Effects of Lime Sulfur and Fish Oil on Pollen Tube Growth, Leaf Photosynthesis and Fruit Set in Apple

Steven McCartney
HortResearch, Hawke’s Bay Research Centre, Private Bag 1401, Havelock North, New Zealand

John Palmer, Sue Davies, and Shona Seymour
HortResearch, Nelson Research Centre, P.O. Box 220, Motueka, New Zealand

Additional index words. fruit abscission, bloom thinning, organic apple production, Malus ×domestica

Abstract. The effect of liquid lime sulfur (LS) and fish oil (FO) application during bloom on leaf photosynthesis (Pn) and pollen tube growth in apple (Malus ×domestica) flowers was investigated in order to determine their mode of action as a bloom thinning agent. LS increased the percentage of flowers with fewer than 10 pollen tubes per flower to more than 64% compared to 5% or less in the control. Pollen tubes were completely absent from 27% to 48% of flowers following LS treatment compared with fewer than 4% of flowers having no pollen tubes on control trees. These data indicate that 30% to 50% of flowers that open on the day of LS application are unlikely to set a fruit due to the complete inhibition of embryo fertilization. Increasing the rate of LS from 0.5% to 4% increased the proportion of flowers with limited pollen tube number in a concentration dependent manner. LS suppressed the rate of light saturated Pn; successive LS sprays during the bloom period had an additive effect on suppression of Pn and fruit set. In one study the reduction in Pn was greatest 12 days after application of LS but Pn recovered by about 19 days after initial treatment. In a second study Pn of primary spur leaves had still not recovered when measured 57 days after the first of three applications. FO had no effect on the number of pollen tubes per flower, but reduced Pn and fruit set by about 10% and 20% respectively. An increase in the proportion of flowers with no pollen tubes, and therefore no embryo development, can account, at least in part, for the thinning response following application of LS to apples during bloom. It is likely that suppression of Pn contributes to the thinning response, although the importance of this mechanism will depend on perturbation of the total carbohydrate supply to developing fruit.

Liquid lime sulfur (LS, calcium polysulfate) is frequently used as a bloom-thinning agent in organic apple production systems in countries where its use is permitted, and is increasingly being used in non-organic production systems for the same purpose. Emulsifed fish oil (FO) is frequently combined with LS to provide greater thinning efficacy (J. McFerson, personal communication). At this time the precise mode of action of LS as a bloom thinning agent is not known. Sulfur sprays may reduce germination (MacDaniels and Furr, 1930) and tube growth (He and Wetzstein, 1994) of pollen grains. The apple flower typically consists of five carpels, each containing two ovules so that there is the potential to develop ten seeds per fruit (Westwood, 1978). Parthenocarpy is seldom observed in most commercial cultivars, indicating that at least some embryo development is necessary for fruit retention. However, for preharvest drop, at least, there does not appear to be any clear relationship between seed number in seeded fruit and their retention (Ward et al., 2001). Since flowers of ‘Summerland McIntosh’ exhibit perfect synchrony, i.e., pollination levels among the five stigmas need not be uniform to obtain full seed development (Sheffield et al., 2005) then the potential seed number per fruit will be related more closely to the number of pollen tubes per flower than per style, and the absence of pollen tubes from all styles in a flower will result in complete ovule abortion. Sulfur may also suppress photosynthesis (Pn) in apple leaves (Hoffman, 1935; Hyre, 1939), and it has been proposed that postbloom LS applications thin apple fruit by inhibiting Pn and decreasing assimilate supply to developing fruit (Noordijk and Schupp, 2003). Palmer et al. (2003) have recently shown that using LS or sulfur as a fungicide over the whole season can reduce Pn of ‘Braeburn’ by almost 50% in mid-summer. The current study was undertaken to quantify the effect of LS applications during bloom on pollen tube growth, spur leaf Pn, and fruit set of apple.

Materials and Methods

Experiment 1: Number of LS applications, Nelson. Four-year-old ‘Braeburn’/M.9 apple trees were selected and blocked into five replications of five trees in 2000. Liquid LS (3%) was applied either 0, 1, 2, 3, or 4 times at 3-d intervals during the bloom period, with the first application made when 30% of spurs were at full bloom (27 Sept. 2000). All treated trees received LS on the first date. Three, two, or one tree within each block received additional LS sprays 3, 6, or 9 d later respectively. Treatments were applied to fully guarded single-tree plots with a Solo backpack sprayer to the point of leaf wetness. Ten flowers at the late-bloom stage were tagged on each of the control trees and on each of the trees to receive a single LS application. The 50 flowers on each of these two treatments were tagged early on the day of the first LS application. U.S. apple treatments were not made until mid afternoon. Tagged flowers were removed 5 d later (2 Oct.) to allow for sufficient pollen tube growth and stored in a 5% sodium sulfite solution until later assessment of pollen tube growth.

The number of pollen tubes present at the mid point of each style was counted using the method of Embree and Foster (1999). Briefly, samples were autoclaved at 120 °C for 20 min before squashing under a glass cover slip with water soluble 0.01% Aniline Blue stain in 0.067 M K·HPO4. Stained samples were examined under a fluorescent light using a Nikon HB-10101 AF super high-pressure mercury lamp and a Nikon Optiphot photomicroscope (Nikon, Tokyo, Japan). Pollen tube number was expressed on a per flower basis rather than per individual style.

Leaf gas exchange was measured on two primary spur leaves per tree using an ADC LCA-3 portable leaf gas-exchange system (ADC, Hoddesdon, U.K.) under constant light conditions (>1,000 µmol·m–2·s–1) at 3- to 4-d intervals beginning one day after the first LS application. As the spur leaves of ‘Braeburn’ are small, the leaf cup described by Palmer (1986) was used rather than the broad leaf chamber of the LCA-3.

Treatments effects on initial fruit set (fruit per 100 flower clusters) were determined from flower cluster counts at bloom and fruit counts taken at the completion of fruit drop 38 d after bloom on two sample limbs on each tree. Sample limbs were chosen that were 8 to 10 cm in circumference and bore 40 to 60 clusters in total.

Experiment 2: LS + FO, Nelson. Five-year-old ‘Braeburn’/M.9 apple trees were selected from within the same orchard as the previous study and blocked into six replications of four trees in 2001. Repeat applications of LS (2%), either alone or in combination with FO (2%) (Seald Group, Nelson, New Zealand), were made three times during bloom in 2001 (27 Sept., 1 Oct., and 5 Oct.). Spray treatments containing FO were vigorously shaken during application to ensure the FO stayed in suspension. The treatments were applied with a Swissmix knapsack sprayer to fully guarded single-tree plots. Five flowers at the late-bloom stage of development were tagged on each tree on the first LS application. Tagged flowers were harvested 5 d later (2 Oct.) to allow for sufficient pollen tube growth and stored in a 5% sodium sulfite solution.
until later assessment of pollens tube growth as in the previous study. Treatment effects on fruit set (fruit per 100 flower clusters) were determined from flower counts at bloom and fruit counts on two dates after bloom (initial fruit set counts taken on 5 Nov., 39 d after bloom; final fruit set counts taken on 7 Dec., 71 d after bloom) on two sample limbs on each tree. Treatment effects on leaf gas exchange of primary spur leaves were determined during the period from 1 to 57 d after the initial LS application using the same equipment as that described in the previous experiment.

Experiment 3: LS concentration and pollen tube number. Hawkes Bay. Four uniform mature 'Royal Gala' M26 apple trees were selected in an orchard in 2001. Forty individual spurs were selected on each tree and the flowers removed by hand to leave a single flower on each spur at the full bloom stage of development. Ten individual flowers on each tree were sprayed with a hand sprayer with LS at a concentration of 0.5%, 1%, or 4% and an additional ten flowers were left unsprayed on each tree. Flowers were sampled 8 d after LS treatments for assessment of pollen tube growth as in the previous experiments.

Statistical analysis. The general linear models (GLM) procedure of the Statistical Analysis System (SAS, Cary, N.C.) was used to test for treatment effects on pollen tube number and fruit set. Percent of flowers with a limiting pollen tube number (<10 or 0 tubes per flower) and leaf Pn were tested both between groups and within subjects (Time).

Results and Discussion

Pollen tube growth. Application of LS during bloom increased the percentage of flowers with fewer than ten pollen tubes per flower in all experiments. In Expt. 1, only 9% of flowers that opened on the day of treatment had <10 pollen tubes per flower, compared to 64% of flowers at the same stage on trees sprayed with 3% LS. In the same study, LS increased the percent of flowers with no pollen tubes to 7%, compared to only 2% in the control (Table 1).

Table 1. Effects of lime sulfur (3%) application on the percent of flowers with limiting pollen tube number (<10 or 0 pollen tubes per flower). Flowers at the full bloom stage of development on the day of treatment were sampled 5 d later. Percent data were analyzed after arcsine-square root transformation. Back-transformed weighted means are presented.

| Treatment | <10 tubes/flower | 0 tubes/flower | Control | Lime sulfur | 
|-----------|-----------------|----------------|---------|-------------| 
|           | 8.6             | 2.0            | 64.3    | 27.1        | 
| Significance (P > F) | 0.003            | 0.006          |         |             | 

Table 2. Main effects of repeated applications of lime sulfur (2%) or FO (2%) during bloom in 2001 on the percent of flowers with limiting pollen tube number (<10 or 0 pollen tubes per flower), initial and final fruit set. Flowers were collected for assessment of pollen tube number 5 d after the initial application, 21 d after the final LS application. In contrast, treatment effects on Pn were still evident 57 d after the initial LS application in Expt. 2, 51 d after the final application (Table 4). FO reduced Pn by 10% whereas LS reduced Pn by 20%. The significant interaction of time and LS indicate that leaf Pn changed differently over time for each level of LS. In all cases stomatal conductance was significantly reduced with decreased leaf Pn (data not shown).

The data presented in Table 3 indicate that the inhibitory effect of LS on Pn of primary
spur leaves reached a maximum around 12 to 14 d after application. However, the pattern of recovery of Pn was different in each experiment; Pn rates recovered within 19 d of the initial application in Expt. 1 whereas the inhibitory effect within 19 d of the initial application. All treated trees received lime sulfur on day zero. Trees receiving 2, 3, or 4 LS applications were sprayed 3, 5 and 6, and 3, 6, and 9 d later respectively.

### Table 3. Effects of number of lime sulfur (3%) applications beginning at 30% spur full bloom (2000) on light-saturated Pn (µmol·m–2·s–1) of primary spur leaves on ‘Braeburn’ M.9 apple trees during the 30-d period following the initial application. All treated trees received lime sulfur on day zero. Trees receiving 2, 3, or 4 LS applications were sprayed 3, 5 and 6, and 3, 6, and 9 d later respectively.

| LS sprays (no.) | Time of Pn measurement (days after initial application) | Mean LS sprays (no.) |
|-----------------|----------------------------------------------------------|----------------------|
| 0               | 1 5 8 12 19 30                                          |                      |
| 1               | 11.9 13.4 16.0 15.7 15.0 16.2 14.7                      |
| 2               | 10.8 11.0 13.5 11.6 14.0 15.8 12.8                      |
| 3               | 12.4 12.0 13.4 9.0 13.3 14.8 12.5                      |
| 4               | 12.6 10.0 10.2 9.9 11.1 14.1 11.3                      |
| Mean (time)     | 12.1 11.1 12.7 11.0 12.3 15.0 10.6                      |

Source df P LSD0.05
LS sprays (no.) 4 <0.001 1.3
Linear 1 <0.001
Quadratic 1 0.380
Time 5 <0.001 1.2
No. LS sprays × time 20 <0.001 2.7
Linear × time 5 <0.001
Quadratic × time 5 0.065

*The LSD0.05 = 2.6 when comparing means within the same level of number of LS sprays.

A transient reduction in the supply of carbohydrates to developing fruit may increase fruit abscission. Beruter and Droz (1991) measured a transient reduction in the concentration of glucose in the pedicel of apple fruit immediately after abscission, suggesting that this reduction may provide the signal for triggering abscission. Postbloom shade treatments of only 3 d duration may provide the signal for triggering abscission. Beruter and Droz (1991; 1992; McArtney et al., 2004) measured a transient reduction in the supply of carbohydrates to the growing fruit according to current hypotheses (Beruter and Droz, 1991; Lakso et al., 1999; Stopar et al., 2000; Untiedt and Blanke, 2001).

The interaction term between LS and FO was not statistically significant for fruit set, pollen tube growth, or photosynthesis data in Expt. 2. There was a significant main effect of both LS and FO on Pn, yet only LS had a significant effect on the percent of flowers with limiting numbers of pollen tubes. FO reduced Pn by about 10% percent, with this effect still evident on the final day of measurement, 51 d after the final application. Even though this represents only a mild suppression of Pn, the cumulative effect on whole tree biomass throughout this period must have reduced carbon availability to developing fruit to the extent that fruit abscission was triggered. Commercial identifying the period from 2 to 4 weeks after bloom as a critical time when the probability is highest for fruit production being limited by carbohydrate supply. The extent and duration of inhibition of Pn that were measured in the current experiments following LS applications at bloom are consistent with a thinning effect via reduction of carbon supply to developing fruit for the cultivar ‘Braeburn’ at least. However, if the Pn response to LS is less sensitive in other cultivars then the thinning response would be less sensitive also. We have measured milder suppression and more rapid recovery of Pn in other apple cultivars in response to LS applications (unpubl. data). If the thinning response is due entirely to a reduction in carbohydrate supply to developing fruit then a reduced thinning efficacy would be expected for cultivars other than ‘Braeburn’.

### Fruitset.
There was a significant linear effect of the number of LS applications during bloom on fruit set; each successive application of LS reduced fruit set by an additional 10.2% of the control level (Fig. 2). In Expt. 2 the interaction term between the two main factors (LS and FO) was not significant so only main effect means are presented (Table 2). FO reduced both initial and final fruit set by about 20%. LS had a greater effect on initial and final fruit set than FO, reducing it by 39% and 34%, respectively.

A single application of LS resulted in 27% of the flowers that were at full bloom on the day of treatment having no pollen tubes present in their styles, and therefore unable to develop any embryos or set a fruit (Expt. 1). Subsequent applications of LS would presumably have a similar effect on flowers that were at full bloom on the day of application, and so have a cumulative effect on fruit set. It is not known at this time if subsequent LS applications also have a suppressive effect on embryo development within flowers in which fertilization has been limited by earlier LS sprays. If LS does limit the potential for embryo development in flowers that have been open for only 1 or 2 d, then this could explain the levels of fruit abscission that are commonly observed with thinning sprays of LS. However, repeat applications of LS during bloom also resulted in a cumulative suppression of Pn, the extent and duration of which could arguably have resulted in fruit abscission via a transient reduction in the supply of carbohydrates to the growing fruit according to current hypotheses (Beruter and Droz, 1991; Lakso et al., 1999; Stopar et al., 2000; Untiedt and Blanke, 2001).

The interaction term between LS and FO was not statistically significant for fruit set, pollen tube growth, or photosynthesis data in Expt. 2. There was a significant main effect of both LS and FO on Pn, yet only LS had a significant effect on the percent of flowers with limiting numbers of pollen tubes. FO reduced Pn by about 10% percent, with this effect still evident on the final day of measurement, 51 d after the final application. Even though this represents only a mild suppression of Pn, the cumulative effect on whole tree biomass throughout this period must have reduced carbon availability to developing fruit to the extent that fruit abscission was triggered. Commercial
Table 4. Effects of three applications of 2% lime sulfur (LS) and 2% fish oil (FO) during bloom in 2001, on light-saturated Pn (μmol·m⁻²·s⁻¹) of primary spur leaves on ‘Braeburn’/M.9 apple trees during the 57-d period following the first application. LS and FO were applied alone or in combination. The second and third sprays were applied 3 and 6 d after the initial application respectively.

| Main effects | Time of Pn measurement (days after initial application) | Mean |
|--------------|--------------------------------------------------------|------|
|              | 1 3 11 17 25 36 46 57 |      |
| Lime sulfur  |                                                        |      |
| No lime sulfur applied | 17.9 18.7 16.8 15.5 16.8 14.8 15.0 14.7 16.3 |      |
| Lime sulfur  |                                                        |      |
| No fish oil applied | 19.1 18.7 15.1 13.6 15.9 14.6 13.2 12.9 15.4 |      |
| Fish oil     |                                                        |      |
| No fish oil applied | 16.0 17.7 12.8 12.9 13.6 12.8 13.1 11.9 13.9 |      |
| Mean (time)  |                                                        |      |
| No lime sulfur applied | 17.5 18.2 14.0 13.2 14.8 13.7 13.1 12.4 |      |

Source df P LSD₀.₀₅
LS 1 0.001 1.1
FO 1 0.008 1.1
LS × FO 1 0.388 1.5
Time 7 <0.001 1.2
Time × LS 7 =0.001 1.9
Time × FO 7 0.224 1.9
Time × LS × FO 7 0.946 2.7

* LSD₀.₀₅ = 1.7 when comparing means within the same level of LS or FO.

formulations of FO contain an emulsifying agent to maintain the oil in a stable suspension. Emulsified FO may have different effects on pollination, Pn and fruit set compared to the FO treatments used in Expt. 2.

From these data it appears that fruit abscission following applications of LS to apples over the bloom period is the result of the combined effects of an increase in the number of completely unfertilized flowers and a reduction in carbohydrate supply to fertilized flowers due to a suppression in the rate of Pn in primary spur leaves. While the suppression of spur leaf Pn following LS applications may be permanent, the effect of LS on whole tree carbon balance may be more transitory due to an increasing contribution of newly assimilated carbon from rapidly expanding extension shoot leaves during this period. If the abscission response following bloom thermogenesis of LS is largely due to a transient suppression of whole-tree carbon assimilation, then thinning results would be highly variable, and dependent on a myriad of factors including the photosynthetic response of the individual cultivar, ambient light and temperature conditions, crop load, availability of carbohydrate reserves and current assimilates. Yet personal observation and grower experience have demonstrated that repeated applications of LS + FO over the bloom period are a consistently effective chemical thinning strategy.

Literature Cited

Beruter, J. and P. Droz. 1991. Studies on locating the signal for fruit abscission in the apple tree. Scientia Hort. 46:201–214.
Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young and D.H. Carbaugh. 1990. Apple fruit abscission by photosynthetic inhibition. J. Amer. Soc. Hort. Sci. 115:14–19.
Byers, R.E., D.H. Carbaugh, C.N. Presley and T.K. Wolf. 1991. The influence of low light on apple fruit abscission. J. Hort. Sci. 66:1–7.
Embree, C.G. and A. Foster Jr. 1999. Effects of coatings and pollinicides on pollen tube growth through the stigma and style of ‘McIntosh’ apple blossoms. J. Tree Fruit Prod. 2:19–32.
He, Y. and H.Y. Weiststein. 1994. Pollen degeneration and resumption of pollen development following fungicide sprays applied during microspore development and shoot expansion. J. Hort. Sci. 69:975–983.
Hoffman, M.B. 1935. The effect of lime-sulfur spray on the respiration rate of apple leaves. Proc. Amer. Soc. Hort. Sci. 33:173–176.
Hyde, R.A. 1939. The effect of sulfur sprays on the photosynthesis and transpiration of apple leaves. N. Y. Agr. Extpt. Sta. Mem. 222.
Lakso, A.N., J.N. Wünsche, J.E. Palmer and L. Coelli-Grappadelli. 1999. Measurement and modeling of carbon balance of the apple tree. HortScience 34:1040–1047.
MacDaniels, L.H. and J.R. Furr. 1930. The effect of dusting sulfur upon the germination of the pollen and the set of fruit of the apple. Bul. Cornell Univ. Agr. Extpt. Sta. 490.
McArtney, S., M. White, I. Latter and J. Campbell. 2004. Individual and combined effects of shading and thinning chemicals on abscission and dry-matter accumulation of ‘Royal Gala’ apple fruit. J. Hort. Sci. Biotechnol. 79:441–448.
Noordijk, H. and J. Schupp. 2003. Organic post bloom apple thinning with FO and lime sulfur. Hortscience 38:690 (abstr.).
Palmer, J.W. 1986. Seasonal variation of light saturated photosynthetic rate of Golden Delicious apple leaves as influenced by leaf type and crop load, p. 30–33. In: A.N. Lakso and F.Lenz (eds.). The regulation of photosynthesis in fruit trees. Symp. Proc. N.Y. State Agr. Extpt. Sta., Geneva.
Palmer, J.W., S.H. Davies, P.Shaw, and J.N. Wünsche. 2003. Growth and fruit quality of ‘Braeburn’ apple trees as influenced by fungicide programmes suitable for organic production. N.Z. J. Crop Hort. Sci. 31:169–177.
Sheffield, C.S., R.F. Smith, and P.G. Kevan. 2005. Perfect syncarpy in apple (Malus ×domestica ‘Summerland McIntosh’) and its implications for pollination, seed distribution and fruit production (Rosaceae: Maloideae). Ann. Bot. 95:583–591.
Stopar, M., A. Gregor, and F. Bati. 2000. Apple fruit abscission influenced by assimilate supply. Acta Hort. 527:169–178.
Untiedt, R. and M. Blanke. 2001. Effects of fruit thinning agents on apple tree canopy photosynthesis and dark respiration. Plant Growth Reg. 35:1–9.
Ward, D.L., R.P. Marini, and R.E. Byers. 2001. Relationships among day of year of drop, seed number, and weight of mature apple fruit. HortScience 36:5–48.
Westwood, M.N. 1978. Temperate zone pomology. W.H. Freeman and Co., San Francisco.