Nitrogen Fertilization Strategies for the 'Hass' Avocado that Increase Total Yield Without Reducing Fruit Size

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SUMMARY. Effects of nitrogen (N) fertilizer application times and rates on 'Hass' avocado (Persea americana) yield and fruit size were determined to resolve whether a single dose of soil-applied N [1x N (25 lb/acre)] at each of the five key stages of tree phenology (January, April, July, August, and November) (control) was as efficacious as soil-applied 2x N (50 lb/acre) at one or two key stages or soil- or foliar-applied 3x N (75 lb/acre) at only one stage. All trees received soil-applied N at 125 lb/acre as ammonium nitrate (NH_4NO_3) annually; trees receiving 2x or 3x N received the remaining N divided evenly at the same phenological stages (months) as trees receiving five 1x N applications. The importance of supplying N during the summer, when June drop, exponential fruit growth, vegetative shoot growth, and floral initiation occur, was determined by testing soil-applied 0.8x N in July plus August only (40 lb/acre N as NH4NO3 annually). Application time proved an important determinant of total yield. Yield of commercially valuable size (CVS) fruit was correlated with total yield (r = 0.80, P < 0.0001). Four-year cumulative total yields were equal for trees receiving soil-applied 1x N at five key phenological stages and trees receiving soil-applied 2x N in April and 18.75 lb/acre N at the four other stages (months). However, trees receiving soil-applied 2x N in April plus November and only 8.3 lb/acre N in the three other months, in particular July and August, had significantly lower 4-year cumulative total yields (P = 0.0362). Additional evidence of the importance of meeting avocado tree N demand in the summer is that trees receiving only 40 lb/acre N split in July plus August produced 4-year cumulative total yields equal to trees receiving 25 lb/acre N at the five key phenological stages; lower annual N would reduce fertilizer expense and protect the environment.

A renewed interest in protecting the environment combined with the increasing cost of N fertilizer has resulted in the need for 'Hass' avocado growers worldwide to improve their N fertilization practices to increase yield per hectare, including yield of CVS fruit, to increase net income and sustain this commoditybased industry (Atucha et al., 2013; Lovatt, 2013; Morales-Payan and Candelas, 2013; Sukamto et al., 2014). Despite problems of low yield,

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small fruit size, and alternate bearing, 'Hass' avocado dominates the global avocado industry (Garner et al., 2011). Management of N is complex because both avocado tree nutritional status and orchard soil fertility vary greatly among local avocado-growing areas (Batjes, 2014; Sotelo-Nava et al., 2013a, 2013b). It is further complicated by the fact that in California multiple N fertilizer experiments have repeatedly demonstrated that 'Hass' avocado yield and fruit size are not related to leaf N concentration (Arpaia et al., 1996; Embleton and Jones, 1972; Embleton et al., 1968; Lovatt, 2001; Lovatt and Witney, 2001; Yates et al., 1993). One possibility is that N applications in spring or summer negatively impact avocado yield by stimulating competition between developing vegetative and floral shoots or between exponentially growing fruit and vegetative shoot growth, respectively (Adato, 1990; Huett, 1996; Kalmar and Lahav, 1976; Lovatt, 2001; Whiley et al., 1996; Zilkah et al., 1987). To avoid competition, it was recommended that growers apply their total annual soil N in one application between January and March or two applications in January to March and June or July (Bekey, 1989). When N was supplied through the irrigation, it was suggested that the total annual N be applied in small amounts at the beginning of each month or every other month from March through October (Bekey, 1989). In contrast, the principle guiding the development of fertilizer best management practices to maximize yield and protect the environment is to properly time the application and amount of soilapplied fertilizer to meet the nutrient demand of the crop. This practice increases nutrient-uptake efficiency and reduces the potential for nutrient runoff and leaching (Alva et al., 2006).

In light of the proposed periods of competition for nutrients between vegetative and reproductive development in avocado tree phenology, it would seem logical to supply sufficiently high amounts of N to meet the demands of the competing growth processes so that floral shoot development,

Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
1	%	g/100 g	1
0.3048	ft	m	3.2808
9.3540	gal/acre	L·ha ^{−1}	0.1069
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
0.4536	lb	kg	2.2046
1.1209	lb/acre	kg∙ha ⁻¹	0.8922
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
1	ppm	$\mu g \cdot g^{-1}$	1
6.8948	psi	kPa	0.1450
(°F - 32)/1.8	۰F	°C	$(^{\circ}C \times 1.8) + 32$

fruit set, fruit growth, and vegetative shoot growth would not be compromised. Indeed, supplying a double dose of soil-applied N [56 kg·ha-1 (50.0 lb/acre)] to 'Hass' avocado trees in April or November significantly increased 4-year cumulative yield (kilograms fruit per tree) 30% and 39%, respectively, with more than 70% of the net increase in yield CVS fruit (178-325 g/fruit), compared with control trees receiving five applications of N at 28 kg·ha⁻¹ (25.0 lb/acre) every other month from April through October ($P \leq$ 0.01) (Lovatt, 2001). In addition, the double dose of N in April significantly reduced the severity of alternate bearing.

Supplying double doses of N to the soil raised the concern that the amount of N leaching past the root zone might be increased. The results of a study evaluating nutrient and irrigation management practices in an avocado orchard in Florida documented that doubling N fertilization rates increased the amount of N in the leachate (Kiggundu et al., 2012). Similarly, N fertilization rates were linearly correlated with N concentrations in the soil water and storm water runoff for California avocado orchards in Ventura County (Mangiafico et al., 2009). A potential solution would be to replace part of the soil-applied N with foliar-applied N, but the ability of avocado leaves to take up N, especially as urea, varies among avocado-growing countries (Nevin et al., 1990; Zilkah et al., 1987). However, other organs can be targeted. A single foliar application of low-biuret urea [28 kg·ha⁻¹ (46% N, $\leq 0.25\%$ biuret)] at the cauliflower

stage of inflorescence development (March in California) increased 3-year cumulative net yield by 55 kg/tree compared with trees receiving soilapplied N (Jaganath and Lovatt, 1995).

Due to the possibility that double doses of soil-applied N might increase the potential for nitrate pollution of groundwater, the objective of the research reported herein was to determine whether it was necessary to supply double or triple doses of N to the soil at key stages of 'Hass' avocado tree phenology or whether single doses of N would be sufficient if each dose was supplied at a key stage in the phenology of the tree and not simply every other month. The efficacy of supplying additional N through the foliage at key stages tree phenology was also investigated. In this experiment, all trees received the same amount of annual total N (125 lb/acre), so trees receiving double or triple doses of N received their remaining N divided equally at the same phenological stages (months) the control trees were fertilized. This is in contrast to the prior research of Lovatt (2001), in which trees receiving a double dose of N received 28 $kg \cdot ha^{-1}$ more N than the control trees, which received 140 kg·ha⁻¹ (125.0 lb/acre) annual N. In addition, in the research reported herein, a treatment was included to test the importance of meeting tree N demand during the summer, when June drop, exponential fruit growth, vegetative shoot growth, and transition from vegetative to floral development for next spring's bloom, occur concurrently (Garner and Lovatt, 2008; Lovatt, 2011; Salazar-García et al.,

1998). The treatment provided 40 lb/acre (44.8 kg·ha⁻¹) annual total N to the soil only in July plus August, the major period of N uptake by 'Hass' avocado fruit (Rosecrance et al., 2012). As part of the current research, the effect of fertilization strategy on the amount of N leaching past the root zone as nitrate-N (NO₃⁻-N) and ammonium-N (NH4+-N) was determined. The goal of the research was to develop a best management practice for N fertilization (N-BMP) of 'Hass' avocado that maximized production, including the yield of CVS fruit (178-325 g/fruit), and reduced the potential for nitrate pollution of groundwater.

Material and methods

PLANT MATERIAL AND N FERTILIZER TREATMENTS. The research was conducted in a commercial orchard of 17-year-old 'Hass' avocado trees on 'Duke 7' clonal rootstock located in Somis, CA (lat. 34°15'N, long. 118°59'W, elevation 93 m). The orchard soil was Salinas day loam, with 35.7% sand, 30.8% silt, 34.5% clay, and a soil depth greater than 200 cm (U.S. Department of Agriculture, 2013). The N treatments were initiated 1.5 years before year 1 of the experiment. All trees received soil-applied NH₄NO₃ at 125 lb/acre per year, with the single exception noted below. In treatment 1, trees received soilapplied 1x N (25 lb/acre) at five stages of tree phenology considered important to crop production based on the results of prior research (mid-month in January, April, July, August, and November) (Table 1). These application times corresponded to the

Table 1. Annual nitrogen (N) fertilizer application times and rates for 'Hass' avocado trees for the four crop years of the research (one crop year is 16 months).

			N application (lb/acre)						
No.	N Treatment ^z		January	April	July	August	November	Annual total	
1	lx N January, April, July, August + November (control)	Soil	25	25	25	25	25	125	
2	2x N April	Soil	18.75	50	18.75	18.75	18.75	125	
3	2x N April + November	Soil	8.3	50	8.3	8.3	50	125	
4	2x N August	Soil	18.75	18.75	18.75	50	18.75	125	
5	2x N November	Soil	18.75	18.75	18.75	18.75	50	125	
6	3x N April	Soil	12.5	75	12.5	12.5	12.5	125	
7	3x N April	Foliar	12.5	75	12.5	12.5	12.5	125	
8	0.8x N July + August	Soil	—		20	20		40	

 z Soil = soil-applied N was ammonium nitrate (NH₄NO₃). Foliar = foliar-applied N was low-biuret urea (granules, 46% N, $\leq 0.25\%$ biuret) at 75 lb/acre in 200 gal/acre (1,870.8 L·ha⁻¹) of water (pH 5.5) applied with a 400-psi (2757.9 kPa) handgun sprayer; 1 lb/acre = 1.1209 kg·ha⁻¹.

following stages of tree phenology in the northern hemisphere: January: early bud swell, initiation of flower organ development (Salazar-García et al., 1998); April: anthesis, fruit set, and initiation of spring vegetative shoot growth, including the apical vegetative shoot of indeterminate floral shoots (Salazar-García et al., 1998); July: period of "June" drop for the current crop (Garner and Lovatt, 2008; Garner et al., 2011), initiation of exponential fruit growth and summer vegetative shoot growth (Lovatt, 2001); August: period of exponential fruit growth (Garner and Lovatt, 2008) and initiation of floral development (phase transition) for next spring's bloom (Salazar-García et al., 1998); and November: floral buds are committed to floral development (meristem determined) and end of fall vegetative shoot growth (Salazar-García et al., 1998). For additional reference, fruit size (diameter × length and weight) ranged as follows for each month of N fertilizer application for the setting crop: July— $10-25 \times 14-35$ mm, <10 g/fruit; August: 26–43 × 38–60 mm, 25–70 g/ fruit; November: $45-61 \times 65-84$ mm, 75–175 g/fruit; and January: $48-65 \times$ 69-90 mm, 80-180 g/fruit (Wang et al., 2016). Although anticipated to be a successful fertilization strategy, treatment 1 was designated the control. Additional treatments included 2x N (50 lb/acre) as NH₄NO₃ applied to the soil in (2) April, (3) April plus November, (4) August, and (5) November, (6) 3x N (75 lb/acre) as soilapplied NH_4NO_3 in April, or (7) 3x N as foliar-applied low-biuret urea (granules, 46% N, ≤0.25% biuret) [75 lb/acre in 200 gal/acre of water (5.5 final pH), sprayed with a 400-psi handgun sprayer] in April. Trees in treatments 1 to 7 received 125 lb/acre total annual N, with the remaining annual N for treatments 2 through 7 divided into equal amounts applied to the soil at the same stages of tree phenology (months) the control trees were fertilized. Treatment 8 was soil-applied 0.8x N (20 lb/acre) as NH_4NO_3 during July plus again in August (40 lb/acre total annual N) (Table 1). Treatments were initiated in January. Soil-applied fertilizer was distributed evenly along the intersection of the drip line of the tree and the wetting pattern of the sprinklers in an area ≈ 12 inches wide on each side of the tree. Fertilizer applications were made toward the end of the irrigation cycle so that the amount of water

applied was sufficient to move the fertilizer into the root zone but not significantly beyond it. The experiment was a randomized complete block design with 20 individual trees per treatment. The research was conducted for four crop years (four harvests); the length of time from full bloom to harvest (one crop year) was 16 months.

LEAF NUTRIENT ANALYSES. In September of each year, 20 sixmonth-old spring flush leaves from nonfruiting terminals were collected uniformly around each data tree at 4.5 ft above the ground. Leaves were washed with soapy water and rinsed thoroughly with distilled water, oven dried at 60 °C for 72 h, and ground in a Wiley mill to pass through a 40-mesh (0.025-inch) screen (Embleton et al., 1973). The ground samples were sent to Albion Laboratories (Clearfield, UT) for mineral nutrient analysis. For N, samples were combusted at 1050 °C and N was determined by thermal conductivity (Leco Corp., St. Joseph, MI). In addition, the concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), boron (B), copper (Cu), molybdenum (Mo), chloride (Cl), and sodium (Na) were determined after nitric acid-hydrogen peroxide microwave digestion by inductively coupled plasma atomic emission spectrometry (Meyer and Keliher, 1992).

YIELD ASSESSMENT. Fruit were harvested annually in August, 16 months after full bloom. The dry matter content of the fruit was greater than the required 20.8% (Dixon, 2013). Total vield was determined as kilograms per tree by removing and weighing all fruit produced by a tree. In addition, at harvest, a randomly selected sample of 100 to 150 fruit/tree, representing $\approx 30\%$ to 100% of the mean total number of fruit on a tree for each year of the experiment, was collected for each data tree and the fresh weight of each fruit in the subsample was determined as grams per fruit. These data were used to calculate pack-out, i.e., the kilograms of fruit of each packing carton size per tree and to estimate the total number fruit and number of fruit in each packing carton size category per tree. The following packing carton fruit sizes (grams per fruit) were used 84 (99 to 134 g), 70 (135 to 177 g), 60 (178 to 212 g), 48 (213 to 269 g), 40 (270 to 325 g), 36 (326 to 354 g), and 32 (355 to 397 g).

For fruit quality analysis, at each annual harvest, two fruit were selected randomly per tree and allowed to ripen to "eating soft" at 18 to 21 °C. When ripe, external (exocarp) and internal (mesocarp, edible portion) quality was evaluated for decay and discoloration. Vascularization (presence of vascular bundles and associated fibers) of the mesocarp was also determined. The above fruit quality parameters were rated visually on a scale from 0 (normal) to 4 (high incidence of decay, discoloration, or vascularization).

To determine treatment effects on the severity of alternate bearing, the alternate bearing index (ABI) was calculated for each data tree for each pair of consecutive harvests using the following equation: ABI = (year 1 yield – year 2 yield)/(year 1 yield + year 2 yield) in which yield is total kilograms of fruit per tree and the difference in yield between years 1 and 2 is expressed as an absolute value. An ABI of zero means no alternate bearing, whereas an ABI of one is complete alternate bearing (Pearce and Dobersek-Urbanc, 1967).

TEMPERATURE AND RAINFALL DATA. Monthly average maximum and minimum air temperatures and rainfall for the four crop years of the research were downloaded from the California Irrigation Management Information System website (California Department of Water Resources, 2009) for the closest station #152, Camarillo, CA (lat. 34°23'W, long. 118°99'N, elevation 76 m). Differences in average maximum or minimum temperatures and rainfall for the months corresponding to the key stages in 'Hass' avocado tree phenology were determined for each crop year (January preceding bloom to harvest in August the following year). The relationship of these climate factors to yield was also determined.

N LEACHING ASSESSMENT. Bags of anion-exchange resin Dowex 1-X8 (Sigma Life Sciences, St. Louis, MO) and cation-exchange resin Dowex 50-W-X8 (Sigma Life Sciences) were made of nylon cloth (24 cm²). Each bag contained 5.4 g resin as dry weight. The anion and cation resin bags were prepared in advance in three successive washes with 0.5 M sodium bicarbonate or 0.5 M hydrochloric acid, respectively, centrifuged in a salad spinner (Tokig), placed in individual zippered plastic bags to prevent contamination, and refrigerated at 4 °C until used. Just before the application of N to the soil, resin bags (one each of both anion- and cationexchange resin were placed at the lower end of separate polyvinyl chloride pipes, 2.5 inches i.d.) that were placed in the ground at a 45° angle with the soil surface and at a depth of 75 cm. The pipes were located at the intersection between the drip line of the tree and the wetting pattern of the sprinklers on both sides of each of 10 trees per treatment. The following treatments were sampled at each soil fertilizer application for 2 years: control; 2x N in April, August, or November; 3x N in April; and no N fertilization to determine N background levels. The bags were collected 2 d after N application and irrigation. New resin bags were used for each fertilizer application. Each resin bag was retrieved from its tube, placed in a labeled individual zippered plastic bag and taken immediately to the laboratory in a cool box. Collected resin bags were rinsed with deionized water to remove adhering soil and the excess water was removed by centrifugation in a salad spinner. Ions were removed from the resin by submerging intact bags in 100 mL 2.0 M potassium chloride overnight with shaking followed by filtration through Whatman no. 42 filter paper (Thermo Fisher Scientific, Waltham, MA). The sample filtrates were then sent to the University of California Agriculture and Natural Resources Analytical Laboratory (Davis, CA) and analyzed for NO₃⁻-N (Wendt, 1999) and NH₄⁺-N (Switala, 1999). The concentration of NO₃-N was determined, after reduction to nitrite via a Cu-cadmium column, by diazotization with sulfanilamide followed by coupling with N- (1naphthyl)-ethylenediamine dihydrochloride. The absorbance of the product was measured at 520 nm (QuickChem Method 10-107-04-1-A; Lachat Instruments, Milwaukee, WI). The NH4⁺-N samples were heated with salicylate and hypochlorite in an alkaline phosphate buffer in the presence of ethylenediaminetetraacetic acid to prevent precipitation of Ca and Mg; sodium nitroprusside was added to enhance the sensitivity of the assay. The absorbance of the reaction product was measured at 630 nm (QuikChem Method 10-107-06-1-A; Lachat Instruments). The sensitivity of each method was $\approx 0.05 \text{ mg} \cdot \text{L}^{-1} (\text{w/v})$ for NO₃⁻-N and 0.01 mg·L⁻¹ (w/v) for NH₄⁺-N and was reproducible within 7%. Sample filtrates analyzed for NH₄⁺-N were also analyzed for total N and carbon by combustion analysis to quantify the amount of organic matter trapped on the resin bags (Pella, 1990). These results were used to correct the NH₄⁺-N samples for this source of N, which did not originate from the fertilizer applications.

STATISTICAL ANALYSES. Repeated measure analysis was used to test for treatment effects on yield parameters, fruit quality, and leaf N concentrations with year as the repeated measure factor. Repeated measure analysis was also used to test for treatment effects on leachate concentrations of NO₃⁻-N and NH₄⁺-N with sample date as the repeated measure factor. This analysis was performed using the General Linear Models procedure of SAS (version 9.2; SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to test for treatment effects on leaf concentrations of N and other nutrients, year effects on climate parameters, amount of NO₃⁻-N or NH₄⁺-N leaching past the root zone in a specific year and as cumulative leachate, and on all yield and fruit quality parameters for a specific year and for cumulative yield. When ANOVA testing indicated significant differences, post hoc comparisons were run using Fisher's protected least significant difference procedure with a family error rate of $\alpha \leq 0.05$. Pearson correlation coefficients were calculated to determine the strength of the relationships between total yield and fruit size and leaf nutrient concentrations and climate factors. Note that for repeated measure analysis and cumulative yields, a missing datum point for a tree in any year excluded all data for that tree from the statistical analysis.

Results

EFFECTS OF N FERTILIZER TREATMENTS ON ANNUAL AND 4-YEAR CUMULATIVE YIELD. Fertilizer strategy had a significant effect on total yield as kilograms per tree in each year of the 4-year study, but in years 2 and 4 the significance was only at the 10% level (data not shown). In contrast, the fertilizer treatments significantly impacted total yields as number of fruit per tree only in years 1 and 3 (data not shown). Despite this, trees receiving

1x N at five key stages of tree phenology and trees receiving soil-applied 2x N in April or 0.8x N in July plus August produced significantly greater 4-year cumulative yields as kilograms per tree than trees receiving foliarapplied 3x N in April or soil-applied 2x N in April plus November, but not greater than trees receiving soilapplied 3x N in April or 2x N in August or November [P = 0.036](Table 2)]. As number of fruit per tree, trees receiving soil-applied 2x N in April had significantly greater 4-year cumulative yields compared with trees receiving soil-applied 2x N in April plus November, 2x N in November, and foliar-applied 3x N in April, but not greater than trees receiving 1x N at five key stages, trees receiving soil-applied 3x N in April, 0.8x N in July plus August or 2x N in August (P = 0.003). Trees receiving foliar-applied 3x N in April produced the lowest number of fruit per tree, which was significantly less than trees in all treatments, except soil-applied 2x N in April plus November and 2x N in November [P = 0.003 (Table 2)].Fertilization treatments did not influence yield of CVS fruit (packing carton sizes 60 + 48 + 40; 178–325 g/fruit) as kilograms or number of fruit per tree in any year of the research or as 4-year cumulative yield (Table 2). Fertilization treatment had a significant effect on the yield of small fruit (packing carton sizes 84 + 70; 99–177 g/fruit) in years 1 and 3 as both kilograms and number of fruit per tree (data not shown), which resulted in significant differences in the 4-year cumulative yield of small fruit as both kilograms and number per tree (Table 2). Trees receiving soil-applied 2x N in April produced significantly more small fruit as 4-year cumulative yield than trees receiving foliar-applied 3x N in April or soil-applied 2x N in April plus November, 0.8x N in July plus August, and 2x N in November as kilograms per tree (P = 0.0003) and number of fruit per tree (P = 0.0004), but not more small fruit than the control, trees receiving soil-applied 3x N in April or 2x N August (Table 2). As total yield increased, yield of CVS fruit and small fruit increased based on kilograms per tree (r = 0.80, P < 0.0001 and r = 0.63, P < 0.0001, respectively) and number of fruit per tree (r = 0.67, P < 0.0001 and r = 0.81, P < 0.0001, respectively).

RESEARCH REPORTS

Table 2. Effects of nitrogen (N) fertilizer application times and rates on 'Hass' avocado 4-year cumulative total yield and yield of commercially valuable size (CVS) fruit (178–325 g/fruit FW) and small fruit (99–177 g/fruit FW) as kilograms and number per tree.^z

			4-yr cumulative yield ^z						
			Total		CVS fruit		Small fruit		
No.	N Treatment ^y		(kg/tree)	(no./tree)	(kg/tree)	(no./tree)	(kg/tree)	(no./tree)	
1	lx N January, April, July, August + November (control)	Soil	243.2 a ^x	1,241 ab	158.0 a	695 a	77.7 ab	525 ab	
2	2x N April	Soil	249.9 a	1,338 a	144.2 a	646 a	99.8 a	675 a	
3	2x N April + November	Soil	208.0 b	1,062 bc	134.9 a	595 a	65.6 b	446 b	
4	2x N August	Soil	220.2 ab	1,163 ab	134.4 a	596 a	79.3 ab	548 ab	
5	2x N November	Soil	220.1 ab	1,094 bc	152.7 a	671 a	59.5 b	400 bc	
6	3x N April	Soil	235.2 ab	1,196 ab	151.2 a	666 a	74.5 ab	503 ab	
7	3x N April	Foliar	208.1 b	946 c	160.2 a	682 a	33.5 c	223 с	
8	0.8x N July + August	Soil	241.6 a	1,181 ab	173.6 a	764 a	58.6 bc	390 bc	
P valu	ie		0.0362	0.0026	0.1451	0.1465	0.0003	0.0004	

 $^{x}1$ g = 0.0353 oz, 1 kg = 2.2046 lb, 1 lb = 0.4536 kg; commercially valuable size fruit and small fruit correspond to packing carton sizes 60 + 48 + 40 and 84 + 70, respectively; packing carton size is based on the number of fruit per 25-lb box within a tolerance of 0.5 lb. ^yRefer to Table 1.

^xMeans in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$. See table 3 for accuracy.

Table 3. Effects of nitrogen (N) fertilizer application times and rates on 'Hass' avocado leaf N concentrations in year 4 and averaged across the four crop years of the research; one crop year is 16 months.

			Leaf N concn (g/10	0 g leaf tissue DW) ^y
No	. N Treatment ^z		Yr 4	4-yr avg
1	1x N January, April, July,	Soil	2.37 abc ^x	2.56 ab
	August + November (cont	rol)		
2	2x N April	Soil	2.32 bc	2.55 ab
3	2x N April + November	Soil	2.33 bc	2.53 bc
4	2x N August	Soil	2.31 bc	2.57 ab
5	2x N November	Soil	2.37 abc	2.58 ab
6	3x N April	Soil	2.45 a	2.62 a
7	3x N April	Foliar	2.38 ab	2.59 ab
8	0.8x N July + August	Soil	2.28 c	2.46 c
P va	alue		0.0270	0.0284

^zRefer to Table 1.

^y1 g/100 g = 1%. ^xMeans in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$.

EFFECT OF N FERTILIZER TREATMENTS ON TREE NUTRIENT status. Nitrogen fertilization strategy had no effect on leaf N concentration until year 4 of the research. By year 4, trees receiving soil-applied 3x N in April accumulated leaf N concentrations that were significantly greater than trees receiving soil-applied 2x N in April, 2x N in April plus November, 0.8x N in July plus August, and 2x N in August (P = 0.027), but control trees and trees receiving foliar-applied 3x N in April or soilapplied 2x N in November had leaf N concentrations that were intermediate and not significantly different (Table 3). Trees receiving only 0.8x N in July plus August had the lowest leaf N concentration but it was not significantly lower than that of the

control trees or trees receiving soilapplied 2x N in April, 2x N in April and November, and 2x N in August or 2x N in November. Repeated measure analysis documented an annual decrease in leaf N values from year 1 to year 4 (P < 0.0001) (data not shown). Trees receiving soilapplied 3x N in April had the greatest 4-year average leaf N concentration, which was significantly greater than trees receiving soil-applied 2x N in April plus November and 0.8x N in July plus August, but not greater than other treatments [P = 0.028 (Table 3)]. Trees receiving soil-applied 0.8x N in July plus August had the lowest 4-year average leaf N concentration, but not lower than trees treated with soilapplied 2x N in April and November (Table 3). All trees in all treatments in

all years had leaf N concentrations that were greater than the 2.2% recommended by the California Avocado Commission (Lovatt and Witney, 2001). There was a weak but significant relationship between leaf N concentration and total yield as kilograms and number of fruit per tree (r = 0.29, P < 0.0001 and r = 0.22, P < 0.0007, respectively) and yield of CVS fruit as kilograms and number of fruit per tree (for both r = 0.38, P < 0.0001). Interestingly, yield of small fruit was not related to leaf N concentration as kilograms or number of fruit per tree.

Leaf P, K, Ca, Mg, S, Fe, Zn, Mn, B, and Cu concentrations were all within the ranges considered optimal for avocado in California (data not shown) (Jones and Embleton, 1978). There is no recommendation for Mo, but leaf concentrations of Mo were not significantly different across treatments and years. Both Cl and Na were below the levels considered toxic to avocado (Jones and Embleton, 1978). Nitrogen fertilization strategy had a significant effect only on leaf concentrations of S, Mn, and Fe and only in the first 2 years of the research. In year 1, trees receiving 0.8x N in July plus August had a significantly greater leaf S concentration than trees in all other treatments (P = 0.05). In contrast, trees receiving 0.8x N in July plus August or foliar-applied 3x N in April had equal leaf Mn concentrations that were significantly lower than trees in all other treatments (P <0.0001). In year 2, there continued to be an N fertilizer effect on leaf S (P =(0.002) and Mn (P < 0.0001), with an

Table 4. Effects of nitrogen (N) fertilizer application times and rates on the alternate bearing index (ABI) of 'Hass' avocado trees for the four harvests in the research.

			ABI ^y				
No.	N treatment ^z		Yrs 1 and 2	Yrs 2 and 3	Yrs 3 and 4	4-yr avg	
1	1x N January,	Soil	0.47 abc ^x	0.62 ab	0.46 a	0.52 abc	
	April, July,						
	August + November						
	(control)						
2	2x N April	Soil	0.60 a	0.67 a	0.55 a	0.61 a	
3	2x N April +	Soil	0.47 abc	0.43 c	0.40 a	0.43 bc	
	November						
4	2x N August	Soil	0.50 abc	0.46 bc	0.46 a	0.47 bc	
5	2x N November	Soil	0.54 ab	0.47 bc	0.59 a	0.53 ab	
6	3x N April	Soil	0.42 bc	0.41 c	0.36 a	0.40 c	
7	3x N April	Foliar	0.33 c	0.43 c	0.47 a	0.41 bc	
8	0.8x N July + August	Soil	0.52 ab	0.50 abc	0.33 a	0.45 bc	
P val	ue		0.0868	0.0492	0.1251	0.0387	

^zRefer to Table 1.

^yABI = (year 1 yield – year 2 yield)/(year 1 yield + year 2 yield) in which yield is total kilograms of fruit per tree and the difference in yield between years 1 and 2 is expressed as an absolute value; an ABI of zero means no alternate bearing, an ABI of one means complete alternate bearing.

^xMeans in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$.

additional effect on leaf Fe (P = 0.01) concentration. Trees treated with soil-applied 2x N in April plus November had a leaf Fe concentration greater than trees receiving soilapplied 2x N in April, 2x N in August, and 0.8x N in July plus August, but control trees, trees receiving soilapplied 2x N in November, and soilor foliar-applied 3x N in April had intermediate leaf Fe concentrations that were not significantly different from all other treatments. Despite these N fertilizer effects, leaf concentrations of S, Mn, and Fe were always with the optimum range for avocado and there was no significant relationship between the leaf concentrations of S, Mn, or Fe and total yield or yield of CVS fruit or small fruit in years 1 and 2, respectively.

EFFECTS OF CLIMATE AND N FERTILIZER TREATMENTS ON ALTERNATE BEARING AND 4-YEAR AVERAGE YIELD. There were no significant differences in average maximum or minimum temperatures during key stages of 'Hass' avocado tree phenology during the four crop years of the research. Rainfall was significantly greater from prebloom in January to the end of bloom (part of fruit set) in May in year 2 than in years 1 and 3, but only by 2.2 inches over the 5 months, and thus, likely of little consequence in the irrigated orchard; year 4 rainfall during this period was intermediate and not significantly

from the other years (P = 0.259). Annual average rainfall during key stages of avocado tree phenology did not correlate with annual yield parameters. Thus, the ABI for the orchard was unaffected by these climatic factors during the four crop years of the research. The ABI for the orchard was moderate, averaging 0.48, 0.50, and 0.45 across treatments for years 1 to 2, 2 to 3, and 3 to 4, respectively (Table 4). In contrast, N fertilizer strategies tested in this experiment significantly influenced the ABI for crop years 2 to 3 (Table 4). Although the relative effects of each N strategy on ABI varied from year to year, there was a significant effect of N treatment on 4-year average ABI (P = 0.039). Trees receiving soil-applied 2x N in April had the highest 4-year average ABI, which was significantly greater than trees in all treatments, except the control and trees receiving 2x N in November (P = 0.039).

Despite the facts that the orchard was alternate bearing, with significant differences in yield from year to year, and that N fertilizer treatments affected the degree of alternate bearing in the orchard, it is noteworthy that N fertilization treatment had a significant effect on total yield as both kilograms and number of fruit per tree when averaged across the 4 years of the experiment. Results of the repeated measure analysis identified N treatments that had a positive effect

on total yield annually, indicating that significant increases in cumulative vield were not solely the result of the on-crop years due to alternate bearing (Table 5). Control trees and trees receiving soil-applied 2x N in April produced greater 4-year average yields as kilograms per tree than trees receiving foliar-applied 3x N in April and soil-applied 2x N in April plus November (P = 0.031), with trees in all other treatments producing intermediate yields that were not significantly different. Only trees receiving soil-applied 2x N in April produced 4-year average yields as number of fruit per tree that were significantly greater than trees receiving foliar 3x N in April or soil-applied 2x N in April plus November, and 2x N in November (P = 0.002). Trees receiving foliar 3x N in April produced the lowest 4-year average number of fruit per tree, which was significantly lower than that of the other treatments except soil-applied 2x N in April plus November and 2x N in November. Year was a significant factor influencing total yield as both kilograms and number of fruit per tree (P < 0.0001) and there was a significant treatment and year interaction influencing total yield as kilograms per tree (P = 0.032) and number of fruit per tree (P =0.036). Year was the only factor affecting the 4-year average yield of CVS fruit as kilograms (P < 0.0001) and number of fruit per tree [P <0.0001 (Table 5)]. In contrast, there was a significant effect due to N fertilization strategy on the 4-year average yield of small fruit as both kilograms (P = 0.0002) and number (P = 0.0003) per tree (Table 5). In both cases, trees receiving soil-applied 2x N in April produced more small fruit as both kilograms and number per tree than trees receiving soilapplied 2x N in April plus November, 0.8x N in July plus August, 2x N in November, and foliar-applied 3x N in April, but not more than the control trees, trees receiving soil-applied 3x N in April or 2x N in August (Table 5). Although trees receiving foliarapplied 3x N in April produced the least small fruit, it was not less than trees treated with soil-applied 0.8x N in July plus August or 2x N in November. Year strongly influenced the yield of small fruit as both kilograms (P < 0.0001) and number of fruit per tree (P < 0.0001) and there was

Table 5. Effects of nitrogen (N) fertilizer application times and rates on 'Hass' avocado 4-year average total yield and yield of commercially valuable size (CVS) fruit (178–325 g/fruit FW) and small fruit (99–177 g/fruit FW) as kilograms and number per tree.^z

			4-yr avg yield ^z							
			Total		CVS fruit		Small fruit			
No.	N treatment ^y		(kg/tree)	(no./tree)	(kg/tree)	(no./tree)	(kg/tree)	(no./tree)		
1	lx N January, April, July, August + November (control)	Soil	61.4 a ^x	318 ab	38.8 a	171 a	20.8 ab	142 ab		
2	2x N April	Soil	62.5 a	335 a	36.0 a	162 a	25.0 a	169 a		
3	2x N April + November	Soil	52.0 b	266 bc	33.7 a	149 a	16.4 b	112 b		
4	2x N August	Soil	55.7 ab	297 ab	33.7 a	150 a	20.6 ab	143 ab		
5	2x N November	Soil	55.0 ab	274 bc	38.2 a	168 a	14.9 bc	100 bc		
6	3x N April	Soil	58.8 ab	299 ab	37.8 a	167 a	18.6 ab	126 ab		
7	3x N April	Foliar	52.0 b	236 с	40.0 a	171 a	8.4 c	56 c		
8	0.8x N July + August	Soil	60.4 ab	295 ab	43.4 a	191 a	14.7 bc	98 bc		
	Year									
	1		84.5 a	432 a	61.2 a	280 a	22.8 b	151 b		
	2		42.6 c	161 d	35.7 b	139 b	0.7 d	5 d		
	3		52.7 b	321 b	20.4 c	99 с	32.3 a	221 a		
	4		49.0 bc	244 c	33.6 b	145 b	13.6 c	94 c		
	<i>P</i> value									
	Treatment (T)		0.0305	0.0016	0.1711	0.1824	0.0002	0.0003		
	Year (Y)		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
	$T \times Y$		0.0323	0.0356	0.2113	0.1854	0.0291	0.0366		

 $^{z}1$ g = 0.0353 oz, 1 kg = 2.2046 lb, 1 lb = 0.4536 kg; commercially valuable size fruit and small fruit correspond to packing carton sizes 60 + 48 + 40 and 84 + 70, respectively; packing carton size is based on the number of fruit per 25-lb box within a tolerance of ±0.5 lb. ^yRefer to Table 1.

*Means in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$.

a strong interaction between N fertilization treatment and year that impacted the 4-year average yield of small fruit as kilograms (P = 0.029) and number of fruit (P = 0.037) per tree (Table 5).

EFFECT OF N FERTILIZER TREATMENTS ON FRUIT QUALITY. Vascularization, the presence of vascular bundles and associated fibers in the mesocarp, was the only fruit quality parameter affected by N fertilization treatment when averaged across the 4 years of the research (Table 6). The mesocarp of fruit from control trees had a significantly greater incidence of vascularization than fruit from trees in all other treatments except soil-applied 2x N in November, which had a level of vascularization equal to that of fruit from all other treatments except foliar-applied 3x N in April (P = 0.007). The annual incidence of vascularization, discoloration, and decay were low, but significantly influenced by year (P < 0.0001). It is unknown whether the effect of year was related to alternate bearing and crop load or due to annual differences in climate. There were no significant treatment and year interactions that affected any fruit quality parameter for the 4 years of the research.

EFFECT OF N FERTILIZER TREATMENTS ON N LEACHING PAST THE ROOT ZONE. There were no significant effects of N fertilization strategy on the amount of N leaching past the root zone quantified as NO₃⁻-N or NH4+-N for any sample date in years 1 or 2 of the research or as the cumulative amount for year 1 or 2 (data not shown). There were no N treatment effects on the amount of NO_3^{-} -N or NH_4^{+} -N leaching past the root zone averaged across the first 2 years of the research (Table 7). However, sample date had a significant effect on the amount of NH4+-N leaching past the root zone (P <0.0001).

Discussion

Nitrogen fertilization treatments were tested with the objectives of increasing total yield of the 'Hass' avocado without reducing fruit size or increasing the potential for groundwater contamination by NO_3^--N or NH_4^+-N . The results of this research provide strong evidence that soil N application time is an important factor influencing 'Hass' avocado yield. There were no significant differences in 4-year cumulative or 4-year average total yields or yields of CVS fruit (as kilograms per tree) for trees receiving a single dose of N at the five key stages of tree phenology described in the methods (control) vs. trees receiving a double dose of N (50 lb/acre) at one key stage of tree phenology and only 18.75 lb/acre N instead of 25 lb/acre N at the four other phenological stages. On the basis of this, the five 1x N soil applications of N would be the preferred fertilization practice compared with providing 2x N in a single soil application due to the possible increased potential for nitrate pollution of the groundwater. However, control trees did not have significantly lower amounts of NO3--N or NH4+-N leaching past the root zone. In contrast, 4-year cumulative and 4-year average total yields were significantly lower than the control and trees receiving soilapplied 2x N in April when trees received foliar-applied 3x N in April, but not soil-applied 3x N in April. The results suggest that the high rate of urea had a toxic effect when applied to the canopy during flowering and fruit set that did not occur when 3x N as NH₄NO₃ was applied to the soil during this stage of tree phenology. Yield and fruit size of trees receiving soil-applied 3x N were not significantly reduced, which also suggests that supplying only

				Fruit c	uit quality (0–4 scale) ^y	
No.	N treatment ^z		Time to ripen (d)	Vascularization	Discoloration	Decay
1	lx N January, April, July, August + November (control)	Soil ^y	11.1 a ^x	0.3 a	0.1 a	0.1 a
2	2x N April	Soil	11.0 a	0.1 bc	0.0 a	0.1 a
3	2x N April + November	Soil	10.4 a	0.2 bc	0.1 a	0.2 a
4	2x N August	Soil	11.0 a	0.2 bc	0.1 a	0.1 a
5	2x N November	Soil	10.7 a	0.2 ab	0.1 a	0.1 a
6	3x N April	Soil	10.4 a	0.1 c	0.1 a	0.1 a
7	3x N April	Foliar ^v	10.5 a	0.1 bc	0.1 a	0.1 a
8	0.8x N July + August	Soil	10.6 a	0.2 bc	0.1 a	0.1 a
	Year					
	1		w	0.1 c	0.0 b	0.0 b
	2		_	0.1 c	0.0 b	0.1 b
	3		12.2 a	0.3 a	0.0 b	0.3 a
	4		9.1 b	0.2 b	0.2 a	0.1 b
	<i>P</i> value					
	Treatment (T)		0.7037	0.0073	0.4542	0.9240
	Year (Y)		< 0.0001	< 0.0001	< 0.0001	< 0.0001
	$T \times Y$		0.1111	0.1235	0.9468	0.9829

Table 6. Effects of nitrogen (N) fertilizer application times and rates on 'Hass' avocado fruit quality parameters averaged across the four harvests in the research.

^zRefer to Table 1.

^yFruit quality parameters were rated visually on a scale from 0 (normal) to 4 (high incidence of vascularization, discoloration or decay of the mesocarp, and edible portion of the fruit).

*Means in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$. *Missing data.

Table 7. Effects of nitrogen (N) fertilizer application times and rates on the amount of nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) leaching past the root zone of 'Hass' avocado trees averaged across the first 2 years of the research.

No.	N treatment ^z		$\frac{NO_{3}^{-}-N (\mu g \cdot g^{-1}}{\text{resin DW})^{y}}$	NH4 ⁺ -N (µg·g ⁻¹ resin DW)
1	lx N January, April, July, August + November (control)	Soil	239 a ^x	38 a
2	2x N April	Soil	463 a	40 a
3	2x N August	Soil	547 a	55 a
4	2x N November	Soil	820 a	40 a
5	3x N April	Soil	677 a	38 a
	Sample date			
	April year 1		727 a	94 a
	August year 1		W	_
	November year 1		625 a	18 c
	April year 2		608 a	50 b
	August year 2		709 a	32 bc
	November year 2		251 a	21 c
	<i>P</i> value			
	Treatment (T)		0.9517	0.6363
	Sample date (SD)		0.7589	< 0.0001
	$T \times SD$		0.2976	0.9890

^zRefer to Table 1.

 $^{y}1 \ \mu g \cdot g^{-1} = 1 \ ppm.$

*Means in a vertical column followed by different letters are significantly different by Fisher's protected least significant difference test at $P \le 0.05$.

"Missing data.

12.5 lb/acre N at each of the four other key phenological stages was sufficient, at least over the 4 years of this research. In contrast, the reductions in 4-year cumulative and average total yields associated with soil-applied 2x N in April plus November suggest that supplying 8.3 lb/acre N at each of the remaining three phenological stages was insufficient to maintain

yield and fruit size. In particular, these trees would have received only 8.3 lb/ acre N in July and August. For comparison, trees receiving 0.8x N (20 lb/ acre N) only in July plus August (40 lb/acre total annual N) had 4-year cumulative and 4-year average total yields and yields of CVS fruit equal to those of the control trees and trees receiving soil-applied 2x N in April over the 4 years of the research. This result supports the hypothesis that it is important to meet the N demand of the multiple physiological and developmental process that occur concurrently in 'Hass' avocado tree phenology during the summer, the major period of N uptake by the fruit (Rosecrance et al., 2012). It should also be noted that soil-applied 2x N in August produced similar yield results as 0.8x N in July plus August. June drop for the developing fruit occurs from mid-June through August in California (Garner and Lovatt, 2008; Garner et al., 2011). Summer vegetative shoot growth and exponential fruit growth occur in July through August (Lovatt, 2011). In addition, the end of July to beginning of August is when abscission of the mature fruit begins (Garner and Lovatt, 2008; Garner et al., 2011) and inflorescence initiation for next year's crop takes place (Salazar-García et al., 1998).

Insufficient N to meet the needs of the developing fruit, mature fruit, and summer vegetative shoot growth during this time would not only contribute to yield loss and reduced fruit size, but also contribute to alternate bearing. Summer vegetative shoots contribute $\approx 60\%$ to 70% of the total inflorescences at spring bloom the following year in California (Lovatt, 2011). Taken together, these results indicate that N fertilizer should be applied to the soil during the summer at an adequate rate to mitigate June drop and support the competing growth processes of the fruit and summer vegetative shoots in a N-BMP developed for 'Hass' avocado.

Vascularization of the mesocarp (edible portion of the fruit) was the only fruit quality parameter affected by N treatment. Control trees had significantly more vascularization of the mesocarp than trees in all other treatments, except trees receiving soil-applied 2x N in November. However, the incidence of vascularization of the mesocarp for fruit from control trees was only 0.3 on a scale from 0 to 4 and thus likely physiologically insignificant.

All N fertilization treatments were implemented in the orchard 1.5 years before the start of the experiment. However, in year 1 of the experiment, leaf N concentrations averaged 2.71% across trees in all treatments, suggesting that previous N fertilization rates used in this orchard might have been greater than those used in the research presented herein. By year 4 of the experiment, leaf N values for trees in all treatments decreased to an average of 2.35%. The results indicate that residual soil N was decreasing with time. Consistent with this interpretation, the amount of NO₃⁻-N and NH₄⁺-N leaching past the roots zone tended to be lower by November of year 2. Significant differences due to N treatment were observed in the 4-year average leaf N concentration using repeated measures analysis; trees receiving soilapplied 3x N in April had the highest leaf N values and trees receiving soilapplied 0.8x N in July plus August had the lowest, with all trees in all treatments having slightly greater leaf N concentrations than the industry recommended 2.2% (Lovatt and Witney, 2001). It should be noted that annual total yield and yield

of CVS fruit were only weakly positively related to leaf N concentrations; vield of small fruit was not related to leaf N concentration. The poor relationship between leaf N concentration and yield of the 'Hass' avocado in California is not a new finding; it is well documented that avocado leaf analyses are not sensitive enough to detect changes in tree N status or fertilization rates that cause changes in yield (Arpaia et al., 1996; Embleton and Jones, 1972; Embleton et al., 1968; Lovatt, 2001; Lovatt and Witney, 2001; Yates et al., 1993). In the current research, the poor relationship between leaf N concentration and vield parameters cannot be attributed to deficiencies or toxicities in other nutrients. Leaf concentrations of P, K, Ca, Mg, S, Fe, Zn, Mn, B, and Cu were all within the ranges considered optimal for avocado in California; leaf Cl and Na concentrations were below the values considered toxic (Jones and Embleton, 1978). Fertilization strategy influenced only leaf S, Mn, and Fe concentrations in years 1 and 2 and the resulting differences were not related to total yield or fruit size (Jones and Embleton, 1978). In addition, yield and ABI were unaffected by climate conditions (maximum or minimum temperature or rainfall) during the four crop years of the research.

In light of the findings of this research, although intriguing, caution must be exercised in attempting to sustain total yield and yield of CVS fruit with only 40 lb/acre N applied in July plus August for multiple years. Careful monitoring of tree N status would be required. However, in a situation of high leaf N concentrations, high soil N concentrations or significant amounts of N in the irrigation water where it is desirable to reduce avocado tree N status, reducing the annual total amount of soil N and applying it during the summer (July and August) to mitigate June drop and support exponential fruit growth, summer vegetative shoot growth and next spring's bloom would be a logical approach to solve this problem. This strategy would be cost-effective and contribute to greater net income to the grower, as well as offer increased protection of the environment by reducing the potential amount of N that could occur in the runoff water or pollute the groundwater. Compared with five soil applications of 1x N, which produced

equivalent 4-year cumulative and average total yields and yields of CVS fruit, 0.8x N in July plus August would reduce a grower's fertilizer cost by 68% each year for as many years as it could be used without reducing tree N status below optimal. In addition, the reduced manpower required for two applications compared with five fertilizer applications per year, regardless of application method, would further reduce the cost of fertilizing an avocado orchard. Moreover, since all fertilizer rates were based on the amount of N provided, it is likely that other fertilizer sources of N would be equally effective as NH₄NO₃. In situations of low to optimal leaf N concentrations and the need to supply N fertilize to sustain tree N status due to low residual soil N, applications of 1x N at the five key stages of tree phenology identified herein are justified. However, it is suggested that the January application be eliminated in cold wet winters, because N uptake would be compromised and the potential N leaching past the root zone would be increased. The rate of 1x N should reflect the total annual N required to support the crop load on the trees, taking into consideration that in some growing areas both young and mature fruit might be on the tree simultaneously. Using only the amount of N fertilizer necessary to meet tree demand will help keep N fertilizer cost and the potential for $NO_3^{-}-N$ pollution of groundwater at a minimum. In developing a BMP for N fertilization of the 'Hass' avocado, the results presented herein should prove useful for adjusting the time of N application to match key stages of avocado tree phenology with high demands for N to increase total yield and yield of CVS fruit and to reduce the potential for NO₃⁻-N to leach into the groundwater.

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