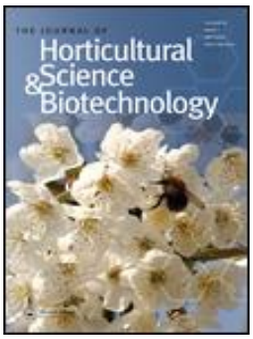

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Foliar urea pretreatment tempers inefficient N recovery resulting from copper chelate (CuEDTA) defoliation of apple nursery plants

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SUMMARY
Copper chelate (CuEDTA) defoliation of deciduous fruit nursery plants, although effective in leaf removal, causes inefficient N recovery from leaves, which results in reduced reserve N levels in woody perennial tissues, compared with natural defoliation. To correct this problem, we sprayed spring bench-grafted Fuji/M.26 apple (*Malus domestica* Borkh.) nursery plants with 3% urea twice, at 5 d intervals, 13 d prior to 1% CuEDTA spray treatment on 31 October, 1997. The effects of urea and CuEDTA on defoliation, reserve N, and later regrowth were determined, as compared with those of hand- or natural-defoliation. CuEDTA resulted in >80% defoliation within 6 d of application. The abscission induced by the CuEDTA treatment, however, did cause significantly less N to be withdrawn from leaves compared with those plants undergoing natural defoliation. This inefficient N recovery was tempered by the foliar-urea pretreatment. The application of urea substantially increased the reserve-N level (total-N or soluble-protein concentrations) in all components of the tree without affecting the efficacy of CuEDTA on defoliation. The extent of regrowth in N-deficient medium was proportional to the reserve-N level. The urea-treated plants, whether hand- or CuEDTA-defoliated, showed better growth performance than the no-urea controls. Therefore, CuEDTA, in combination with pretreatments of foliar urea, proved effective for defoliating apple nursery plants, increasing reserve-N levels, and improving regrowth performance during tree establishment.

Controlled defoliation is essential for the successful management of deciduous nursery plant production, because natural defoliation often occurs later than is desired for an efficient harvest that will escape the effects of low temperatures and high precipitation in the autumn (Knight, 1983; Larsen and Fritts, 1986). Premature defoliation by hand is time-consuming and, therefore, costly, and can cause damage. Hence, a chemical defoliation has long been desired by nurserymen.

Cooper *et al.* (1968) observed that the chelated form of copper also caused leaf abscission when used to enhance citrus fruit abscission. Since then, this compound has been found effective in defoliating various deciduous fruit nursery stocks (Knight, 1983; Larsen and Fritts, 1986). However, there is at present no information on how CuEDTA-induced defoliation affects the reserve-N status in deciduous nursery plants, through leaf senescence/N mobilization. In deciduous trees, reserve-N is essential for early-season growth when N uptake by roots can be restricted by unfavourable soil temperatures (Millard, 1996).

The merits of chemical defoliants can be maximized when they also effectively mobilize N from senescing leaves before abscission for the most efficient internal cycling of N in the autumn. In this context, abscisic acid (ABA) is now used to promote leaf senescence and defoliation (Guak and Fuchigami, 1997; Larsen and Higgins, 1998). Our preliminary study, however, indicated that CuEDTA effectively defoliated fruit nursery stock, but possibly in a manner different from ABA in terms of N recovery from the leaves. CuEDTA defoliation was so rapid that senescence was prevented prior to abscission. This may have decreased the recovery of N from leaves, which resulted in reduced N reserves and poor growth performance the following season.

To correct this problem, we hypothesized that pretreating the plant with foliar urea before applying the CuEDTA could increase leaf-N level and a portion of this N would then be mobilized from the leaves to storage tissues prior to abscission. Foliar-urea sprays have effectively increased reserve-N levels in fruit-tree nursery stock as well as in orchard trees (Oland, 1960; Sanchez *et al.*, 1990). In this study, therefore, we evaluated in apple nursery plants how effectively foliar urea pretreatment tempers CuEDTA-induced inefficient N recovery from the leaves. We also determined the relationship of the resulting increases in the reserve-N level the treatment year to growth performance the following spring. The effects of CuEDTA were compared with hand- or natural-defoliation.

MATERIALS AND METHODS

Spring bench-grafted Fuji/M.26 apple nursery plants were grown in 3.75 dm^3 plastic pots containing 1:1:1:1 peat moss, perlite, vermiculite and sand in a lathhouse at Oregon State University in Corvallis. They were trained to single shoots and fertigated weekly with 100 ppm 20-10-20, from early May until late August, 1997. Fourteen days before the experiment began, 84 plants

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were moved outdoors and grouped, by size, into six blocks. The average size of plants selected was around 95 cm in height and 0.80 cm in stem diameter at 10 cm above the grafting union.

In mid-October, 3% (w:v) urea (reagent grade; Sigma, St Louis, MO, USA) was hand-sprayed to run-off twice on selected plants (5 d apart). The tops of the pots had been covered with plastic to prevent excess urea from falling on the growing medium. Although no rain fell in the 3 d after the final urea application, the weather was usually foggy in the early morning, clear at midday, then foggy again in the late afternoon. Five days after the final urea application, six plants each for the control and urea treatments were harvested and dissected into leaves, stems, and roots to determine total-N and soluble protein concentrations.

A 1% a.i. (v:v) solution of CuEDTA (Hampshire Chemical Corp., NH, USA) was applied to the selected plants on 31 October (13 d after the final urea application) to run-off with a hand-sprayer, when all the plants were held in a glasshouse at ambient temperature. The treated plants were held there for another 3 d and returned outdoors. Percent defoliation was recorded once, 6 d after the CuEDTA treatment.

Twelve days after the CuEDTA application (12 November), half of the untreated and the plants treated with urea only were hand-defoliated. The other half were allowed to defoliate naturally. In total, this experiment comprised six treatments, each with six double-tree replicates in a randomized complete-block design: 1) hand-defoliation control, 2) natural-defoliation control, 3) urea and hand-defoliation, 4) urea and natural-defoliation, 5) CuEDTA only, and 6) urea and CuEDTA.

Plants were depotted on 15 December, washed free of potting media from roots, and divided into two equal groups, then stored at 2°C for chemical analyses (total-N and soluble-protein concentrations) and spring regrowth. One group of stored plants was washed in double-distilled water and dissected into stem bark (including vegetative buds) and wood, and roots. Tissue samples were lyophilized, ground in a Wiley mill, and sieved for determining total-N and soluble-protein concentrations. The second group was planted the following April in pots containing 1:1 perlite and vermiculite (N-deficient media), then grown in a glasshouse (22°C day/18°C night) under natural illumination. After 45 d of regrowth, these plants were harvested for determinations of new biomass production (i.e. total leaf area and dry weight, and total new shoot growth in length and dry matter), leaf-chlorophyll concentration, and total-N and soluble-protein concentrations in various tissues.

Total-N concentrations from all tissues was determined colorimetrically with an autoanalyzer after micro-Kjeldahl digestion (Schuman *et al.*, 1973). Soluble proteins were extracted only from leaves, bark, or roots, with a method modified from that of Wetzel *et al.* (1989). Tissues (approximately 0.5 g dry weight) were homogenized at 4°C in 5 cm^3 borate (50 mM ascorbic acid, 50 mM sodium borate, 1% β-mercaptoethanol, 1 mM fresh PMSF, 200 mg PVPP; pH 9.0) for 1 min with a Tekmar Tissuemizer (Tekmar Corp., Cincinnati, OH, USA) at maximum speed. Samples were then centrifuged at 16,000 *g* for 1 h at 4°C. The resulting supernatant was collected, filtered first through two folds of Kimwipes and next through serially-positioned 0.4 and 0.2 μm syringe filters, and assayed for protein content using a modified Bradford method (Ramagl and Rodriguez, 1985).

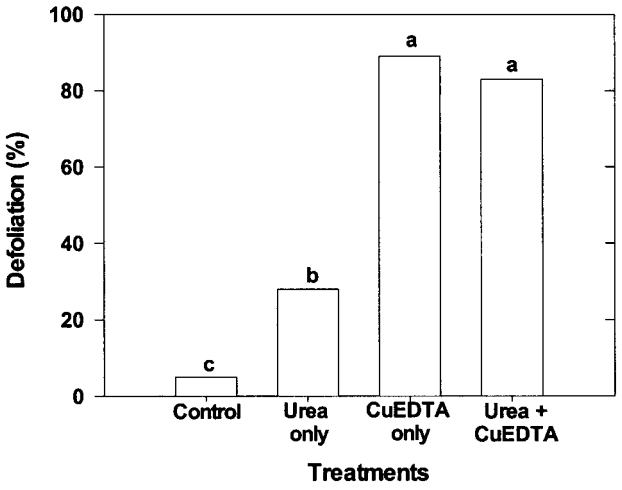

FIG. 1
Percent defoliation of Fuji/M.26 apple nursery plants, measured on 6 November 1997, 6 d after treatment with 1% CuEDTA. Thirteen days before this treatment, some plants were sprayed with 3% urea twice, at 5 d intervals. Means followed by different letters are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Leaf-chlorophyll concentration was indirectly determined from ten mid-stem leaves per tree with SPAD (Minolta Corp., Japan). We used the following equation established between SPAD readings and chlorophyll concentrations from 30 Fuji/M.26 apple leaves of various range of leaf greenness: $Y = -0.02 + 0.00068X + 0.000021X^2$ ($r^2 = 0.94$), where X is the SPAD reading and Y is the chlorophyll concentration (g m^{-2}). The chlorophyll concentration was determined from the same leaf used for the SPAD measurement according to the procedures of Wintermans and Demots (1965).

Because the plants were uniform in each block, the relative magnitude of N reserves in the bark was calculated as the difference in bark total-N concentrations between the time of estimated maximum N concentration (i.e. during winter following leaf abscission) and the time of estimated minimum N concentration (i.e. following 45 d of regrowth in N-deficient media).

TABLE I
The effects of foliar urea application on total-N and soluble-protein concentrations in Fuji/M.26 apple nursery plants, determined on 23 October 1997, 5 d after the second foliar urea spray

Plant part	Treatment	Total nitrogen (% DW)	Soluble proteins (mg g^{-1} DW)
Leaf	Control	2.38 b	18.2 a
	3% urea twice	3.98 a	18.5 a
Bark	Control	0.77 b	9.2 b
	3% urea twice	1.95 a	11.9 a
Roots	Control	0.92 b	5.0 b
	3% urea twice	1.50 a	6.0 a

Each value is a mean of six replicates; within columns, means followed by different letters are significantly different by Duncan's multiple range test ($P \leq 0.05$).

TABLE II
The effects of foliar urea and CuEDTA application on total-N and soluble-protein concentrations in stem bark and wood, and roots of Fuji/M.26 apple nursery plants, determined on 15 December, 1997

	Total nitrogen (% DW)			Soluble proteins (mg g^{-1} DW)	
Treatment	Bark	Wood	Roots	Bark	Roots
Hand-defoliation control	0.85 c	0.32 b	0.85 c	12.3 b	6.2 c
Natural-defoliation control	1.31 b	0.44 b	1.23 b	14.8 a	8.9 b
Urea + Hand-defoliation	1.96 a	0.78 a	2.59 a	15.1 a	10.8 a
Urea + Natural-defoliation	2.03 a	0.77 a	2.72 a	15.4 a	10.9 a
CuEDTA only	1.00 c	0.39 b	1.32 b	13.2 b	9.1 b
Urea + CuEDTA	2.08 a	0.95 a	2.71 a	15.1 a	10.8 a

Each value is a mean of six replicates; within columns, means followed by different lettrs are significantly different by Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Defoliation and leaf senescence

Although CuEDTA induced substantial leaf removal within six days of application (Figure 1), this treatment did not enhance leaf senescence (i.e. chlorophyll breakdown or withdrawal of leaf N). In fact, CuEDTA-induced leaf abscission occurred when leaves were still green. In addition, there were no difference in the total-N concentrations between the leaves that abscinded after the CuEDTA treatment and those still attached to the untreated-control plants (2.15 and 2.20%, respectively; $P = 0.192$), indicating insignificant recovery of N from the leaves of the CuEDTA-treated plants. This was compared with the process during natural defoliation and could limit N mobilization from leaves before abscission.

The CuEDTA-induced abscission seemed to also differ from that induced by ABA. Our previous study with similar plants (spring bench-grafted Fuji/M.26) showed that spraying with ABA at 1000 ppm (w/v) twice in early September significantly enhanced leaf senescence (Guak and Fuchigami, 1997): for example, from late October to late November total-N concentrations in the mid-stem leaves dropped by about 29.8% and 40.2% in control and ABA-treated plants, respectively. Similarly, soluble-protein concentrations during that period declined by about 22.2 and 59.6% in control and ABA-treated plants, respectively.

Total-N and soluble-protein concentrations

Pretreatment with foliar urea substantially increased total-N concentrations in leaves, bark, and roots, by about 67, 153, and 63%, respectively (Table I). However, soluble-protein concentrations increased in bark and roots by only 29 and 20%, respectively, and not at all in leaves (Table I). This indicates that leaf absorbed urea-N existed mainly in a non-protein form. Shim *et al.* (1972) had similar results, finding that the amount of urea absorbed by leaves of young apple seedlings paralleled the increase in soluble N mostly as urea. Our results also demonstrated the rapid transport of N from the leaves to the storage tissues, within 5 d after application.

Consequently, reserve-N concentrations of dormant stem (wood and bark) and root tissues significantly increased in all treatments involving foliar urea applications (Table II). For example, in the urea + CuEDTA treatment, total-N concentrations in bark and roots increased by some 145% and 219%, respectively, over the hand-defoliation control. In contrast, soluble-protein concentrations increased by only 23% and 74% in bark and roots, respectively. This less dramatic increase in soluble-protein concentrations indicates that a large fraction of translocated urea-derived N still existed in non-protein form in the bark and roots tissues. The large increase in reserve N from foliar-applied urea-N into the woody tissues including roots was similarly reported in young apple plants (Shim *et al.*, 1973; Swietlik and Faust, 1984) and in mature bearing peach and nectarine trees (Rosecrance *et al.*, 1998). However, in mature bearing trees of apple (Khemira, 1995) and pear (Sanchez *et al.*, 1990), only limited movement of urea-derived N was reported beyond the tissues subtending the sprayed leaves, and little was translocated to roots.

Despite the insignificant mobilization of N from leaves by CuEDTA, compared with our natural-defoliation treatment, the CuEDTA-only treatment increased total-N and soluble-protein concentrations slightly in bark but significantly in roots, compared with hand-defoliation. We cannot rule out a direct effect of CuEDTA on N mobilization into roots. However, this result can also be explained by N dilution caused by carbohydrate accumulation (c.f. Lemaire and Millard, 1999) in the roots of hand-defoliated control plants, where hand defoliation was delayed until 12 d after CuEDTA treatment, while CuEDTA caused rapid defoliation.

TABLE III
Total-N and soluble-protein concentrations in Fuji/M.26 apple nursery plants treated with foliar urea and CuEDTA, as determined after 45 d of spring regrowth in pots containing N-deficient media

	Total N (% DW)		Soluble proteins (mg g^{-1} DW)	
Treatment	Bark	Roots	Bark	Roots
Hand-defoliation control	0.54 bc	0.62 c	5.4 b	2.5 c
Natural-defoliation control	0.63 b	0.79 b	5.5 b	4.1 b
Urea + Hand-defoliation	1.14 a	1.10 a	7.1 a	5.0 a
CuEDTA only	0.50 c	0.66 c	3.7 c	4.0 b
Urea + CuEDTA	1.10 a	1.08 a	7.3 a	4.9 a

Each value is a mean of six replicates; within columns, means followed by different letters are significantly different by Duncan's multiple range test ($P \leq 0.05$).

TABLE IV

Growth of Fuji/M.26 apple nursery plants treated with foliar urea and CuEDTA, as determined 45 d after spring regrowth in pots containing N-deficient media

Treatment	Leaf chlorophyll conc. (g m^{-2})	Total leaf area per tree (cm^2)	Total leaf dry wt per tree (g)	Total new shoot growth per tree	
				Length (cm)	Dry wt. (g)
Hand-defoliation control	0.24 c	772 c	5.0 c	8.7 d	5.2 c
Natural-defoliation control	0.31 b	931 b	5.9 b	16.8 bc	6.2 b
Urea + Hand-defoliation	0.34 a	1087 a	6.6 a	19.7 b	7.2 a
CuEDTA only	0.24 c	843 bc	5.3 c	14.1 c	5.7 c
Urea + CuEDTA	0.36 a	1141 a	6.7 a	25.0 a	7.6 a

Each value is a mean of six replicates; within columns, means followed by different letters are significantly different by Duncan's multiple range test ($P \leq 0.05$).

Regrowth

Following 45 d of regrowth in a N-deficient medium, total-N and soluble-protein concentrations decreased in bark and roots (Table III). Urea-treated plants, whether hand- or CuEDTA-defoliated, used much more reserve N for regrowth than did the no-urea treatments, although the minimum values were still significantly higher in the urea-treated plants (compare Tables II and III).

The urea-treated plants, whether hand- or CuEDTA-defoliated, consistently produced better growth, as evaluated by total leaf area and dry weight per tree, total new shoot growth per tree, and leaf chlorophyll concentration (Table IV). Such a positive urea effect was closely associated with the increased concentrations of bark reserve N (Figure 2). This supports earlier work on the importance of reserve N for early growth in deciduous fruit trees (Neilsen *et al.*, 1997; Titus and Kang, 1982; Weinbaum *et al.*, 1984).

No injury was observed in the CuEDTA-treated plants, i.e. all their buds grew normally, and the percent bud break was similar to that seen in the natural-defoliation control (data not shown). Damage sensitivity to this compound apparently depends on cultivar or species. As shown by Knight (1983), 2.1% sprays of CuEDTA caused little or no damage to 'Cox's Orange Pippin' apple trees. In contrast, even a 0.75% CuEDTA induced some bark damage below the buds on 'Bartlett' pears at tree harvest (Larsen and Fritts, 1986).

In conclusion, CuEDTA effectively and rapidly defoliated apple nursery plants, while causing inefficient recovery of N from leaves to woody tissues including roots, compared with plants that underwent natural defoliation. Therefore, CuEDTA-induced leaf abscission does not appear to enhance the leaf-senescence process as does natural defoliation or the use of defoliants such as ABA. However, combining the CuEDTA treatment with a foliar urea pretreatment effectively improved the reserve-N level, while still retaining its defoliation effect. Likewise, the increased reserve-N level resulted in better tree growth during establishment the following spring. However, further experimentation in the field conditions is necessary before the potential of foliar urea and CuEDTA treatment can be fully defined.

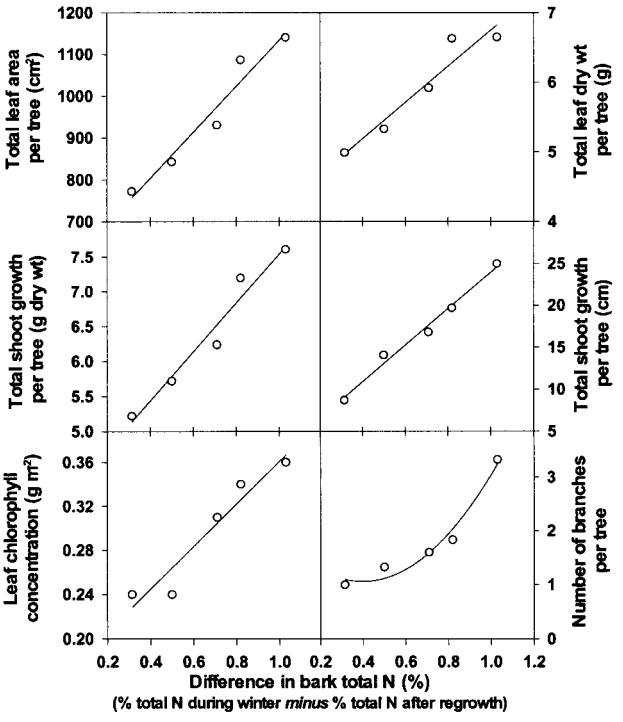

FIG. 2

Relationship between the relative amount of reserve N (on concentration basis) in stem bark and spring regrowth in Fuji/M.26 apple nursery plants. The relative magnitude of reserve N was determined by the difference in bark percent total N between the time of maximum concentration (i.e., during winter) and the time of minimum concentration following the spring regrowth in N-deficient media.

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