition is initially retarded (Fig. 2). In leaf samples taken 2 weeks after spray application, the ratio was approximately 0.93. This situation appears to be overcome with time, as leaves sampled later in the experiment exhibited by Pb:As ratios approaching that initially calculated (Fig. 2).

Similar results were obtained from the 1400 ppm LA spray experiment involving 'Duncan' grapefruit. Leaves accumulated As to levels averaging approximately 11 ppm, while Pb levels averaged 25 ppm over the experiment (Fig. 3). According to the Pb:As ratio determined for this experiment (2.62), Pb deposition was again initially retarded but increased the experiment progressed (Fig. 4).

In conjunction with the 'Duncan' 1400 ppm spray experiment, a low concentration, low pH LA treatment (700 ppm, pH 4.5) was monitored to compare element deposition characteristics and effectiveness of acidity reduction to the neutral pH spray. Pb and As levels for the 700 ppm experiment were reduced compared to 1400 ppm spray residue levels (Fig. 3), although a direct comparison of uptake cannot be made since this study did not include either a 1400 ppm low pH or a 700 ppm, pH 7.0 treatment. Interestingly, the discrimination against Pb deposition appeared to be enhanced as a result of the low pH spray, and Pb:As ratios increased less rapidly over the experiment compared to the 1400 ppm neutral pH treatment (Fig. 4).

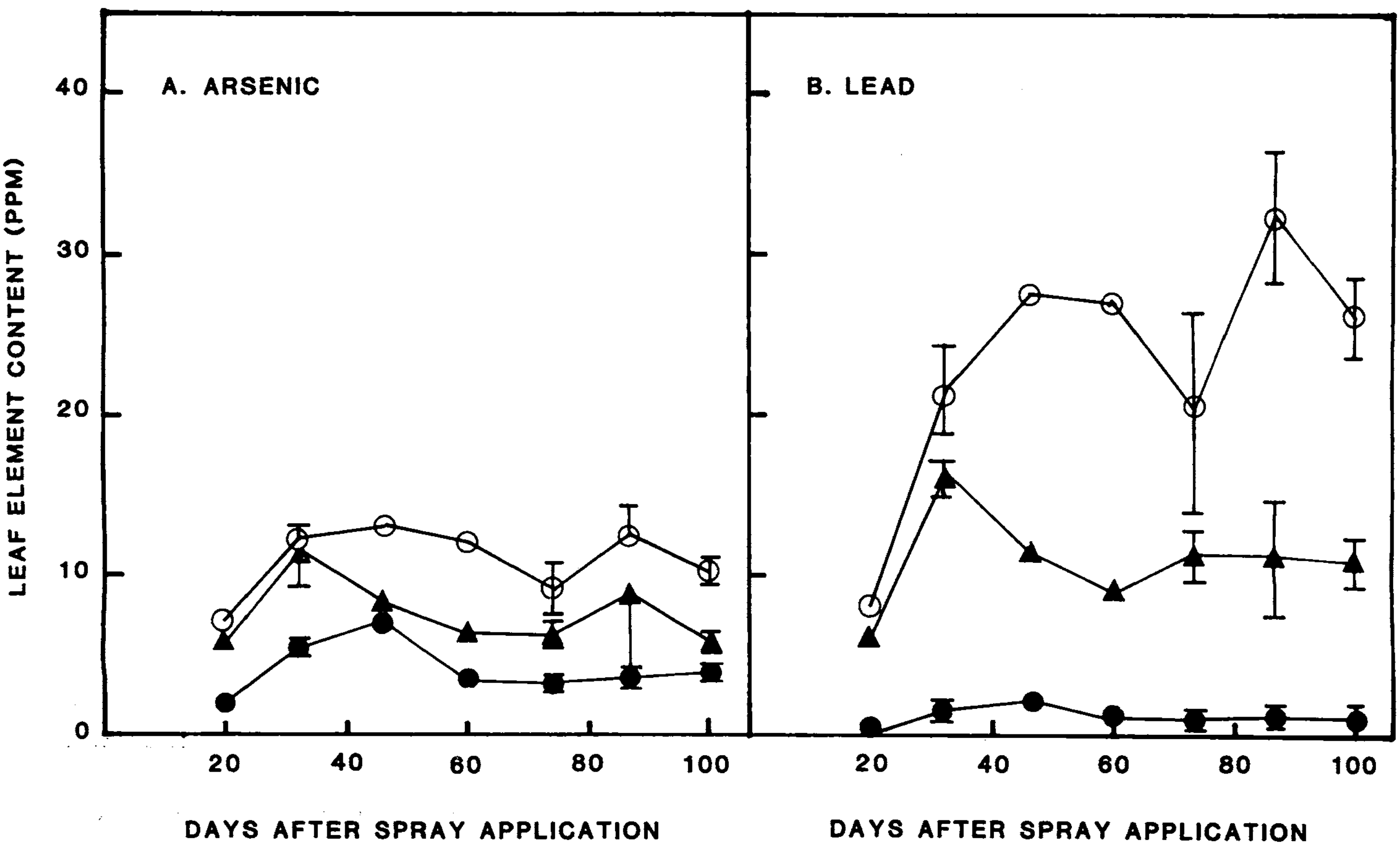

Fig. 3. A. Arsenic content of leaves of 'Duncan' grapefruit trees as a result of lead arsenate (LA) spray application. (●—●), control; (▲—▲), 700 ppm high acid LA spray; (o—o), 1400 ppm neutral pH LA spray. B. Lead content. (●—●), control; (▲—▲), 700 ppm high acid LA spray; (o—o), 1400 ppm neutral pH LA spray. Points without vertical bars are from single sample determinations.

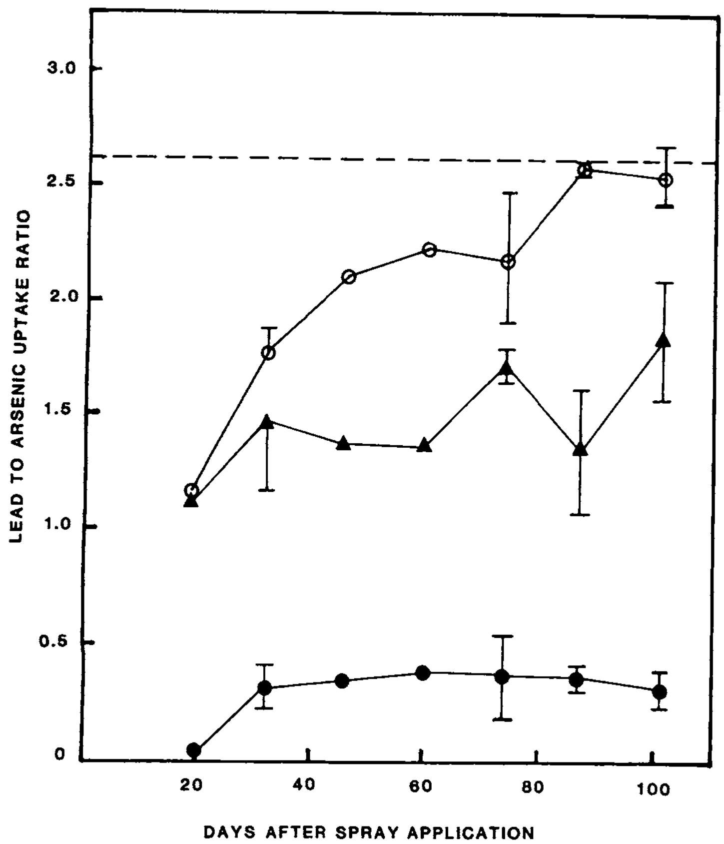

Fig. 4. Ratio of lead to arsenic content of leaves of 'Duncan' grapefruit trees following lead arsenate (LA) spray application. (●— ●), control; (▲—▲), 700 ppm high acid LA spray; (o—o), 1400 ppm neutral pH LA spray. Points with vertical bars represent the mean +/– SD of 3 samples. Points without vertical bars are from single sample determinations. Dashed line represents the lead to arsenic ratio in the initial spray solution.

It should be noted that leaves from unsprayed trees also showed detectable Pb and As levels (Fig. 1 and 3). It is not known at this time whether these levels reflect background levels resulting from spray applications in previous seasons, or cross contamination during the current spray application procedure. However, similar residue background levels were observed in leaf tissues analyzed from both experiments, which were initiated in successive years at locations over 100 miles apart. Furthermore, control trees were separated from sprayed trees by several buffer rows and spray applications were made on clear, windless days. Finally, Pb and As were not present in leaves from 'Valencia' orange trees, a variety to which LA sprays are not applied in Florida (S. Nikdel, unpublished data). Thus, it is more likely that element contents of the control leaves were the result of spray applications in previous seasons.

In spite of detectable levels of the elements in the control leaves, the additional levels in the sprayed trees were sufficient to elicit the desired response as evidenced by the juice acidity determinations made during these experiments. The pattern generally observed was reduced acid accumulation in the juice of developing fruit from sprayed trees as compared to fruit from control trees (Fig. 5 and 6). The low pH, LA low concentration was as effective in reducing acid accumulation in the juice of 'Duncan' fruit as the neutral pH, 1400 ppm LA spray (Fig. 6). As further evidence that accumulation rates were affected by the lead arsenate spray, regression lines were determined for percent acid content versus fruit weight, in grams, for the individual trees in the 'Marsh' experiment (Table 1). There was high linear correlation between acid content and fruit weight for the developing fruit from both control and lead arsenate treated trees. However, rates of acid accumulation, given by the slope of the regression lines, were greater for fruit from control trees than sprayed trees. Similar results were observed with the 'Duncan' experiment (data not shown). These results indicate a low level, continual response which is initiated soon after spray application.

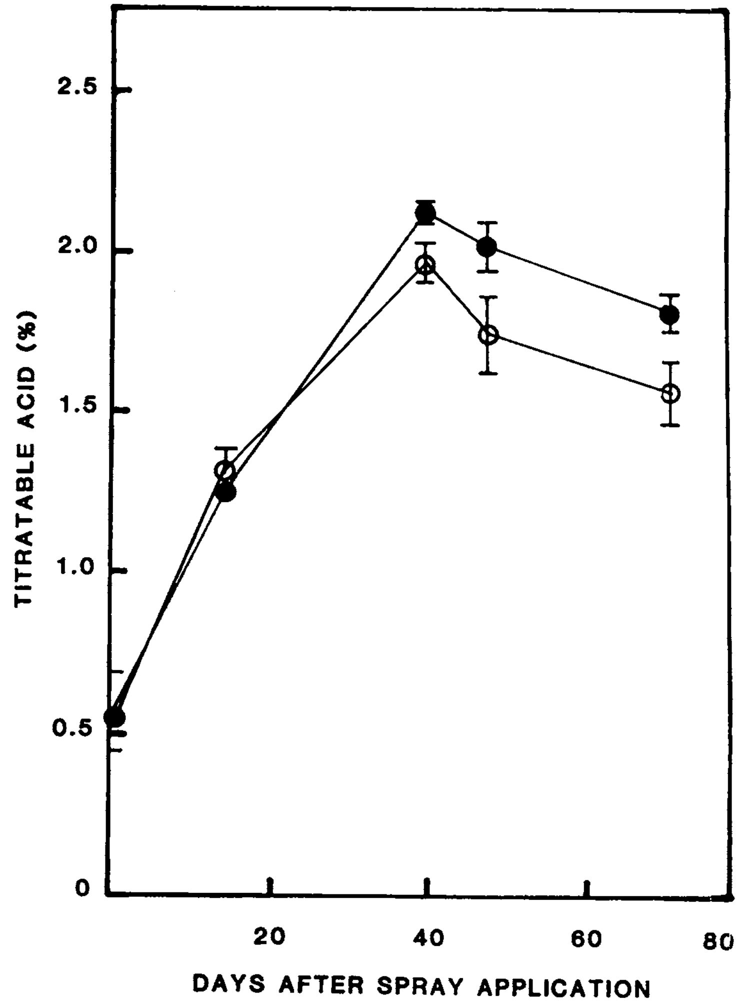

Fig. 5. Titratable acid levels in the juice of developing 'Marsh' grapefruit after lead arsenate (LA) spray application. (●—●), control; (o—o), 1400 ppm LA spray. Points represent the mean +/– SD of 3 samples.

In this study, whether or not Pb and As were actually internalized by the leaves following spray application and

Table 1. Regression of percent acid content on weight of developing 'Marsh' grapefruit from unsprayed trees and trees sprayed with 1400 ppm LA.

Treatment		Regression equations	Correlation coefficient (r)	Overall treatment slopes
Unsprayed[z]	1	y = –0.28 + 0.016x	0.998	0.0168
	2	y = –0.48 + 0.018x	1.000	
	3	y = –0.39 + 0.017x	0.981	
Sprayed	1	y = –0.08 + 0.013x	0.959	0.0129
	2	y = –0.16 + 0.014x	0.922	
	3	y = –0.01 + 0.012x	0.977	

[z]Treatments were significantly different at the 1% level using the F test.

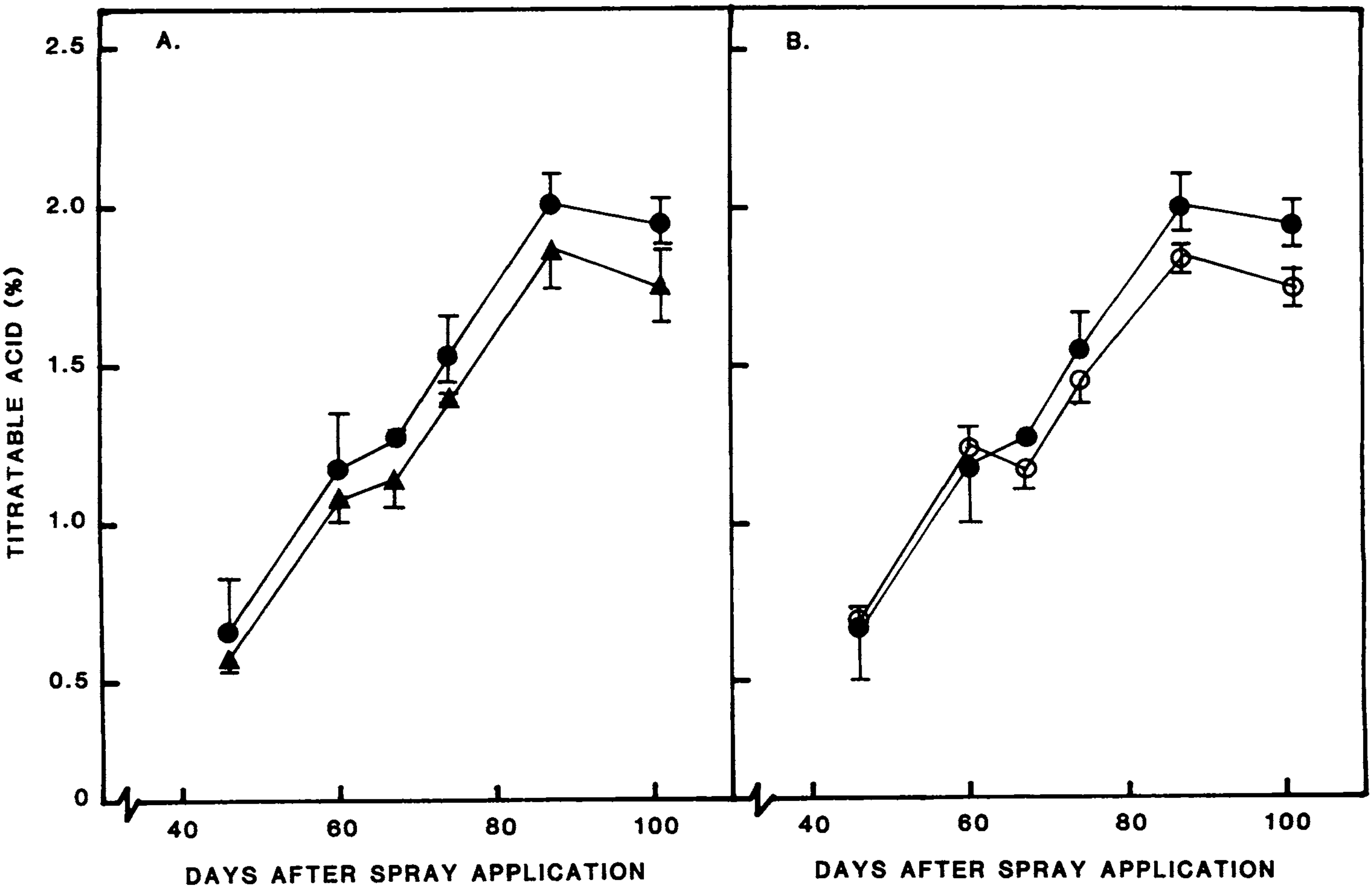

Fig. 6. Titratable acid levels in the juice of developing 'Duncan' grapefruit after lead arsenate (LA) spray application. A. (●—●), control; (▲—▲), 700 ppm high acid LA spray. B. (●—●), control; (o—o), 1400 ppm neutral pH LA spray. Points represent the +/– SD of 3 samples.

the relative contribution of each element towards acidity reduction following spray application were not determined. However, lack of residue accumulation in the juice, and results from previous studies showing that citrus peel accumulates negligible quantities of residue following spray application (8) indicate that the primary effect of the sprays may be elicited in the leaves. Secondly, the use of nonlead arsenates have been observed to elicit similar effects to lead arsenate (3, 6), indicating that As is the major contributor to acidity reduction. However, if subsequent investigations show Pb to become internalized following spray applications, the physiological effects of this element may also need to be considered.

Conclusion

The application of LA maturity sprays affected the rate of acid accumulation in the juice of developing grapefruit. Acidification of the maturity spray allowed as effective juice acidity reduction as the neutral pH spray of higher LA concentration. Arsenic and Pb did not accumulate in the juice of developing fruit as the result of LA spray application. However, high levels of As and Pb associated with the leaves of sprayed trees indicates that the effects of LA maturity sprays may be initiated in the leaves. In this case, further investigation concerning the possible physiological effects of LA on citrus leaves is warranted.

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