FINAL PROJECT REPORT **PERIOD:** 2 year of 2 years

Project Title: WA 38: understanding green spot origin, timeline, and development

WTFRC Project Number: AP-20-103A

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Total Project Request: **Year 1:** $108,875 **Year 2:** $111,790 **Total request: $220,665**

Budget 1
Primary PI: Stefano Musacchi
Organization Name: Washington State University
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Item	(Year 1 (2020)	Year 2 (2021)
Salaries	$54,000	$56,160
Benefits	$18,875	$19,630
Plot Fees	$3,000	$3,000
Consumables	$14,000	$14,000
Travel	$4,000	$4,000
Total	**$93,875**	**$96,790**

Budget 2
Organization Name: USDA
Contract Administrator: Chuck Myers
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Item	(Year 1 (2020)	Year 2 (2021)
Good and Service (LCMS annual service)	$12,000	$12,000
Consumables	$3,000	$3,000
Total	**$15,000**	**$15,000**

RE-CAP OBJECTIVES:

1. Determine the effect of bagging fruit on the intensity of green spot appearance
2. Verifying the effect of netting on green spot appearance along the season

SIGNIFICANT FINDINGS:

1. Determine the effect of bagging fruit on the intensity of green spot appearance.

- GS symptoms started to appear at the end of July and became clearer within 4 weeks from onset.
- The GS area showed damage to lenticels, and the chemistry of sunken GS revealed signs of localized osmotic stress in the peel. This seems to be a reaction to the lenticel damage.
- Differences in average daily humidity were found inside bagged treatment (higher avg. %RH from June to August) for both years. These variations in the microenvironment may have contributed to a reduction in the incidence of GS in the bagged treatment compared to the control.
- Significantly higher GS incidence in WA 38/G41 and WA 38/NIC29 control trees with respect to the corresponding bagged treatments in both years.
- All apples, regardless of the rootstock, if closed in the bags before July 2, showed 0-1% GS incidence.
- In both years, WA 38/G41 proved to be more prone to GS than WA 38/NIC29.

2. Verifying the effect of netting on green spot appearance along the season.

- Comparing the GS incidence between "netted" and control apples within each rootstock did not reveal any significant differences in 2020. In 2021, we analyzed the four treatments together. We found apples belonging to G41 control trees had higher and statistically different GS incidence than apples picked from netted G41 and NIC29 control and netted trees.
- WA 38-netted/NIC29 and control-NIC29 were the two combinations showing the lower incidence of GS across the two years, indicating a rootstock effect.

RESULTS AND DISCUSSION:

1. Determine the effect of bagging fruit on the intensity of green spot appearance

Bagging

In 2021, the time point bagging experiment was repeated in the same WA 38 block planted in 2013 [Sunrise Research Orchard (SRO), Rock Island, WA] as in 2020 but selecting fewer time points along the season. WA 38 trees grafted on Geneva 41 (G41) or M9-NIC29 (NIC29) and trained to spindle [10 ft × 3 ft (=1499 trees/A)] were selected for similar fruit load in early June 2021. Then, trees were randomly assigned experimental groups, either as *control* (fruit never bagged) or *bagged* at a designated time point over the season. Whereas in 2020, we performed bagging at six different time points (starting the first week of June and ending the last week of August), in 2021, the bagging experiment focused only on the time points before the onset of green spot (GS) (usually the end of July). Beginning 6/18/21, the apples of 3 trees per time point per rootstock (2) were bagged approximately two weeks apart until 7/16/21. Time points were named T1, T2, and T3, corresponding to the dates 6/18/21,7/2/21, and 7/16/21, respectively. For each rootstock combination, 3 trees were not bagged and designated as controls. The present trial was carried out on a total of 24 trees (18 bagged and 6 control). Before bagging, each cluster was hand-thinned to a single fruit. Then, each singularized apple was inserted into a two-layer commercial fruit bag and secured with a twist-tie. On 9/24/21, all 24 trees were harvested, and apples were stored in RA at 33 °F until grading. Across the 24 trees, yield ranged between 27 and 99 apples/tree with a productivity range between 8 and 26 kg/tree. Seven weeks after harvest, all apples were sized and categorized from <65 to 105 mm (5 mm increment scale), and visually graded for color, defects, GS incidence and types, and general packout, keeping the protocol consistent with the 2020 grading. GS was graded by increasing severity on a scale from 1 – 4, with GS1 including fruit with few green spots, small in diameter (less than the diameter of a pencil eraser), or pronounced green halos around lenticels, progressing in severity up to GS4 consisting of severe GS symptoms with cracking. Fruits were scored GS5 if the green spots appeared to be a darker brown color, even if cracking was not present. Finally, GS6 was reserved for fruit showing the superficial "flecking" GS symptoms regardless of severity.

Fruit grading

In general, the bagging technique significantly reduced the percentage of culled apples in WA 38/G41 (25%) compared to control trees (56%); however, bagging WA 38/NIC29 apples did not statistically reduce the percentage of culled apples (22%) with respect to the control (35%, data not shown). Regarding size, the distribution of apples harvested from WA 38/G41 bagged trees reported a smaller proportion of small apples (70-75 mm category; 10%) than WA 38/G41 control trees (21%). Average fruit weight also significantly differed between WA 38/G41 treatments, with control apples averaging 237 g while bagged apples averaged 276 g (data not shown).

GS incidence at harvest for each experimental treatment was reported as the number of apples presenting the disorder (regardless of the type and severity) over the total apples graded per tree in percentage. In general, GS incidence in 2021 control trees (22%) was lower than in 2020 control trees (41%, data not shown). The comparisons of the GS incidence in bagged trees versus control trees within each rootstock and year are reported in Table 1. Results showed a significantly higher GS incidence in the control trees for both rootstocks in 2021; GS incidence was 29% in WA 38/G41 control trees compared to 8% in WA 38/G41 bagged trees, and 15% in WA 38/NIC29 control trees compared to 3% in WA 38/NIC29 bagged trees.

Table 1. *WA 38 GS incidence (%) in 2020 and 2021: comparison between apples inside the bags and apples grown in control trees (no fruit bags) within each rootstock. Significance by treatment (2020 and 2021) within each rootstock: *=p<0.05, **=p<0.01 and NS=no significant difference.*

Rootstock	trt	*N trees 2020=*	*2020 GS incidence (%)*[1]		*N trees 2021=*	*2021 GS incidence (%)*[1]	
G41	Bagged	*18*	*27.9*	*B*	*9*	*7.6*	*B*
G41	Control	*9*	*60.6*	*A*	*3*	*29.4*	*A*
Significance			**			*	
NIC29	Bagged	*18*	*27.2*		*9*	*3.2*	*B*
NIC29	Control	*9*	*21.8*		*3*	*15.2*	*A*
Significance			*NS*			*	

[1] Apples presenting one or more types of GS were counted just once (presence/absence of GS regardless of the type)

The "bagged" treatment in Table 1 for 2021 was represented by 3 separate time points (6/1/21, 6/18/21, and 7/2/21) averaged together. Comparing the GS incidence across the time points for each rootstock allows us to partially confirm results obtained in 2020. Figure 1 shows the incidence of GS (%) on WA 38 for each of the times of bagging in both years; all apples, regardless of the rootstock, if closed in the bags before July 2, showed 0-1% GS. Starting from the first week of July (7/6/2020 and 7/2/2021), the disorder frequency increased in the bagged treatments (mainly as type GS1 and some GS5 cases), suggesting that early-season bagging can mitigate but not suppress GS development.

Data analysis of GS incidence by type and time point in 2021 revealed GS6 was found only in apples harvested from control trees on both the rootstocks but absent in the 3 timepoints of the bagged trees.

The most frequently identified types of GS inside the bags were GS1, GS5, and GS2 but only in the WA 38/G41 trees (data not shown).
GS accounted for only 6% of the total cullage by a defect in bagged fruit. In contrast, misshapenness, sunburn inside the bag, limb rub, bruising, or mechanical damage accounted for most of the cullage (data not shown). On the other hand, 20% of the culled control apples were judged unmarketable regardless of the rootstock due to GS damage. Aside from green spot, the most common cause of cull for control apples in 2021 was general mechanical damages, including stem punctures from harvest fruit handling and picking.

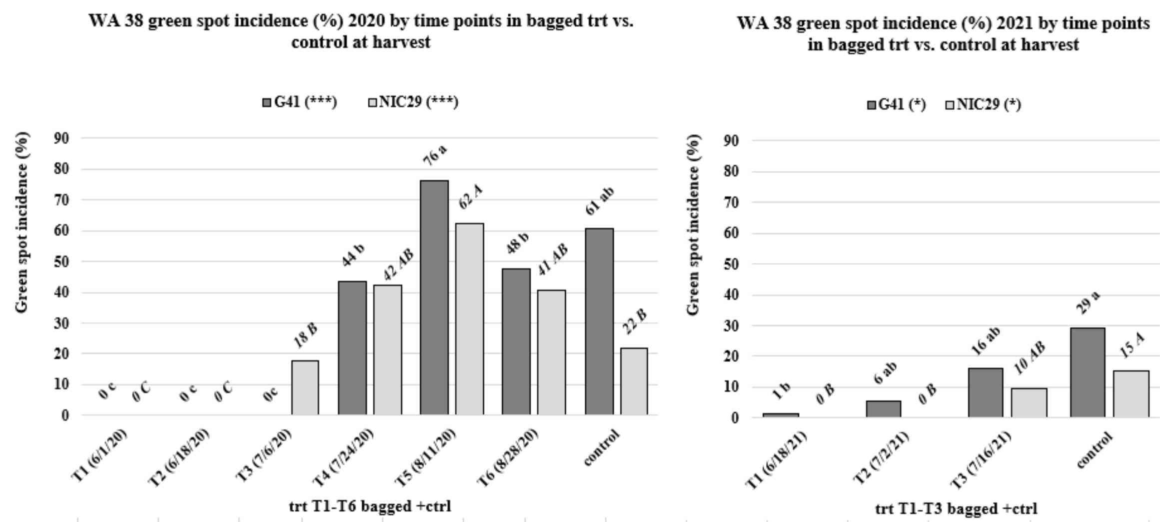


Figure 1*. Comparison between GS incidence (%) in WA 38 apples inside the bags at each time point and apples grown in control trees within each rootstock: G41 and NIC29 after harvest 2020 and 2021. Significance by time points within each rootstock: *=p<0.05 and ***=p<0.001, capital letters distinguish the incidence for NIC29 in each year and lowercase letter in G41 in the 2 years.*

Microclimate monitoring

On 6/18/21, twelve dataloggers (iButton®, Maxim Integrated) were deployed in 6 WA 38/G41 spindle trees to monitor temperature and humidity over the course of the growing season as done in 2020. Three trees were selected in the T1 bagging time point, and three control trees were selected for microclimate monitoring. Dataloggers were placed inside the fruit bags in the "bagged" treatment or secured to a branch in control trees. All sensors collected temperature and relative humidity data throughout the season. In each of the six trees, 2 dataloggers were installed: one located in the "upper canopy" (avg. height from the ground = 225 cm) and one in the "lower canopy" (avg. height from the ground = 132 cm). Dataloggers were removed at harvest on 9/24/21.
Daily average temperatures, daily minimum temperatures, daily maximum temperatures, daily average percent relative humidity (%RH), daily minimum %RH, and daily maximum %RH were averaged by month and analyzed in SAS 9.4 using proc GLM and SNK test for both years (Table 2A and B). In 2021, the analysis indicated no significant differences between the bagged and control treatments in daily average temperatures, daily minimum temperatures, and daily maximum temperatures for June and August 2021; while in July 2021, the daily temperatures (avg., min, and max) were always significantly higher in the bagged treatment an average difference of less than 1°C. The only significant difference that emerged in September was the daily minimum temperature, where the minimum temperature inside the bags was higher than the unbagged control (Table 2B). These results are slightly different from the 2020 results (Table 2A), which showed higher daily max temperatures in the control

than bagged in July and August 2020. The current year, 2021, was reported as a peculiar year for Western North America climate history because of the heatwave that affected the early summer season. This climatic event could be implicated in the different results we obtained for control vs bagged fruit temperature parameters, mainly in July 2021 (Table 2B). The bagging treatment seemed to keep daily average temperatures, daily minimum temperatures, daily maximum temperatures more similar between high canopy location with respect to medium canopy (data not shown). When looking at the same parameters in the control trees, averaging all the months together, the temperatures resulted significantly higher in the high canopy level than the medium one (data not shown). Comparison of relative humidity parameters between treatments within each month in 2021 confirmed the trend observed in 2020 (Table 2A and B). Significantly higher values for daily average %RH, minimum daily %RH, and maximum daily %RH were reported in bagged fruit in June and July 2021 compared to control fruit humidity parameters (Table 2B). However, in August and September 2021, significant differences between bagged and control groups only emerged when comparing daily average %RH and/or minimum daily %RH, with the former treatment showing always greater average values than the latter (Table 2B). The largest difference in daily average %RH in 2021 between the bagged and control treatments was found in June with 43.4%RH and 35.6%RH, respectively.
These results from two consecutive seasons highlighted the effect of bagging on retaining humidity. These differences in the microenvironment may have contributed to a reduction in the incidence of GS in the bagged treatment compared to the control.

Table 2. *Comparison of temperature and relative humidity measurements inside WA 38 fruit bags versus outside fruit bags in 2020 (A) and 2021 (B). Average daily temperatures, minimum daily temperatures, maximum daily temperatures, average daily percent relative humidity (%RH), minimum daily %RH, and maximum daily %RH were averaged by month and statistically analyzed using SAS 9.4 proc GLM/SNK test.*

A)					**2020**									
Months	**trt**	**N**	**Average Temperature (°C)**		**Min Temperature (°C)**		**Max Temperature (°C)**		**Average RH (%)**		**Min RH (%)**		**Max RH (%)**	
Jun	bagged	180	20.7		14.5		28.4		54.0	A	38.3	A	71.3	
Jun	CONTROL	180	20.9		14.4		29.0		47.6	B	28.6	B	69.1	
Significance			NS		NS		NS		***		***		NS	
Jul	bagged	186	24.6		17.4		32.8	B	45.1	A	29.7	A	62.9	
Jul	CONTROL	186	24.8		17.1		33.7	A	41.7	B	24.0	B	62.4	
Significance			NS		NS		*		***		***		NS	
Aug	bagged	186	24.3		17.3		32.2	B	45.2	A	30.3	A	62.5	
Aug	CONTROL	186	24.5		16.8		33.5	A	41.7	B	23.6	B	62.8	
Significance			NS		NS		***		***		***		NS	
Sep	bagged	132	20.1		13.9		26.9		58.8		42.3	A	75.5	B
Sep	CONTROL	132	20.2		13.5		28.1		57.8		37.4	B	78.5	A
Significance			NS		NS		NS (0.059)		NS		**		*	
B)					**2021**									
Months	**trt**	**N**	**Average Temperature (°C)**		**Min Temperature (°C)**		**Max Temperature (°C)**		**Average RH (%)**		**Min RH (%)**		**Max RH (%)**	
Jun	bagged	78	30.4		22.2		39.5		43.4	A	31.5	A	57.9	A
Jun	CONTROL	78	29.9		21.8		39.2		35.6	B	19.9	B	53.5	B
Significance			NS		NS		NS		***		***		**	
Jul	bagged	186	28.3	A	20.8	A	37.1	A	41.7	A	28.7	A	56.4	A
Jul	CONTROL	186	27.7	B	20.3	B	36.2	B	37.4	B	21.9	B	54.4	B
Significance			**		*		***		***		***		**	
Aug	bagged	186	24.5		18.5		32.2		48.9	A	34.0	A	63.7	
Aug	CONTROL	186	24.0		17.7		31.8		46.6	B	28.5	B	65.4	
Significance			NS		NS		NS		**		***		NS	
Sep	bagged	144	18.7		12.6	A	26.3		59.4		41.0	A	77.6	
Sep	CONTROL	144	18.2		11.9	B	25.6		57.6		35.8	B	79.9	
Significance			NS		*		NS		NS		***		NS	

N= 2 positions in the canopy *n days recorded in the month * 3 experimental trees per each trt. Significance: NS=not significant, *= $p<0.05$, ** $p<0.01$, *** $p<0.001$. Different letters on the right of the means indicate significant discrimination by SNK ($p<0.05$)in column.

Green spot scouting (2020-2021)

Our first observations of GS in 2020 occurred in late July and were photographed on 7/29/20. In July, appearing symptoms were typically minor discolorations that turned out to be more easily visible by contrast with developing overcolor in the following weeks. GS symptoms typically worsened until harvest. In some cases of superficial GS symptoms, the intense red overcolor can result in covering up the disorder by harvest time; however, this did not happen with sunken-type GS symptoms. Beginning in June 2021, the experimental block was scouted weekly for signs of GS onset. Examples of apples with emerging signs of the GS were photographed and tagged for development tracking. We started tracking specific apples on 7/22/21 for 10 consecutive weeks taking pictures of the area of the fruit interested by the disorder to monitor the evolution of the green spot. In 2021, the GS disorder symptoms became evident around the end of July, with clear manifestation within the first 10 days of August; this is consistent with the timeline of GS emergence observed in the same block in 2020. From the visual evolution of the disorder in apples tracked in 2021 (Figure 2), we can notice that at harvest, the disorder seemed to appear as a worsened stage (i.e., GS4; cracking over green spots) of milder GS symptoms (i.e., GS1) that emerged in July.

Moreover, some dark brown-spotted areas (classified as GS5) appeared at the beginning of August 2021; note the brown spots originated as green spots earlier in the season (Figure 2). A possible involvement of lenticels as weaker structures within GS-impacted areas has been hypothesized during this study (Figure 3). The red pigmentation of the peel appeared disrupted in the GS areas early in the season with clear contrasted margins (green/red), but at harvest, the majority of the apples presented the typical intense red coloration covering up the majority of the areas, although green spots were still visible.

Figure 2. *WA 38 apple tracking of GS development from onset to harvest 2021 in a weekly interval. Seven apples displayed here are representing a subsample of the fruit we tracked for 10 weeks from 7/22/21 to 9/23/21 to describe the GS evolution along the season.*

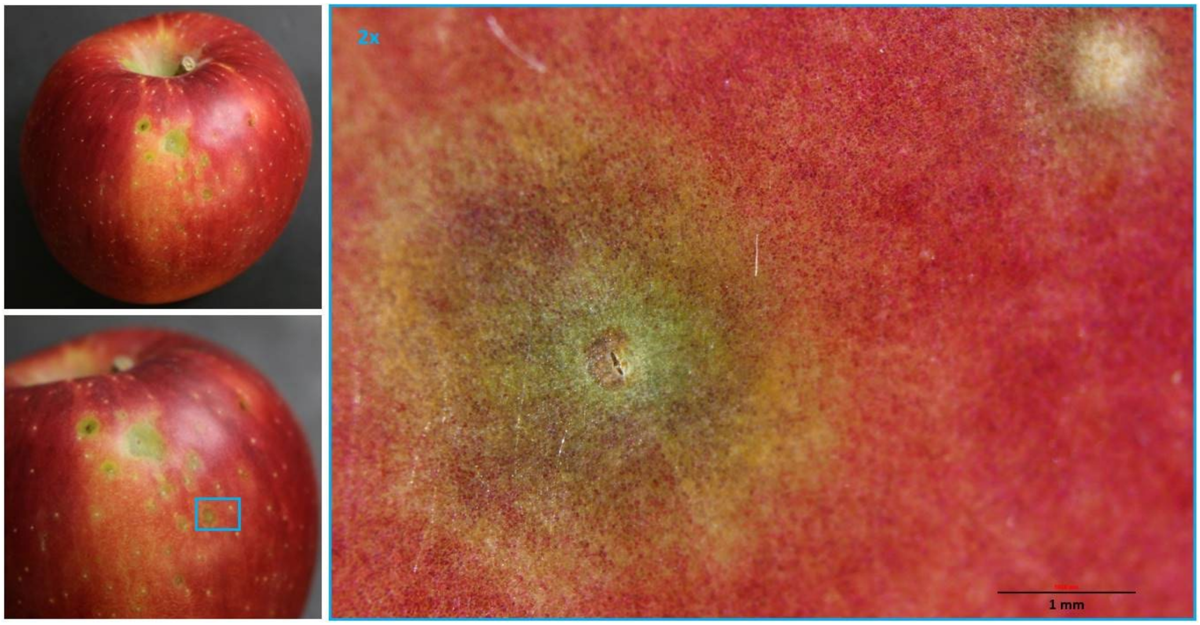


Figure 3. *Details of green spot and lenticel damage on WA 38 apple.*

“**Chemistry of green spot”**

An initial study was conducted in 2019 to determine the effects of time point bagging on GS incidence. Visual assessment at harvest showed GS incidence was reduced in some bagging treatments, and bagging appeared to reduce GS when done earlier in the season more effectively. We collected apple peel and cortex tissue from WA 38/G41 trees at harvest for LCMS (liquid chromatography-mass spectrometry) analysis to understand how metabolic profiles of healthy tissues differ from those tissues affected by either superficial GS (GS6) or severe, sunken GS (GS3, GS4). Apples picked at harvest were brought to the WSU-TFREC lab, where peel and cortex samples were prepared for metabolomic analysis. Peel and cortex tissue samples were immediately frozen in liquid nitrogen and stored at -112 ^{0}F until LCMS analysis.

Our broad chemical analysis of green spot (GS) has indicated key differences among different symptomatic peels, cortex, and surrounding healthy tissue categories. As may be expected, given differences in appearance, the chemistry of both cortex and peel differed depending upon the symptoms (sunken or superficial) or which symptoms the tissue was surrounding (Figures 4-5). This may point to differences in chemistry resulting from maturity, genetics, or interaction with an environmental factor that triggers the more severe necrosis of the cortex underlying the GS. Different amounts of natural chemicals elevated in different tissues can often tell us about interactions between the fruit and the environment that may lead to (or protect from) symptom development and the mechanisms the fruit uses to cope with the stress.

Often “symptoms” are merely the physical manifestations of plant defenses. For instance, levels of two compounds, glucose, and sorbitol, that can accumulate in tissues to relieve osmotic stress, were elevated in sunken tissue and tissue surrounding sunken tissue, indicating a response triggered by light, water, or heat stress associated with the development of the more severe symptoms (Figure 5). Other evidence of stress includes elevated levels of chemicals associated with photoprotection, barrier formation, nitrogen metabolism, and cell wall chemistry. While other photoprotective pigments accumulate, related compounds responsible for red peel color disappear. It is also interesting to note that these typically light-related peel compounds are also elevated in the sunken symptomatic cortex. Our ongoing analysis points to a transformation of the flesh (or “parenchyma”) cells to produce chemicals typically only found on the peel surface (epidermis). In fact, some of the compounds that are most indicative of this response are a relatively unstudied class of large molecules called pentacyclic triterpenes that are loosely similar to steroids in animal systems but, in plants, may help improve the properties of the epidermis and, in this case, barrier layers. A common plant defense mechanism is to form a barrier to prevent pathogen invasion or water loss. This is what appears to be happening in the case of sunken green spot—localized osmotic stress in the peel, eventually leading to a barrier-forming defense response in deeper tissue (Figure 5). Determining precisely when these chemical changes occur, if they are pre-symptomatic changes leading to the disorder, and what environmental cues provoke them could lead to defining the disorder's causes.

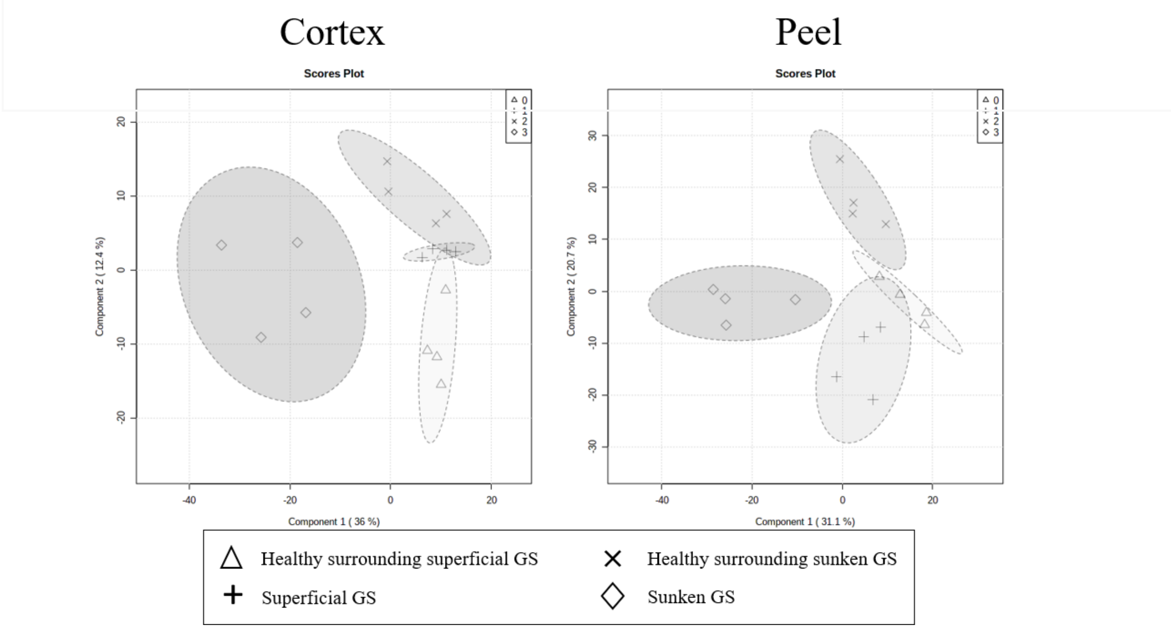
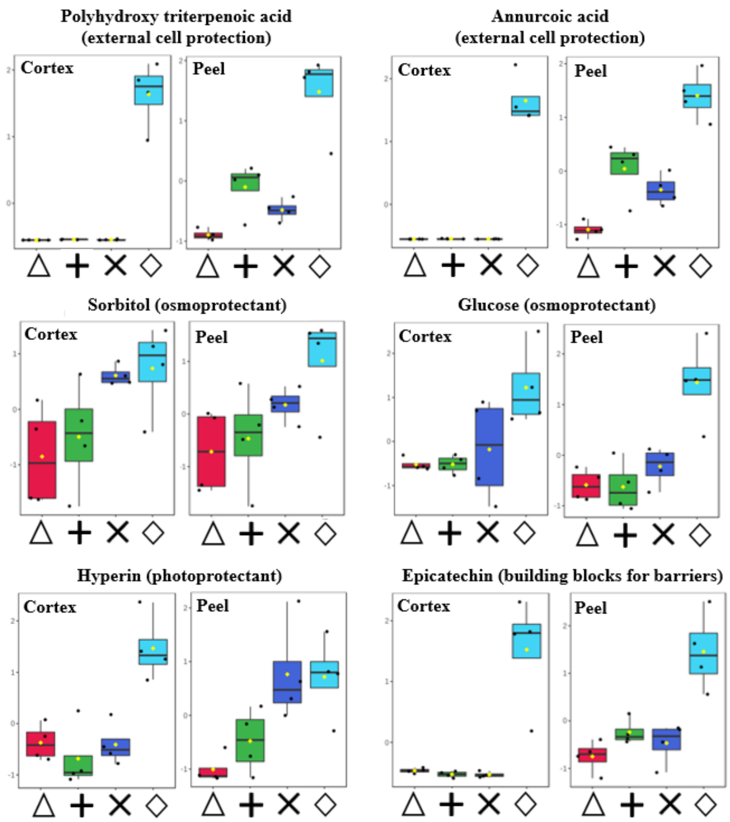


Figure 4. *WA 38 cortex and peel chemistry are different depending upon green spot (GS) symptom category. In 2019, an analysis of over 800 natural peel and cortex chemicals from symptomatic and surrounding tissue reveals different amounts of many chemicals. Different shaded ellipses represent the degree of these differences as these ellipses' relative position indicates how closely chemistries of each tissue are related to each other. This analysis can be used to rank each natural chemical difference with respect to GS severity.*

In 2020, a time point bagging approach was combined with time point sampling throughout the season to understand how peel chemistry changes over the course of a season, how it is influenced by bagging, and how we can identify chemical signatures associated with GS onset in WA 38. Full bloom in the present block was 4/18/2020. A select group of trees was enclosed in drape netting the first week of June, coinciding with the first bagging time point. The second bagging time point occurred on 6/18/20, and for each subsequent sampling time point, peel samples were collected from each of the previous bagging time points in addition to control samples (never bagged). This enabled us to study the chemistry of WA 38 peels as the fruit develops and matures throughout the season as well as the influence of time point bagging and netting. In 2020, GS symptoms emerged at the end of July and early August, and 8/11/20 (116 days after full bloom (DAFB)) was considered the GS onset time point for sampling. At this time point, we sampled an additional set of replicates with GS symptoms that could only be considered superficial GS (GS6) at the time sampling. This time point was used as a focal point to study metabolomic differences between GS and healthy fruit, and the effect of time point bagging on peel chemistry. Sampling was conducted by collecting 5 fruit replications from each treatment, transporting them to the WSU-TFREC lab in Wenatchee, and sampling whole peels from independent fruit replicates into liquid nitrogen. Frozen samples were stored at -112 °F until they were processed for liquid chromatography–mass spectrometry (LCMS) analysis.



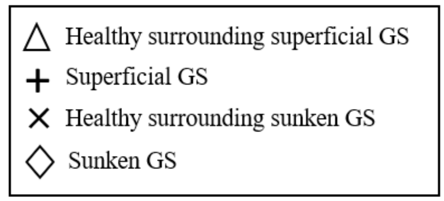


Figure 5. *Examples (out of over 800 screened to date) of natural peel and cortex chemicals associated with different types of green spot (GS) symptoms and tissue immediately surrounding the disorder at harvest 2019. These examples appear to outline a process by which osmotic stress may provoke a multifaceted defense response, including photoprotection and barrier formation and, eventually, more severe, sunken symptoms. In 2020 we sampled at multiple time points during the season to help determine which cultural and environmental events trigger these chemical changes to detect the event before symptoms occur.*

To find changes in levels of chemicals associated with events that may lead to symptom development, we tracked levels of these chemicals in peel from 62-153 DAFB to check for trends different between bagged peel (a treatment that lessens green spot incidence) and control (Figure 6). Peel chemistry differed between bagged and unbagged, even at the earliest sampling date (approximately 62 DAFB after the first bagging time point). Not surprisingly, chemicals associated with peel color and light exposure, such as chlorophylls and carotenoids, were different between the two treatments. However, other chemicals associated with symptoms but not commonly associated with light exposure were also different. For instance, natural chemicals associated with barrier formation found in higher levels in symptomatic peel and cortex were produced most in peels of control apples (not bagged), where production began around the same time the first green spot symptoms were recorded on control peel (116 DAFB) (Figure 7). Elevated levels of these chemicals were also associated with the onset of GS in peels sampled at 116 DAFB (Figure 6).

Because symptoms typically appear on or near the shoulder and not on every fruit, chemicals with the most variable levels may be those of interest early when the conditions and initial metabolic response actually occur. This makes it difficult to pinpoint that metabolic event. Further work will focus on techniques that will help us determine only metabolic events around those lenticels.

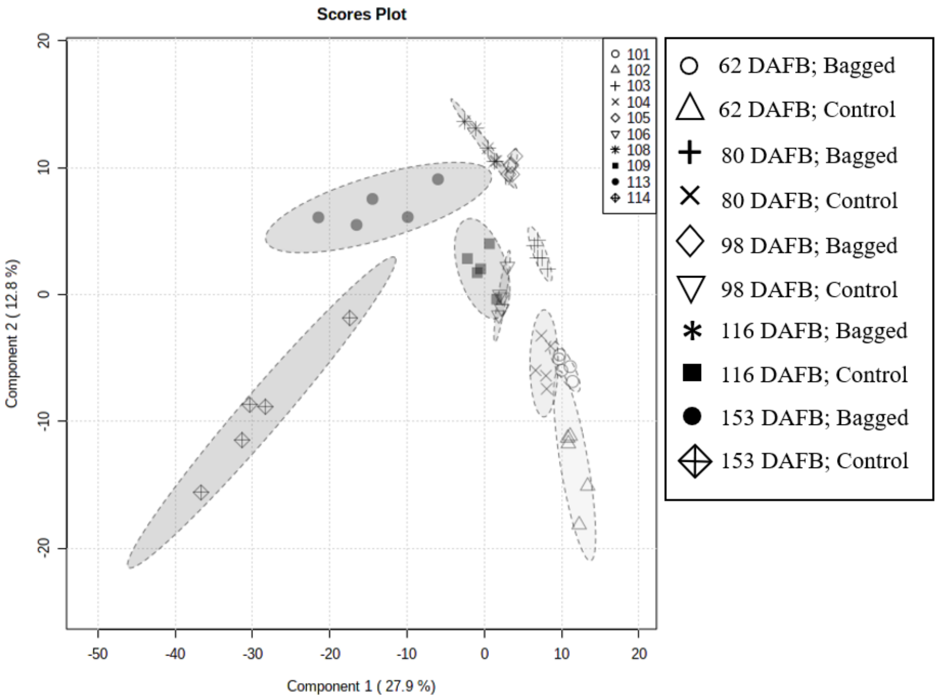


Figure 6. *WA 38 fruit bagging reduces or eliminates green spot symptoms. In 2020, the chemical profiles of peels from apples bagged 45 DAFB (June 1, 2020) and unbagged apples (Controls) diverged over the growing season. Peel chemistries differentiated as early as a few weeks (62 DAFB) after bagging and differences widened until harvest 153 DAFB (September 17, 2020). Different shaded ellipses and icons indicate different sampling date and treatment combinations as denoted in the legend.*

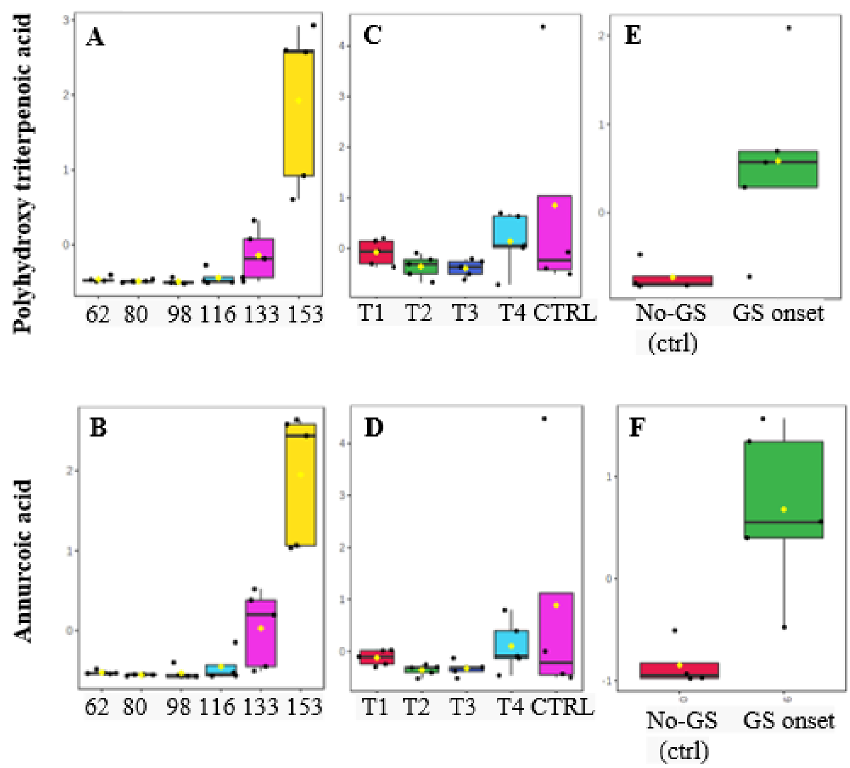


Figure 7. *Annurcoic acid and unidentified polyhydroxy triterpenoic acid are both natural chemicals found in elevated levels in symptomatic peel and cortex. These compounds may help form protective barriers around cells. Levels of these chemicals in peel changed during the growing season (A and B), with bagging as indicated here at the timepoint (116 DAFB) when green spot was first detected on control peel (C and D), and symptomatic versus asymptomatic tissue at 116 DAFB (E and F).*

A, B) Time points correspond to sampling at 62, 80, 98, 116, 133, and 153 DAFB.
C, D) Time points correspond to bagging treatments imposed on 6/1/2020 (T1), 6/18/2020 (T2), 7/6/2020 (T3), 7/24/2020 (T4), and not-bagged control (CTRL) sampled at GS onset (116 DAFB).
E, F) Comparison between asymptomatic (No-GS ctrl) and symptomatic (GS onset) WA 38 peels.

2. Verifying the effect of netting on green spot appearance along the season

On 6/4/2021 two entire rows of WA 38 trees in a block were enclosed inside drape netting (Diamond V5® Monorang, 10% shading, 2.8 mm x 4.0 mm weave, Helios® anti-hail systems, Bergamo, Italy) to test the netting practice as a potential strategy to mitigate GS. Each row comprised randomized plots of WA 38 grafted on either G41 or NIC29 rootstocks. For each rootstock combination, 9 trees were selected under the net and another 9 trees were chosen outside the nets in adjacent rows to serve as controls for a total of 36 trees in trial for this objective. On 9/27/21, trees were harvested and all apples from each tree were boxed in the field and moved into RA storage at 33 °F until grading. Yield data from these trees showed a significant difference in the number of apples per tree at harvest across the 4 combinations in trial (p=0.0319); with netted WA 38/G41 and control WA 38/G41 trees producing significantly higher numbers of apples per tree and yield (20 and 19 kg/tree, respectively) than control WA 38/NIC29 trees (13 kg/tree), while netted WA 38/NIC29 reported intermediate productivity (17 kg/tree) (data not shown). No significant differences were reported for average apple weight across the 4 combinations.

Fruit grading

The color grading of WA 38 apples grown under the net from June 4 to harvest showed differences across the combinations on the two different rootstocks, confirming results obtained in previous studies on this variety. The significantly higher proportions of apples (98-100%) in the most colored category (color1=50-100% red overcolor) was found in control and netted WA 38/NIC29 trees, while control and netted WA 38/G41 reported significant lower percentages of fruit in this category (96 and 93%, respectively). Analysis of the percentages of apples in all the size categories revealed that 10-12% fruit in both G41 combinations (control and netted) were sized in the ≤65mm diameter category (considered unmarketable for size), while both combinations on WA 38/NIC29 (control and netted) had only 2-4% of incidence in ≤65mm diameter category (data not shown). The incidence of GS in 2021 was also evaluated on the apples picked under the net for both rootstocks and compared to fruit harvested from the un-netted control. This was a similar protocol as in 2020; however, in 2021, the number of replications of the netted trees was increased to equal the control trees for a more balanced analysis and a larger data set to enable the evaluation of more apples. When the four combinations were analyzed together, we found that apples belonging to G41 control trees had higher and statistically different GS incidence than apples picked from netted G41 and NIC29 control and netted trees (Table 3). The difference in GS incidence between WA 38/G41 control and WA 38/NIC29 control was 39% in 2020 and 15% in 2021 (Table 3). The type of GS most present in control G41 apples in 2021 was GS1 (29%) followed by cases of 2 types of GS present in the same apple (17%) and then GS5, which represented approx. 9% of the fruit affected by GS in that specific combination with respect to control WA 38/NIC29 apple showing a 46% of GS1, 16% of 2 types of GS in the same apple, and only 1.4% of GS5 (p<0.05 for only GS5 class, data not shown).

Table 3. *WA38 GS incidence (%) in 2020 and 2021: comparison between apples grown under net treatment and in control trees (no net) within each rootstock. Significance by treatment (2020 and 2021) within each rootstock: ***=p<0.001.*

Combinations	**N trees 2020=**	***2020 GS incidence (%)***[1]		**N trees 2021=**	***2021 GS incidence (%)***[1]	
Control WA 38/G41	9	60.6	A	9	26.9	a
Netted WA 38/G41	3	64.9	A	9	16.8	b
Control WA 38/NIC29	9	21.8	B	9	11.6	b
Netted WA 38/NIC29	3	26.7	B	9	9.7	b
Significance		***			***	

[1] Apples presenting one or more types of GS were counted just once (presence/absence of GS regardless of the type)

In general, both netted and control WA 38 on G41 trees produced significantly higher proportions of apples in the cull category compared to netted and control WA 38 on NIC29 (data not shown). Interestingly, netting in 2021 helped reduce the culled apples due to sunburn in G41 and the proportion of fruit discarded due to splits in NIC29, but no statistically significant differences in the percentages of apples culled for GS were found between the control and netting treatments in either rootstock combination.

EXECUTIVE SUMMARY

Project Title: WA 38: understanding green spot origin, timeline, and development

Keywords: Humidity, temperature, lenticel, glucose, sorbitol, hyperin, epicatechin.

Abstract:

Our preliminary research determined when the onset of green spot (GS) symptoms appears (last ten days of July) and identified a possible physiological reason (damage to lenticels).

Fruit protected in bags during June did not develop green spot symptoms, while fruit bagged in July showed an increasing trend of damaged fruit. Bagging fruit late in the season (August) was ineffective in controlling GS symptoms.

A difference in the appearance of the symptoms has been noticed with different rootstocks: in both years of the project, G41 showed a higher percentage of fruit affected by GS compared to NIC29.

A significant increase in the average humidity level inside the bags was recorded in June-July-August in both years. This percentage increase of humidity in the bag ranged from 6.4% in June 2020 to 3.5% in August 2020 and from 7.8% in June 2021 to 2.3% in August 2021. A high and constant humidity level is a well-known condition that helps reduce lenticel damage. The temperature of fruit in the bag compared to the control was not significantly different in 2020, while in 2021, differences were significant in July due to the heatwave. The fact that bags can protect the fruit from GS onset focuses our attention on the role of lenticels in this disorder. The images taken under the microscope showed damage in the lenticels—further, the tissue surrounding them displayed the typical green discoloration of the peel. Peel and cortex on the sunken GS areas exhibit the presence of chemical compounds related to stress protection. An accumulation of glucose sorbitol, hyperin, and epicatechin in sunken green spot tissue and tissue surrounding sunken green spot have been identified. Also, compounds associated with photoprotection, barrier formation, nitrogen metabolism, and cell wall chemistry have been detected. In 2020 netting did not reduce the incidence of fruit showing GS symptoms, while in 2021, the net seems to reduce the percentage of fruit affected by GS only in combination with G41 rootstock.

We know that bags are not a feasible option for growers due to the high cost and availability of labor. Bags are just a research tool to investigate the origin of green spot.

PROJECT OUTCOMES

Presentations:

- Musacchi S. Annual report. Apple Research Review, Holiday Inn Express, Pasco January 27 – 28, 2021
- Musacchi S. Presentation at the WA 38 field day. September 17, 2021.
- Musacchi S. The key findings from 8 years of WA 38 research. Washington State Tree Fruit Association Annual meeting December 8, 2021.

FUTURE DIRECTIONS

What do bags do to the enclosed fruit? Bags increase the level of air humidity around the fruit and minimize the effect of diurnal humidity variation. As a future activity, we are starting a collaboration with a private farm to test the use of fog system early in the season (May-June) to mimic the conditions of high humidity induced by the bags. Another possible future investigation will be a study on the role of the lenticel anatomy to determine why they are susceptible to damage in WA 38. Due to the mixed results of netting on green spot incidence over the previous two years, further studies are required to understand if this practice (and related variables, i.e., shading factor, net color, etc.) can be a cost-effective measure in reducing cullage associated with green spot.