FINAL PROJECT REPORT
WTFRC Project Number: PH-04-0443

Project Title: Regulation of apple fruit ripening

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Budget History:

Item	**Year 1: 2004**	**Year 2: 2005**	**Year 3: 2006**
Salaries	$26,800*	$13,232**	$23,282***
Benefits	$13,200	$6518	$11,468
Wages	$0	$1675	$0
Benefits	$0	$825	$0
Equipment	$0	$0	$0
Supplies	$1500	$1500	$1500
Travel	$0	$0	$0
Miscellaneous	$0	$0	$0
Total	$41,500	$23,750	$36,250

*0.5 FTE Postdoctoral Research Associate
**0.25 FTE Biological Science Technician
***0.44 FTE Biological Science Technician

Objectives:

1. Characterize apple fruit production of nitric oxide during fruit development and ripening.
2. Characterize apple fruit response to exogenous nitric oxide at harvest and during storage.
3. Characterize apple fruit response to activators/inhibitors of nitric oxide metabolism.

Summary Findings:

1. Nitric oxide (˙NO) production increases during apple maturation.
2. Whole apples exposed to ˙NO or NO_2^- can have reduced ethylene production and respiration rate.
3. Development of superficial scald, CO2-induced internal browning, and softening were inconsistently altered by whole fruit exposure to ˙NO or NO_2^- at harvest or during storage.
4. Apples convert NO_2^- to ˙NO in low O_2 or air sufficient to reach ˙NO treatment target concentrations without development of phytotoxicity.
5. Treatment of whole apples with a strobiluron fungicide (a compound reported to stimulate ˙NO production) inconsistently impacts fruit ethylene production and respiration rate.
6. Treatment of cut apple slices with ˙NO donors results in reduced ethylene production relative to non-treated control slices.

Results and Discussion:

Fruit Maturity and ˙NO production

Delicious apples harvested at weekly intervals were assessed for maturity including ethylene and ˙NO production. An increase in ˙NO production between the last two harvest dates accompanied increases in starch score and ethylene production. The increase in ˙NO production, while significant, occurred after the onset of ripening as indicated by increased ethylene production. The increase in ˙NO also is in contrast to published reports indicating an inverse relationship between ethylene and ˙NO production at the onset of fruit ripening. Based on these results with Delicious as well as results with other cultivars, the increase in ˙NO production occurs after ripening has initiated and as such may not be an early indicator of harvest maturity.

Harvest Date	starch	lbs	watercore %	ethylene ppm	NO ppb
Sep 19	1.6	16.0	0	0.0	0.07
23	1.7	17.0	0	1.5	0.00
Oct 1	1.9	17.0	0	7.1	0.23
5	2.0	16.6	0	7.2	0.17
19	3.9	15.9	50	43	0.68
$LSD_{0.05}$	0.4	ns	-	11.3	0.35

LSD: least significant difference; ns: not significant

Table 1. Progression of Delicious maturity including ethylene and ˙NO production. Fruit were evaluated the day after harvest (n=20).

Responses of fruit ethylene production and respiration to exogenous $^{\cdot}$NO and NO_2^-

Delicious apples held in 0.5% O_2 were exposed to 0,1,5,10, or 50 ppm $^{\cdot}$NO or 50 ppm NO_2^- for 2 hours the day of harvest (16.6 lbs, 2.0 starch). During holding in air for 5.5 days at 68 ^oF after treatment, fruit respiration and ethylene production were monitored, and analyses of fruit quality were performed on day 7. All $^{\cdot}$NO and NO_2^- treatments delayed and/or reduced ethylene production with the largest impact from 10 ppm $^{\cdot}$NO. Respiration rate was also reduced by most $^{\cdot}$NO and NO_2^- treatments with the largest impact after exposure to 50 ppm NO_2^- . These results were the basis for subsequent $^{\cdot}$NO and NO_2^- treatments at harvest and during storage.

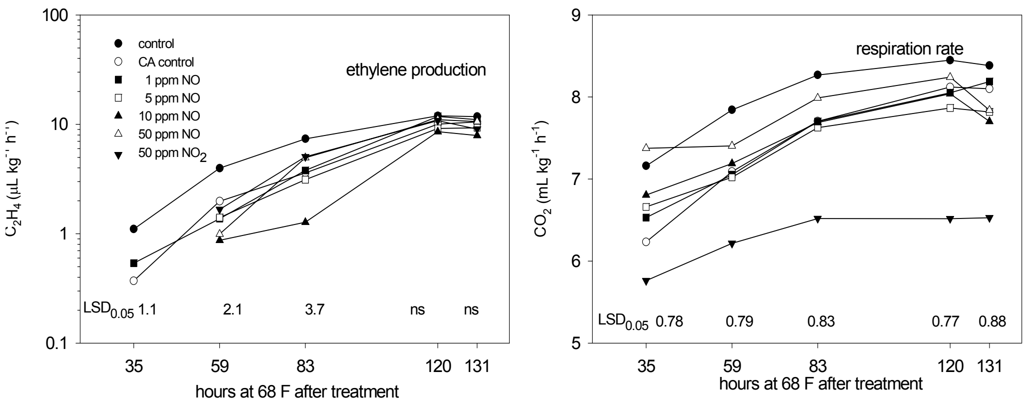

Figure 1. Delicious apple ethylene production and respiration rate. Fruit treated at harvest with $^{\cdot}$NO or NO_2^- then held in air at 68 ^oF .

Impacts of $^{\cdot}$NO and NO_2^- treatments on fruit quality and physiological disorder development

Internal CO_2 injury: Braeburn apples were treated with 1 ppm 1-MCP, 2000 ppm DPA, 1-MCP + DPA, 10 ppm $^{\cdot}$NO , 1-MCP + $^{\cdot}$NO, then stored at 33 ^oF in air or CA (1.5% O_2, 3% CO_2). The only fruit stored in air that developed internal disorders was treated with 1-MCP and stored for 6 months. For fruit stored in CA, controls and 1-MCP-treated apples had the highest rates of injury. DPA treatment prevented injury on control as well as 1-MCP-treated fruit. The $^{\cdot}$NO treatment reduced injury incidence in control and 1-MCP-treated fruit through 4 months, however, after 6 months differences between control, 1-MCP, $^{\cdot}$NO , and 1-MCP+$^{\cdot}$NO were less than at 2 or 4 months.

Months CA	control	1-MCP	DPA	1-MCP/DPA	NO	1-MCP/NO
2	61%	50	0	0	11	11
4	50	44	0	0	6	17
6	28	39	0	0	17	22

Table 2. Incidence (%) of internal browning+cavities in Braeburn apples treated at harvest in 2004. Fruit were stored in CA (1.5% O_2, 3% CO_2) and evaluated 1 day after removal.

A study conducted in 2005-06 did not confirm the 2004 results. $^{\cdot}$NO treatments (10 ppm) at harvest or during CA storage did not effectively control disorder development during CA (1.5% O_2, 3% CO_2) storage. Fruit from two orchards were used in this study, Orchard A was harvested at an earlier date (Oct 8 vs. 18) with greener peel ground color (2.1 vs. 3.9, 1-5 scale; 1=green, 5=yellow). Orchard B was fruit source for 2004 study as well.

Months	control	˙NO at harvest	˙NO weekly	˙NO monthly
4	14	22	28	22
8	67	83	61	56

Orchard A near Royal City, WA.

Months	control	˙NO at harvest	˙NO weekly	˙NO monthly
4	56	35	44	56
8	68	49	47	55

Orchard B near Manson, WA.

Table 3. Incidence (%) of internal browning + cavities in Braeburn apples treated with 10 ppm ˙NO at harvest or during CA (1.5% O_2, 3% CO_2).

A similar lack of internal browning control in ˙NO-treated fruit was observed for Delicious apples in 2005-06. Apples were held for two hours at harvest in sealed jars in air (control), 0.5% O_2, or O.5% O_2 with 10 ppm ˙NO, then stored in RA (5 months) or CA (1% O_2 1% CO_2).

treatment	Orchard 1		Orchard 2		Orchard 3	
	RA	CA	RA	CA	RA	CA
control	0	0	22	41	28	94
0.5% O_2	0	0	33	39	44	83
0.5% O_2+˙NO	0	0	28	59	22	63

Table 4. Incidence of internal browning in Delicious apples treated with 0 or 10 ppm ˙NO at harvest. Fruit stored in air (5 months) or CA (8 months) and evaluated after 7 days at 68 ^oF .

The lack of effective disorder control in 2005-06 indicates the potential for ˙NO treatments to manage internal browning is not consistent across seasons. Factors that may be influencing efficacy remain to be determined.

Superficial Scald

Delicious apples harvested at CA maturity (starch 1.9, 17.7 lbs) in 2004 were exposed to 10 ppm NO while held in 0.5% O_2 for two hours at harvest. Fruit were then stored at 33 ^oF in air or CA (1% O_2, 1% CO_2). No scald developed on fruit stored in CA. For fruit stored in air, scald developed on controls and fruit held in 0.5% O_2 for 2 h at harvest, however, no scald was present on apples exposed to ˙NO. After 6 months, peel analysis for accumulation of conjugated trienes (CT), indicative of α-farnesene oxidation and putatively related to scald development, showed the amount of CT in peel from fruit with scald symptoms was greater compared to ˙NO-treated fruit. ˙NO treatment did not impact fruit ripening as measured by firmness and acidity loss.

treatment	scald %	CT	lbs	TA %	% red
control	28	74	10.1	0.162	89
0.5% O_2	61	75	10.6	0.150	81
0.5% O_2+˙NO	0	30	9.8	0.148	92

CT: conjugated trienes; TA: titratable acidity; % red: visual rating of amount of fruit surface with red color

Table 5. Poststorage quality and scald incidence of Delicious apples. Fruit treated at harvest with 10 ppm ˙NO then stored in air for 6 months. CT: conjugated trienes

Scald incidence was too low to evaluate treatment effects in a similar experiment conducted with Delicious in 2005-06, however, results with Granny Smith did not show a significant ˙NO treatment response for scald reduction.

treatment	Orchard 1		Orchard 2		Orchard 3	
	5M RA	8M CA	5M RA	8M CA	5M RA	8M CA
control	94 %	6	56	33	17	78
0.5% O_2	83	0	67	33	22	89
0.5% O_2+˙NO	89	6	50	44	17	78

Table 6. Granny Smith scald incidence 7 days after removal from storage. Fruit were held in air or 0.5% O_2 with or without 10 ppm ˙NO for 2 hours at 70 ^oF, then stored in air at 33 ^oF or CA (1.5% O_2, 1.5% CO_2) for 5 or 8 months, respectively.

NO treatment during storage

Delicious and Granny Smith apples exposed to 0 or 10 ppm NO at harvest in 2004 then stored in CA (0.5% O_2, 1% CO_2) were exposed to 10 ppm NO or NO_2 twice a week, or 10 ppm NO once or twice a month. ˙NO accumulated in CA chambers following injection of NO_2^- indicating likely conversion of NO_2^- to ˙NO by fruit. Through 6 months, effects of ˙NO or NO_2^- treatment on Delicious firmness, respiration rate and ethylene production were detected, but no other treatment effects were apparent. No impacts from any of the treatments on Granny Smith fruit quality were detected, and no phytotoxicity was observed on either cultivar. There was no incidence of superficial scald on any fruit (NO/NO_2-treated or controls) through 6 months plus 7 days at 68 ^oF .

Delicious: 6 months CA plus 7 days at 68 ^oF.

Treatment	lbs	CO_2 ppm	C_2H_4 ppm	SSC (%)	TA (%)	decay (%)
control	13.2	315	1.88	13.7	0.188	11
˙NO@harvest	13.4	263	2.01	13.0	0.214	6
˙NO monthly	14.4	267	1.62	13.2	0.211	0
˙NO 2Xmonth	15.1	217	0.76	13.2	0.207	11
˙NO 2Xweek	14.4	228	1.16	13.5	0.205	0
NO_2^- 2X week	15.2	277	1.19	13.2	0.178	0
$LSD_{0.05}$	1.8	50	0.74	ns	ns	ns

LSD: least significant difference; ns: not significant

Table 7. Impact of repeated ˙NO and NO_2^- treatments on Delicious quality. ˙NO and NO_2^- concentrations were 10 ppm for 2 hours during treatments.

Similar experiments conducted in 2005-06 using 3 lots each of Delicious and Granny Smith with treatments limited to weekly or monthly ˙NO addition did not result in observable impacts on scald development, internal disorders, or fruit quality throughout an 8 month storage period. The lack of responses in 2005-06 to either ˙NO treatments at harvest or during storage indicates minimal likelihood of development of ˙NO gas treatments as performed in these studies as an effective means to manage fruit quality in the postharvest environment.

Fruit responses to activators of ˙NO production

Studies related to Objective 3 were conducted initially using cortex disks prepared from Golden Delicious apples. Treatments were applied as aqueous solutions to disks contained in 50 mL Erlenmeyer flasks. Solutions contained either potassium nitrite (NO_2-), *S*-nitrosoglutathione (GSNO), a ˙NO donor, oxidized glutathione (GSSG), a GSNO control, sodium nitroprusside (SNP), also a ˙NO donor, sodium ferrocyanide [$NaFe(CN_6)$], a SNP control, or potassium nitrite (NO_2^-). NO_2^- can be metabolized to form ˙NO in plant tissues. Following a 30 min equilibration period, flasks were sealed for an additional 30 min then sampled to measure evolved ethylene and ˙NO.

Treatment with NO_2^- resulted in increased ˙NO production and, likewise, decreased ethylene biosynthesis. Generation of ˙NO increased linearly while ethylene generation decreased exponentially with increasing NO_2^- treatment concentration. Treatment with solutions containing the ˙NO donors GSNO and SNP reduced ethylene biosynthesis compared to treatments containing equimolar concentrations of GSSG or sodium ferrocyanide, respectively. GSSG and sodium ferrocyanide did not affect ethylene biosynthesis. These results indicate ethylene production in apple disks can be manipulated by the presence of ˙NO. These results may have commercial potential for sliced apples if the reductions in ethylene production and respiration rate are sufficient to slow quality deterioration. No studies to investigate that potential have been conducted.

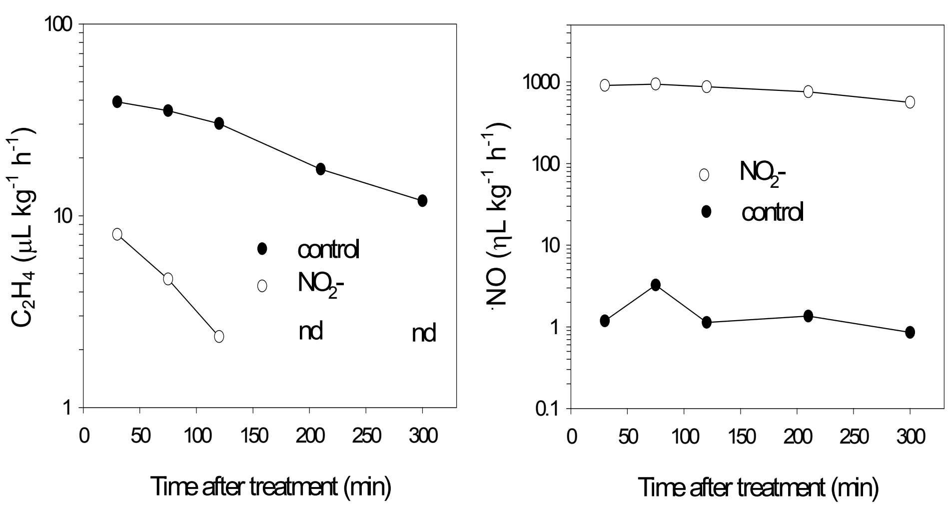

Figure 2. Ethylene and ˙NO production by Golden Delicious apple fruit disks exposed to nitrite. nd: not detected.

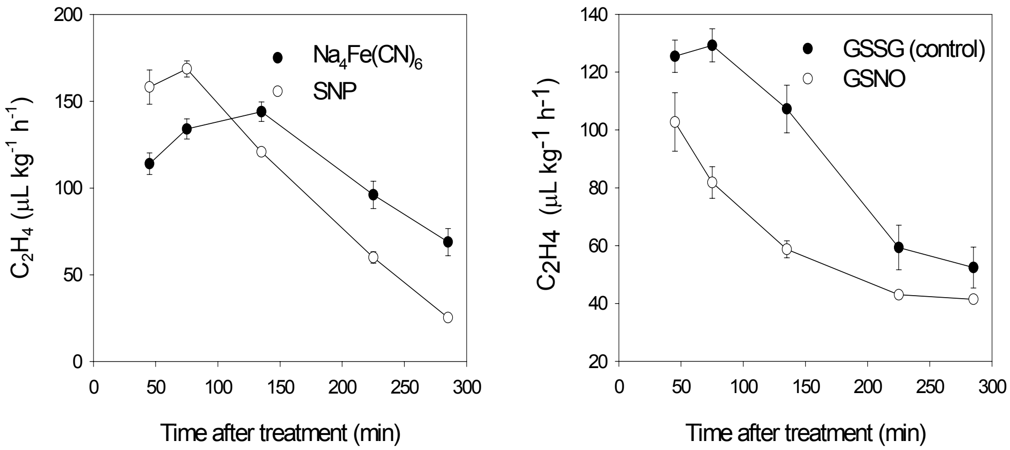

Figure 3. Ethylene (C_2H_4) production by 'Golden Delicious' apple fruit disks. Disks were exposed to solutions containing $^{\cdot}$NO releasers sodium nitroprusside (SNP), S-nitrosoglutathione (GSNO), or controls containing sodium ferrocyanide [$Na_4Fe(CN)_6$]or oxidized glutathione (GSSG).

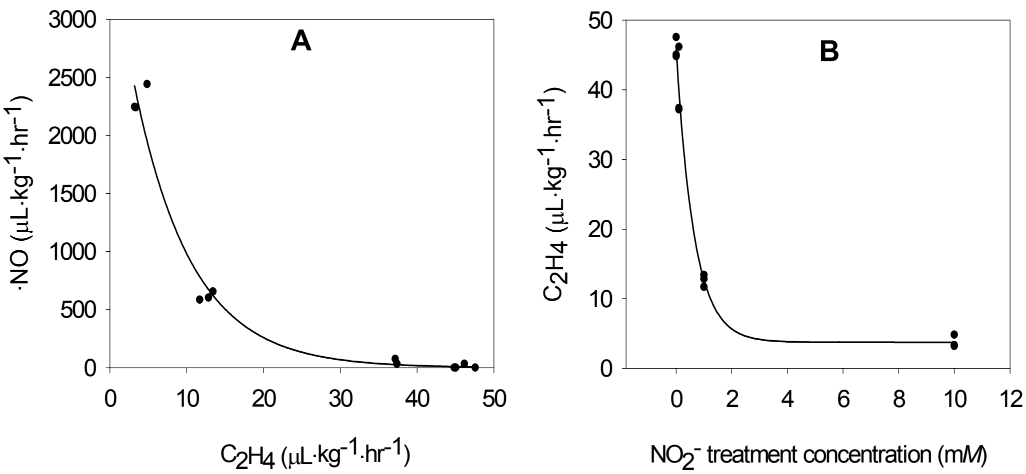

Figure 4. Relationships between $^{\cdot}$NO and ethylene production in response to treatment of 'Golden Delicious' apple disks with increasing concentrations of nitrite.

The reactive nature of $^{\cdot}$NO also prompted a study examining whether a direct reaction between $^{\cdot}$NO and ethylene occurs that results in reduced ethylene concentration. Both compounds were injected into sealed glass jars to a concentration of 10 ppm. One jar contained air, the other was purged with nitrogen prior to introduction of $^{\cdot}$NO and ethylene to reduce the rate of $^{\cdot}$NO oxidation by O_2 in air. Ethylene concentration did not change over a 3 hour incubation period in either jar indicating no reaction between these compounds is favored under the conditions of the experiment. In light of this result, changes in ethylene production by apple disks in response to exposure to $^{\cdot}$NO donors or nitrite may be the result of metabolic rather than chemical changes promoted by $^{\cdot}$NO.

Studies with pyraclostrobin, a strobiluron fungicide, and putative stimulant of plant ˙NO production

Strobilurons are a class of compounds with fungicidal activity that have been demonstrated to stimulate plant ˙NO production. Strobiluron fungicidal activity is thought to occur primarily via inhibition of mitochondrial electron transport and respiration rate. A commercial material (Cabrio, BASF, 20% a.i.) was used in 2006 to evaluate the potential of this water dispersible compound to impact fruit ripening via alteration of fruit ˙NO metabolism. Studies were conducted with several cultivars (Early Gold, Gala, Golden Supreme, Delicious, and Granny Smith) where fruit were treated at harvest with Cabrio at concentrations up to 4000 ppm with or without surfactant. Apples were held in air at 68 ^oF or in cold storage and evaluated for treatment effects on fruit quality and disorder development. Consistent positive impacts on fruit quality or decreased disorder development have not been observed with any cultivar.

In an experiment with Early Gold apples, fruit treated with up to 1000 ppm Cabrio in water had lower ˙NO production relative to untreated controls after 7 days at 70 ^oF.

treatment	lbs	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	5.8	938	13.9	2.7
Cabrio: 10 ppm	6.1	914	12.5	1.8
100	6.1	823	12.8	1.6
1000	5.9	935	13.0	1.5
$LSD_{0.05}$	ns	ns	ns	0.5

Table 8. Early Gold firmness, respiration rate, and ethylene and ˙NO production after 7 days at 68 ^oF. Fruit were treated at harvest with water or Cabrio in water without surfactant.

Golden Supreme apples treated with 4000 ppm Cabrio had higher color ratings (yellower), higher ethylene and ˙NO production and higher respiration rate compared to controls 7 days after treatment.

treatment	green-yellow 1-5	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	2.3	215	1.8	0.27
Cabrio:4000 ppm	3.0	348	5.8	0.43
$t\text{-test}_{0.05}$	0.4	85	2.2	0.11

Table 9. Golden Supreme peel color, respiration rate and ethylene and ˙NO production after 7 days at 68 ^oF after treatment. Fruit were treated at harvest with water or Cabrio in water with surfactant.

Gala apples treated with up to 4000 ppm Cabrio had lower firmness, and higher respiration and ethylene production compared to controls 7 days after treatment.

treatment	lbs	CO_2 ppm	C_2H_4 ppm	˙NO ppb
control	16.5	466	1.9	0.27
Cabrio: 100 ppm	16.7	469	1.9	0.3
1000	15.4	539	2.7	0.3
4000	15.4	536	3.2	0.23
$LSD_{0.05}$	1.0	48	0.6	ns

Table 10. Gala responses to pyraclostrobin (Cabrio). Fruit treated at harvest in water plus surfactant with or without pyraclostrobin, then held in air for 7 days at 68 ^oF.

Cabrio treatment effects on Delicious apples were concentration dependent. Fruit respiration rate was enhanced following treatment at 100 or 1000 ppm Cabrio, but reduced by 4000 ppm treatment relative to controls. Similar effects on ethylene or ˙NO production were not observed, nor were impacts on fruit quality detected.

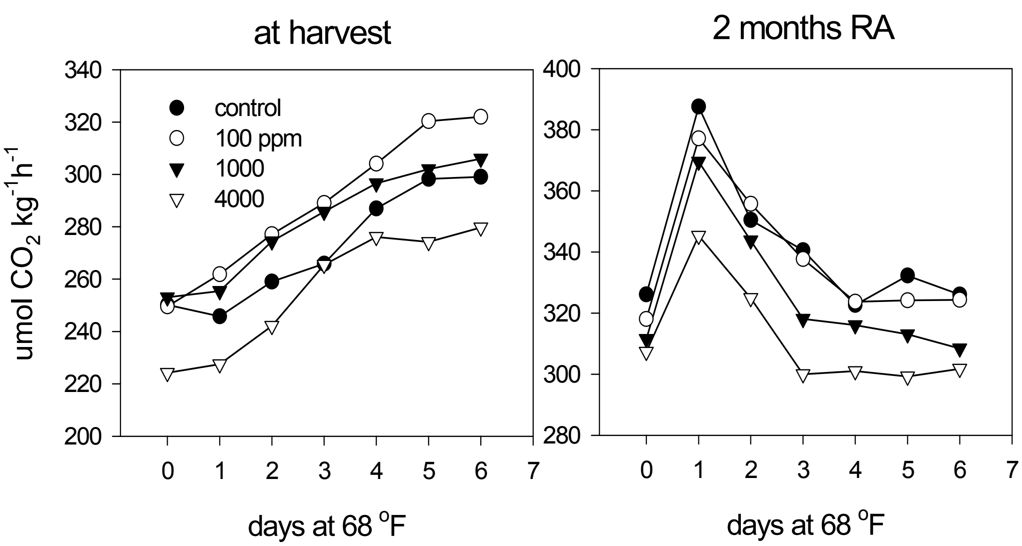

Figure 4. Delicious respiration following treatment with Cabrio with surfactant. Fruit were held at 68 °F after 0 or 2 months cold storage in air.

No Cabrio treatment have been observed to date for Granny Smith apples from two harvest dates (commercial, commercial plus 3 weeks) treated with up to 4000 ppm Cabrio then stored in RA.

The results with this strobiluron fungicide do not support an active role of the material for altering postharvest ripening of apples in a manner consistent with commercial development. Where physiological impacts on respiration or ˙NO production were observed, no accompanying quality or disorder development effects have been observed. The lack of consistent alterations in fruit ˙NO production is in contrast to published reports with vegetative plant tissues where increased ˙NO emission occurs following treatment. Whether formulation issues contributed in part to the lack of consistent treatment effects (the material used is labeled for field use) is unknown.

General Summary

Results of studies conducted over 3 crop years show apple fruit produce ˙NO during development and ripening (ripening data not presented). The pattern of endogenous ˙NO production in relation to fruit maturation is not consistent with previous reports indicating ˙NO and ethylene have an antagonistic relationship (i.e. increased ˙NO accompanied by lower ethylene production). This trend was observed in cut apple slices treated with ˙NO donors and in some experiments where whole fruit were exposed to ˙NO or NO_2^- , but consistent impacts of these treatments on fruit quality or disorder development have not been apparent. Factors that could possibly influence treatment responses (˙NO, NO_2^- gas diffusivity, maturity, seasonality, storage conditions) require further investigation.

However, no continuation of this project is proposed due to a perceived high risk of success based on results to date. While roles for nitrogen radicals as effectors of developmental regulation as well as plant responses to stress are increasingly well documented in plant tissues, an obvious horticultural utility in the postharvest system for apple fruit is not apparent based on the studies conducted in this 3 year project.