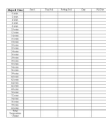# Boulder Apple Tree Project Survey Protocol

P1a Developed August 2018 by: Deidre Jaeger, Eric Johnson, Lisa Corwin, Katie Suding, Dan Haykin

Thank you so much for volunteering to help with ecological research for the Boulder Apple Tree Project! The information collected about the tree and fruit characteristics will aid in cultivar identification and how fruit ripening compares among trees. Identifying cultivars is the first step to understanding the diversity of apple trees we have in Boulder and will allow us to explore research questions about how differences in the local environment and climate change may affect these historical trees in our community.

Ecological research goals for this project:
1) Determine what cultivars are present and at which locations
2) Understand the range in timing of fruit ripening of different trees to inform a spring study of the timing of leaf budburst and flower emergence
3) Identify tree individuals or cultivars that may be good candidates for future grafting and orchard planting projects

Supplies needed to survey the tree and assess fruit:

Survey data sheet	Dot paper	Ripening guide	These three documents are included in this protocol, you can also download them from Boulder Apple Tree Project Website:
			https://www.colorado.edu/sudinglab/boulder-apple-tree-project/get-involved

Smartphone, or other camera and gps	Knife and cutting board	Tape measure and thumb tack	Pencil and chalk	String (10 foot piece and 5 foot piece)	Small post-it	Envelope for 3 leaves	Optional: Sharpie and paper bag if collecting fruits

Apple Tree Survey Instructions:

1. **Setup:** Ensure that you have all the necessary supplies for surveying. For each tree, you will need a separate data sheet, as well as an envelope, and paper bag for each tree if collecting ripe fruits.

2. **Safely locate the tree:** As you approach the tree site keep observant of any safety hazards such as barbed wire fences that may be around the tree and plan to work around them.

3. **If collecting on public property: please feel free to describe what you are doing with the apple tree project.** Other community members may be curious about what you are doing. We encourage you to explain the project aims and the data you are collecting from the tree as we continue to build enthusiasm in the community for the project.

4. **Collect tree photos and data**: On the survey data sheet, answer data fields numbered 1-18, collecting a leaf sample from smaller, green, healthy leaves.

5. **Collect fruit photos and data**: If there are fruits, get your apple Ripening Guide handy! Fruit is ripe when seed coats are dark brown or black. For a summer ripening apple, the seeds may not be dark brown or black, instead look for dropped apples that are full sized or don't have any worm damage. *If fruit is ripe, answer all remaining data fields, optionally collecting 3-5 whole fruit samples to be sent to our lab at CU.* If fruit is NOT ripe, answer data fields 19, 20, and 21 and plan to revisit the tree using the Ripening Guide about once per week to track fruit development, and store your ripe fruits in the refrigerator until you drop them off if possible.

6. **Take a photo of your data sheet and pack up:** Take a photo of the full survey data sheet and apple ripening guide so we can keep it connected to the other photos you took. Gather up your tools and data sheet and ensure you have your a labeled envelope of leaves and a labeled bag of uncut fruits if collected.

7. **Email data sheet, ripening guide, and photos to** appletreedata@colorado.edu. In a single email with the GPS lat/long coordinates in the subject line, send a photo of the Survey Data Sheet, the last page of the Ripening Guide, the tree, the fruit on the tree, and the cut fruit on the dot paper.

8. **Optional: Drop your data sheet, leaf, and ripe fruit samples at CU:** If you are able, bring your data sheets, leaf, and fruit samples in a single paper grocery bag labeled with your name, lat/long, and collection date to the INSTAAR main office, Room N202, CU East Campus Sustainability, Energy and Environment Complex: 4001 Discovery Drive, Boulder, CO 80303, (303) 492-6387. Or the CU Museum of Natural History front desk: Henderson Building, 15th and Broadway, Boulder, CO, 80309, (303) 492-6892. If you are *unable* to drop your samples, email boulderappletreeproject@gmail.com and we may be able to arrange a pick-up. Thank you!

Data to be collected:

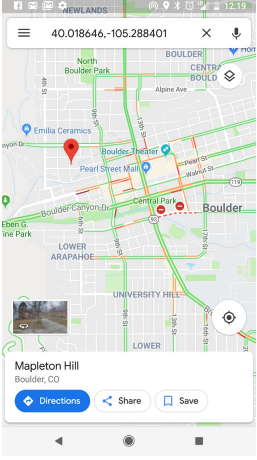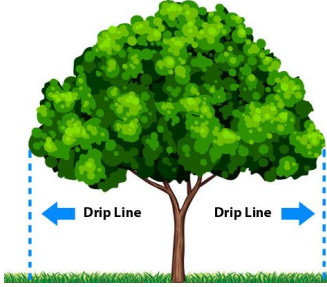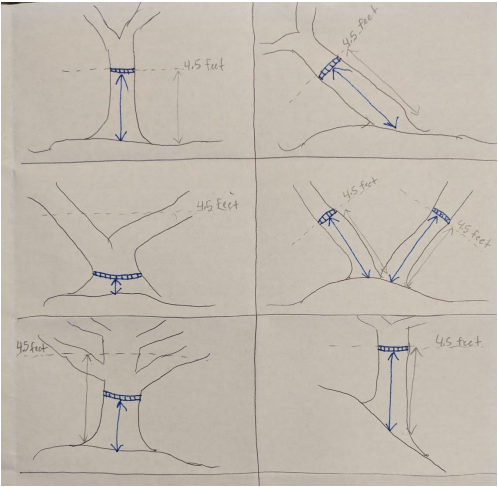| Data field	Definition and Instructions	Format Example	Ref Page
1_collector_ names	***Record your name(s) as the data collector(s).***	Deidre Jaeger Lucy Hansen	
2_email_ address	***Record one person's email address in case of questions about the data.***	deidre.jaeger@ colorado.edu	
3_phone_ number	***Record one person's phone number in case of questions about the data.***	303-492-5798	
4_date	***day, month abbreviation, and year of survey***	15-Aug-2018	
5_tree_tag_id	Leave blank until a tree identification # has been assigned		
6_tree_cultivar	Cultivars are accepted genetic variety of apples that have been bred. ***List cultivar name if known*** from a homeowner, or ***if not known, list unknown.***	honeycrisp	
7_property_ owner	Indicate what type of land ownership the tree is on. ***Answer choices are public, private, and unknown.***	private	
8_tree_site_ location	***Record the house address if the tree is on private property, or the trail or site name if the tree is on public land***	889 Larkspur Ave, Boulder	
9_tree_latitude	The distance north of the equator. Decimal degrees (DD) for latitude in Boulder will start with 39-40. Open Google Maps and zoom in to your blue location dot as close as you can. Then hold your finger over your blue dot until a red marker appears and displays the GPS coordinates and ***record latitude in DD.*** Alternatively, use another GPS app or device.	39.123578	6
10_tree_ longitude	The distance west of the prime meridian. Decimal degrees (DD) for longitude are negative in the western hemisphere and should start with -105 in the Boulder area. Open Google Maps and zoom in to your blue location dot as close as you can. Then hold your finger over your blue dot until a red marker appears and displays the GPS coordinates and ***record longitude in DD***. Alternatively, use another GPS app or device.	-105.889237	6
11_tree_height	The distance from the base of the trunk to the top of the uppermost branch. Using a tape measure, make a mark with chalk at the 5-foot mark on the trunk. Then back away from the tree and estimate the height to the tip of the tallest branch using your 5 foot mark as a distance reference. ***Record height and round to nearest whole foot.***	12	

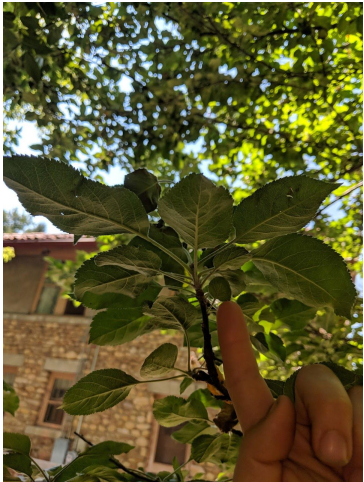| Data field	Definition and Instructions	Format Example	Ref Page
12_tree_drip_line	The widest distance of the tree canopy. Back away from the tree and observe where the distance of a cross section line of canopy is the widest. Place one of your strings under each branch outer tip, then use your measuring tape to measure the distance between the colored tape strips. **Record distance and round to nearest whole foot.**	10	7
13_tree_circumference	Distance around the trunk at a height of 4.5 feet from the ground. Wrap your measuring tape parallel to the ground around the main trunk and record the distance. If you don't have a flexible measuring tape, wrap your string around the trunk and use the tack to hold one string end. If the trunk is not perpendicular to the ground, measure the central axis of the trunk in the direction the tree is growing. If trunk is on sloping ground, measure the central axis that is mid-way between the tree edges that are on the upper and lower ends of the slope. If the main trunk is branched into 2 or more trunks at 4.5 ft, measure the circumference on the main trunk below the lowest branch. **Record circumference and round to nearest quarter inch.**	18.25	7
14_leaves_collected	Collect 3 small, healthy leaves from different parts of the canopy. The younger, thin and small leaves are best to extract DNA from. Place leaves in a yellow coin envelope and label with the GPS lat/long coordinates and date. **Answer options are yes/no** if the leaf sample was collected.	yes	7
15_trunk_rot	Determination of whether the wood of the trunk is rotting. Several fungi species induce wood rotting and may be visible on the trunk. Characteristics of rotting include soft, damp or hollowing wood that may be colored black or dark brown, or sections of wood loss with an irregular perimeter. **Answer options are present/absent** if the trunk has rot.	absent	7
16_fire_blight	Determination of whether the tree has flight blight, a bacterial disease that first attacks the tips of branches. Look for browning and curling of leaves within about 1 foot from the tips of branches, and drooping leaf stems. **Answer options are present/absent** for if the tree has fire blight.	present	8

Data field	Definition and Instructions	Format Example	Ref Page
17_fire_blight_ intensity	Determination of the percent intensity of fire blight leaf browning symptoms. ***Answer options are none/low/medium/ high.*** None = there are no patches of leaf browning, however there may be single isolated leaves that are brown. Low = 5% of total branch tip areas are showing clusters of leaf browning. Medium = 6-15% of total branch tip areas are showing clusters of leaf browning. High = >15% of total branch tip areas are showing clusters of leaf browning.	medium	8
18_tree_photo	Take a photo of the full tree in the center of the frame. If possible face your camera to the North, but if there are obstructions, any direction works. ***Answer options are yes/no*** if photo was taken.	yes	8
19_fruit_on_tre e_ photo	Take a close up photo of the side of a representative fruit hanging on a branch. If no fruit on tree, you can take a photo of fruit on the ground. If no fruits produced, indicate that in the notes. ***Answer options are yes/no*** if photo was taken.	yes	8
20_fruit_drop	Estimate what amount of the tree's fruit has dropped or been removed from the branches. ***Answer options are none/low/medium/high/all.*** None = no fruit has dropped. Low = 1-25% of the total fruits have dropped. Medium = 26-50% of total fruits have dropped. High = 51-75% of total fruits have dropped. All = > 95% of all fruits have dropped.	low	
21_fruit_on paper_photo	If apples are present, select 5 apples from the tree that are representative of the majority of apples on the tree based on size, shape, color, and damage level. Label a post-it with the GPS lat/long coordinates and date and place in the lower right corner of the dot paper. Using a cutting board, cut one apple lengthwise and one apple widthwise, scape 3 intact seeds out from the two cut apples. Arrange the 2 cut halves, 3 whole fruits, and seeds on labeled dot paper according to the diagram, <u>please take the photo in the shade</u>. Work safely while trying to minimize browning of the cut apple flesh before they are photographed. ***Answer options are yes/no that a photo was taken.***	yes	9
22_moisture	Touch an inside surface of one your cut apples to determine moisture. ***Answer options are moist/dry.*** Moist = juicy, sticky, sappy, wet or damp liquid felt by fingers. Dry = devoid of liquid, fabric-like, feels smooth and fibrous like a dry sponge.	moist	
23_seed_coat_ color	The seed coat is the outermost covering of the seeds. Use the seeds from the two apples you cut for the fruit photographs. <u>Determine the color of all nine seeds, and record what color</u>	dark brown	10

	the majority (>3) of the seeds are, or if even split between two colors list both. **Answer options are white/light brown/dark brown/black.** Dark brown = color of a Hershey's milk chocolate bar or darker.		
24_mature_ seeds	Number of seeds with a dark brown or black outer seed coat. **Record the fraction of ripe seeds over total seeds examined.**	5/6	10
25_insect_use_ fruit	Presence of signs that an insect is using or has used the fruit. Evidence includes presence of insect larvae or adults, brown or mushy spots, burrowing lines, or exit holes. Codling larvae create a red "sting" or ring on the apple skin with a crumbly brown substance in the center. **Answer options are present/absent/unsure** if there is insect use in 1 or more of the 5 fruits you collected for the photo.	present	10
26_rotten_fruit_ outside	Flesh and skin has visibly started to degrade and decompose. Rotten fruit may be squishy and brown, moldy, or have irregular hollow chambers. Could be due to insect use or other pathogens. **Answer options are present/absent** if there is any outside rotting in 1 or more of the 5 fruits you collected for the photo.	present	11
27_rotten_fruit_ inside	Flesh has started to degrade and decompose under the apple skin and may or may not be visible while apple is on tree. Rotten fruit may be squishy and brown, moldy, or have irregular hollowing chambers. Could be due to insect use, or other pathogens. **Answer options are present/absent** if there is any inside rotting in 1 or more of the 5 fruits you collected for the photo.	absent	11
28_lab_fruits_ collected	OPTIONAL: Gather 3-5 whole fruits from different areas on the tree that are representative of the majority of apple trees on the tree based on size, shape, color, and damage level. Place inside a paper bag labeled with the GPS lat/long coordinates and date. **Answer options are yes/no** that whole fruits were collected**.** Keep the fruits in a cool dark area until our lab can receive them.	yes	
29_color	The dominant color shade of the apple skin. If striped or spotted, the majority of the skin is this color. If a 50/50 mix, list both colors. **Answer options are: red, pink, green, yellow, cream.**	green	11

Data field	Definition and Instructions	Format Example	Ref Page
30_texture	Qualities of the fruit as determined by sense of feel. ***Answer options are crisp/soft/mealy.*** Examine one of your cut fruits. Crisp = flesh firm and crunchy, resisting dimpling when squeezed in hand, juicy sensation when biting because cells rupture and release liquid. Soft = flesh flexible and moldable, quickly dissolving when eaten and can create dimples if squeezed in hand. Meal = distinctly granular in addition to flexible and moldable flesh, dry sensation when biting.	crisp	11
31_flavor	Qualities of the fruit as determined by sense of taste. ***Answer options are sweet/tart/ bitter/astringent for the primary flavor, but list all that apply in notes.*** Sweet = sugary, honeylike, tongue may feel the same or more moist. Tart = acidic with mild to sharp sensation yet still pleasant, tongue feels a zing of citrus-like sour but remains moist. Bitter= sharply acidic, or soap-like taste with a biting sensation, tongue remains moist and aftertaste may linger. Astringent = dry, chalky, or puckering sensation on the tongue similar to unripe banana or red wine, abruptly drying tongue as mucus membranes contract.	tart	
32_variety_analog	Fruit taste, texture, and moisture is characteristic of a known type of cultivar, or has similarities that remind you of a variety. ***Answer options are open-ended***.	courtland	12
33_fresh_eating_uses	Is this apple tasty when eaten fresh? ***Answer options are yes/no/unsure.***	yes	
34_juice_uses	Do you know if apples from this tree have been pressed into a fresh juice? ***Answer options are yes/no/unsure.***	yes	
35_baking_cooking_uses	Do you know if apples from this tree may have used for baking/cooking? ***Answer options are yes/no/unsure.***	yes	
36_cider_uses	Do you know if apples from this tree may have been fermented into cider? ***Answer options are yes/no/unsure.***	no	
37_dehydrating_uses	Do you know if apples from this tree may have been dehydrated into dried fruit? ***Answer options are yes/no/unsure.***	no	
38_wildlife_uses	Do you know if apples from this tree are eaten by wildlife? ***Answer options are yes/no/unsure.***	yes	

Diagram References

9_tree_latitude, 10_tree_longitude	12_tree_drip_line	13_tree_circumference
	\nPhoto credit: Good Earth Plant Company, Inc	\nDiagram credit: Deidre Jaeger, CU Boulder

14_leaves_collected	15_trunk_rot
\nPhoto: credit: Deidre Jaeger, CU Boulder\nAim to collect small, thin leaves!	\nPhoto credit: Dale Bergdahl, Univ of Vermont , Deidre Jaeger, CU Boulder

16_fire_blight	17_fire_blight_intensity
 Photo credit: Ontario ministry of agriculture and rural affairs	 Photo credit: Deidre Jaeger, CU Boulder Despite many green leaves, this tree has greater than 15% of branch tips infected

18_tree_photo	19_fruit_on_tree_photo
 Photo credit: Marta Hamilton, Boulder	 Photo credit: Deidre Jaeger
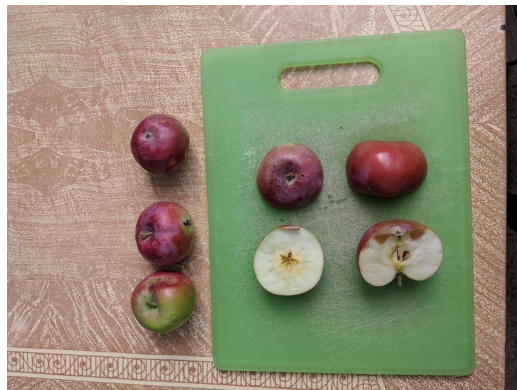
Gather 5 apples, cut 1 widthwise and 1 lengthwise. Scrape 3 intact seeds out from each cut apple. Arrange 3 whole apples, 2 half apples, 6 seeds, and a location/date label according to example on dot paper. Take the photo in the shade and try to minimize browning between cut and photo.
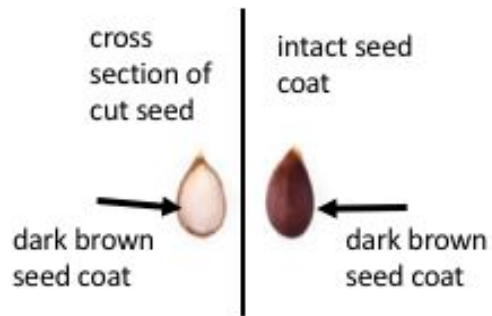
23_seed_coat_color	

Diagram credit: Deidre Jaeger, CU Boulder

24_mature_seeds		
dark brown	light brown	White (and 1 light brown)

Photo credit: garden guides

Photo credit: health orange blog

25_insect_use_fruit	

Photo credit: Missouri botanic garden

26_rotten_fruit_inside	27_rotten_fruit_outside
Photo credit: apple best practice guide	

29_color
Green Yellow Red (slight russeting at stem end) Diagram credit: Eric Johnson, Widespread Malus

30_texture		
Crisp : smooth, even flesh	Soft : medium granules	Mealy: large granules
Photo credit: Kelley and Cricket	Photo credit: Kameron Peace, Washington State University	Photo credit: meals and moves blog

11

Guide to Apples
on PocketChangeGourmet.com

Rome Beauty | Granny Smith | Pink Lady | Winesap | Braeburn

Baking
Fresh Eating

Gala | Golden Delicious | Fuji | Cameo | SweeTango

Pies
Cooking

Rome Beauty | Jonagold | Jazz | Honey Crisp | Mutsu

Salad
Sauces

Granny Smith | Jonathan | Braeburn | McIntosh | Cortland

Survey Data Sheet:			
1_collector_names		3_phone_number	
		4_date	
2_email_address		5_tree_tag_id	
6_tree_cultivar		23_seed_coat_color	
7_property_owner		24_mature_seeds	
8_tree_site_location		25_insect_use_fruit	
9_tree_latitude		26_rotten_fruit_ outside	
10_tree_longitude		27_rotten_fruit_ inside	
11_tree_height		28_lab_fruits_ collected	
12_tree_drip_line		29_color	
13_tree_ circumference		30_texture	
14_leaves_collected		31_flavor	
15_trunk_rot		32_variety_analog	
16_fire_blight		33_fresh_eating_ uses	
17_fire_blight_ intensity		34_juice_uses	
18_tree_photo		35_baking_cooking_ uses	
19_fruit_on_tree_ photo		36_cider_uses	
20_fruit_drop		37_dehydrating_uses	
21_fruit_on paper_photo		38_wildlife_uses	
22_moisture			
Notes:			
	Staff use: data entered? P1a		

After you have completed the survey of your ripe apples:

In a single email to appletreedata@colorado.edu with the GPS lat/long coordinates in the subject line, send a photo of the Survey Data Sheet, the last page of the Ripening Guide, the tree, the fruit on the tree, and the cut fruit on the dot paper.

If you are able, please bring your data sheet, leaf, and fruit samples for each tree in a single paper grocery bag labeled with your name, lat/long, and collection date to one of two drop off locations:

INSTAAR main office
Room N202
CU East Campus Sustainability, Energy and Environment Complex
4001 Discovery Drive
Boulder, CO 80303
(303) 492-6387.
Monday- Friday 8am-5pm

CU Museum of Natural History front desk
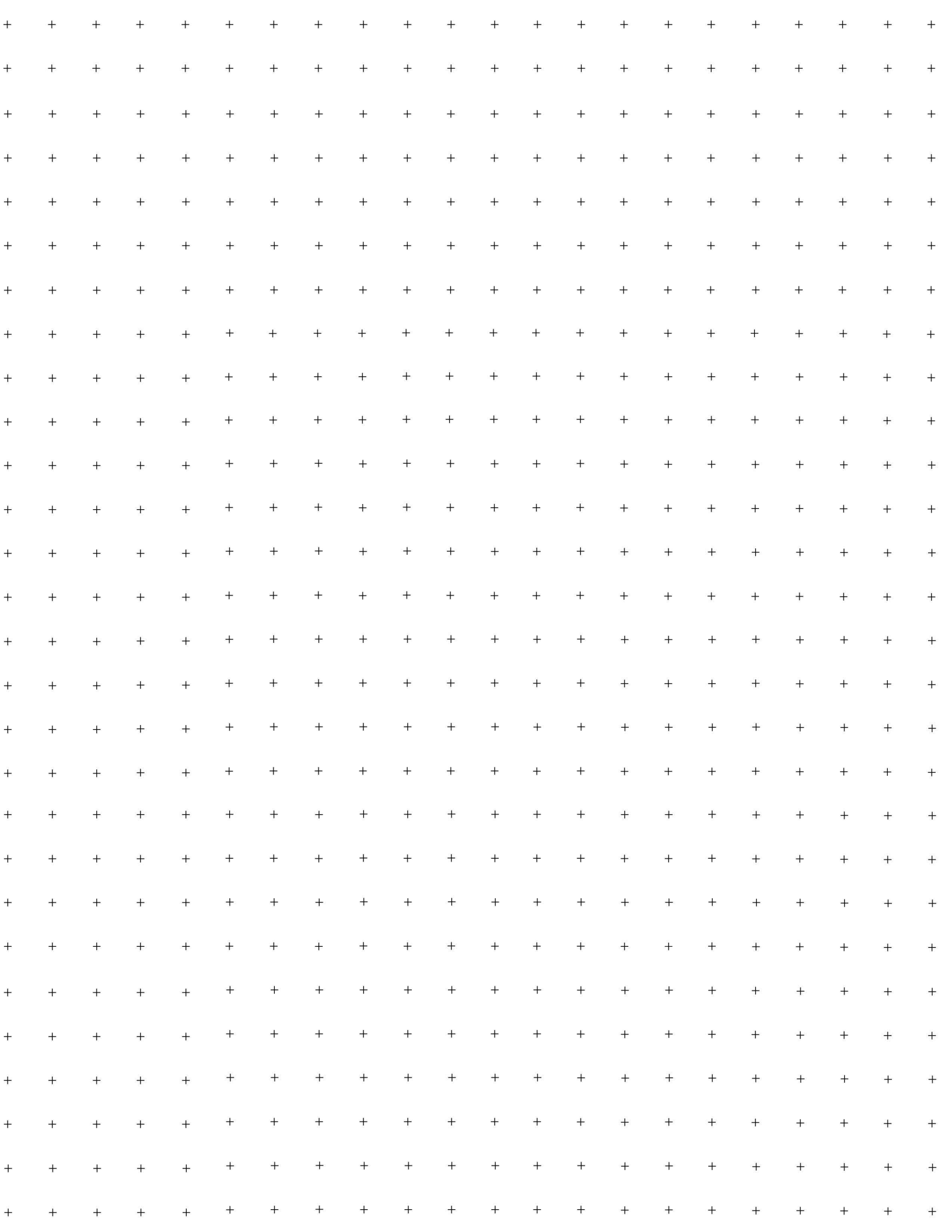Henderson Building
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Monday- Friday 9am-5pm
Saturday 9am-4pm
Sunday 10am-4pm

If you are *unable* to drop your samples, email boulderappletreeproject@gmail.com and we may be able to arrange a pick-up. Thank you!
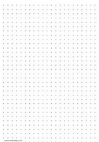
Apple Ripening Guide (P2a)

Artist: Jane Smith, CU Boulder

We are interested in learning more about the diversity of fruit ripening of Boulder apple trees. In addition to leaf DNA, ripe fruit characteristics are helpful for identifying the apple cultivar. Fruit is ripe when seed coats are dark brown or black, however for a summer ripening apple, the seeds may not be dark brown or black. Instead you may have to judge ripeness based on number of dropped apples, especially looking for dropped fruits that don't have worm damage.

If the seeds aren't yet dark and the majority of apples are still remaining on the tree, you can use this guide to monitor the fruits as they continue to ripen. About once per week, check on the fruits by cutting them open and taking a photograph of the cut fruits on dot paper each time you revisit the tree. When the majority of the seeds are dark brown or black, then you can finish up the main survey data sheet.

Supplies Needed:

Knife and cutting board	Pencil/pen	Dot paper	Smartphone, or other camera and gps	Small post-it

20_fruit_drop	Estimate what amount of the tree's fruit has dropped or been removed from the branches. ***Answer options are none/low/medium/high/all***. None = no fruit on ground below tree. Low = 1-25% of the total fruits are on the ground. Medium = 26-50% of total fruits on ground. High = 51-75% of total fruits are on ground. All = > 95% of all fruits have dropped.
21_fruit_photo	If apples are present, select 5 apples from the tree that are representative of the majority of apples on the tree based on size, shape, color, and damage level. Label a post-it with the GPS lat/long coordinates and date and place in the lower right corner of the dot paper. Using a cutting board, cut one apple lengthwise and one apple widthwise, scape 3 intact seeds out from the two cut apples. Arrange the 2 cut halves, 3 whole fruits, and seeds on labeled dot paper according to the diagram, <u>please take the photo in the shade</u>. Work safely while trying to minimize browning of the cut apple flesh before they are photographed. ***Answer options are yes/no that a photo was taken.***
23_seed_coat_color	The seed coat is the outermost covering of the seeds. Use the seeds from the three apples you cut for the fruit photographs. <u>Determine the color of all nine seeds, and record what color the majority (>4) of the seeds are.</u> ***Answer options are white/light brown/dark brown/black.*** Dark brown = color of a Hershey's milk chocolate bar or darker.
24_mature_seeds	Number of seeds with a dark brown or black outer seed coat. ***Record the fraction of ripe seeds over total seeds examined.***

21_fruit_photo_on_paper

Gather 5 apples, cut 1 widthwise and 1 lengthwise. Scrape 3 intact seeds out from each cut apple. Arrange 3 whole apples, 2 half apples, 6 seeds, and a location/date label according to example on dot paper. Take the photo in the shade and try to minimize browning between cut and photo.

24_seed_coat_color

cross section of cut seed

intact seed coat

dark brown seed coat

dark brown seed coat
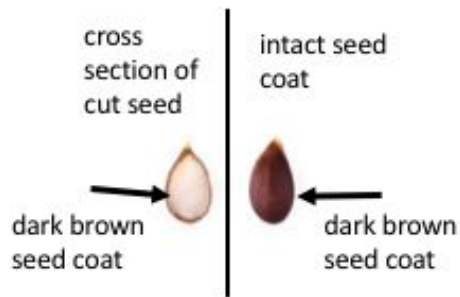
Diagram credit: Deidre Jaeger, CU Boulder

25_mature_seeds		
dark brown	light brown	White (and 1 light brown)
Photo credit: garden guides	Photo credit: health orange blog	

Apple Ripening Guide P2a			
1_collector_names		3_phone_number	
2_email_address			

9_tree_latitude	
10_tree_longitude	

Date		Date	
20_fruit_drop		20_fruit_drop	
21_fruit_on paper_photo		21_fruit_on paper_photo	
23_seed_coat_color		23_seed_coat_color	
24_mature_seeds		24_mature_seeds	

Date		Date	
20_fruit_drop		20_fruit_drop	
21_fruit_on paper_photo		21_fruit_on paper_photo	
23_seed_coat_color		23_seed_coat_color	
24_mature_seeds		24_mature_seeds	

Date		Date	
20_fruit_drop		20_fruit_drop	
21_fruit_on paper_photo		21_fruit_on paper_photo	
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24_mature_seeds		24_mature_seeds	

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If you are able, please bring your data sheet, leaf, and fruit samples for each tree in a single paper grocery bag labeled with your name, lat/long, and collection date to one of two drop off locations:

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Sunday 10am-4pm

If you are *unable* to drop your samples, email boulderappletreeproject@gmail.com and we may be able to arrange a pick-up. Thank you!