

*Camellia sinensis* is an evergreen plant whose leaves and leaf buds are used to produce tea. Tea plants generally flourish in tropical climates (e.g., India), but there exist cultivars (*C. sinensis* and *C. assamica* x *C. sinensis* hybrids) that are well suited for production in Mediterranean climates (e.g., western Washington). The U.S. is the fourth largest importer of tea in the world after France, Malaysia, and Pakistan, and the estimated total wholesale value of the U.S. tea industry as of 2021 was \$13.47 billion (Statista, 2022). Although tea is the second most widely consumed beverage in the world (Tea Association of the U.S.A., 2022), there is essentially no research to support its production and marketing in the U.S. Located in a favorable growing climate, Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU NWREC) is well positioned to take a leadership role in researching tea as a new crop for the Washington nursery industry. This project developed methods for propagating tea from cuttings, established a tea planting, and developed a tea propagation and planting guide, thereby supporting a sustainable emergence of tea production in Washington. This project addresses two of the nursery RFP priorities: 1) Establishment of new cultivar trials conducted within the state and relevant to the climate and hardiness zones of the Washington nursery industry; and 2) Nursery best practices.

### Objectives

1. Test and develop methods for propagating tea from cuttings.
2. Establish tea plants at Washington State University Mount Vernon Northwestern Washington Research and Extension Center to provide the nursery industry with performance information as well as cuttings for development of their own propagation blocks.
3. Develop a tea propagation and planting guide for the nursery industry to support sustainable sales of tea nursery stock.

Three experiments were conducted in 2021 to test and develop methods for propagating tea from cuttings. In Expt. 1, cuttings collected on 1 and 15 Sep and 1 Oct 2021 did not survive while those collected on 5 Nov and 1 Dec 2021 and 12 Jan and 18 Feb 2022 had more than 80% survival on average. In Expt. 2, survival after 7 months was similar for shoots that had been stored in a box for 3 days with and without cold packs (approximately 60%). In Expt. 3, after 8 months, the overall survival of cuttings was approximately 8% for a standard potting media and 48% for a fir mulch media. However, we believe these experiment results were not due to treatment effects as some mismanagement practices occurred due to our learning curve. In response, we assigned Ph.D. student Srijana Shrestha solely to this project and designed new experiments that we carried out in 2022-23, which are explained in detail below. In our current experiments we have achieved 77–100% survival for tea shoot cuttings and we have very high confidence in these results. We will be repeating these experiments in 2023-24, and these experiments and results will be included in the dissertation of the Ph.D. student designated to this project as well as journal articles that we will write.

### Research site, methods and results

The WSU Mount Vernon NWREC field site is located in the maritime Pacific Northwest, with a Mediterranean climate. The summer (June through September) is mild and humid with 16.0 °C average daily temperature, 78% relative humidity, and 134 mm rainfall (20-year average; AgWeatherNet, 2022). The fall (October and November) has 8.5 °C average daily temperature, 85% relative humidity, and 218 mm rainfall; the winter (December through February) has 4.6 °C average daily temperature, 86% relative

humidity, and 262 mm rainfall; and the spring (March through May) has 9 °C average daily temperature, 79% relative humidity, and 199 mm rainfall (20-year average; AgWeatherNet, 2022).

**Objective 1.** Test and develop methods for propagating tea from cuttings. In general, clonal propagation (cuttings) of tea is recommended because of the high level of heterozygosity in seeds (Zee et al. 2003). In 2022, we carried out 2 experiments to test propagation methods for tea plants under environmental conditions of northwest Washington: 1) evaluate the time of year and section of shoot to collect cuttings for successful tea plant propagation; and 2) evaluate the number of nodes per cutting for successful tea plant propagation.

**Materials and Methods.** We collected shoots of tea cultivar Minto Pacific (previously called '1-2') from 21-year-old plants, grown by John Vendeland in Burlington, WA (Fig. 1). This cultivar is originally from Ilahee Hills Tea Farm in Salem, OR, which was discontinued at the end of 2020 and the breeding block is now part of Minto Island Growers in Salem, OR. Shoots collected from this cultivar were used for both experiments at WSU NWREC. The following methods were used for both experiments except where differences are noted.

Shoot collection and cutting methods. Shoots collected for experiments were healthy with an initiated axillary bud but before initiation of flowering from the axillary bud. The cut shoots were placed in a 5-gallon bucket with approximately 5 inches of water (Fig. 2) and brought to the WSU NWREC greenhouse where shoots were immediately processed into cuttings in a shaded, cool area. The cuttings were scored using a knife, to remove 1-2 inches of bark on one or both sides of the cutting's base to encourage adventitious root formation (Kumar, 2016). After scoring, the bottom end of the cutting was dipped into rooting hormone (Dip'n Grow, Clackamas, OR) for 5-10 seconds, placed into a treepot filled with propagation media (peatmoss, vermiculite and perlite in 5:3:2 ratio, pH 4.5; J. Vendeland, personal communication) such that the node with leaves rested on the soil line, and watered well.

**Expt. 1:** Expt. 1 had a randomized complete split plot design with 9 main plot treatments, 3 subplot treatments, and 3 replications. The main plot treatment was different time of shoot collection: 8 and 21 Sep, 6 and 24 Oct, 3 and 21 Nov, and 8 Dec 2022, and 18 Jan and 8 Feb 2023. The subplot treatment was the section of the shoot taken as a cutting: top, mid, and bottom section (Fig. 3). Each subplot had 10 cuttings.

Cuttings were 3-node in which the cut was made 3-4 cm below the third node, and the leaf attached to the third (bottom) node was removed, keeping the first two leaves intact. The treepots were placed in the mist chamber (model #549, Mist Timer II, Drips Inc., Concho, AZ) on the greenhouse bench (Fig. 4). The chamber was set to mist for 20 seconds every 30 minutes and supplemented with bottom heat (Jump start seedling heat mat, 20 x 48, 107 Watts, Johnny's Selected Seeds, Fairfield, ME). The mist chamber had 50% shade and about 70-80% relative humidity.

**Expt. 2:** Expt. 2 had a randomized complete block design with 3 treatments, 3 replications, and 10 cuttings per plot. Treatments included 1-node, 2-node, and 3-node cuttings. For 1-node cuttings, the leaf was not removed, and for 2-node cuttings, the second/bottom leaf was removed (Fig. 5). The 3-node cuttings were processed as described in Expt. 1.

**Data collection.** For Expts. 1 and 2, data were collected for plant survival, plant height, number of new leaves, and overall plant health of the cuttings. Plant survival was measured as the total number of alive cuttings per sub plot/plot indicated by the presence of either old, mature leaves intact or with new buds/leaves. Plant height was measured as the length of the cuttings from the node resting on the potting media surface to the tip of the cuttings. Number of new leaves were counted as the number of

buds that developed into a completely new leaf. Overall plant health was recorded on a 5-point scale where 1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good. Data were collected once a month except for the number of new leaves, on the 15<sup>th</sup> of each month: 1) for Expt. 1, from Sep 2022 to Jun 2023; and 2) for Expt. 2, from Dec 2022 to Jun 2023. Data on the number of new leaves were collected starting Jun 2023 for both experiments.

**Data analysis.** All data were analyzed using statistical analysis software (R version 1.4.1106-5 for Windows; RStudio, Boston, MA; SAS version 9.4 for Windows; SAS Institute, Cary, NC). The assumptions of normality and homogeneity of variance were tested using the Shapiro-Wilk test ( $\alpha = 0.05$ ) and the Levene's test ( $\alpha = 0.05$ ), respectively, and the data were subjected to analysis of variance. A nonparametric transformation was applied using PROC RANK in SAS to the data that did not satisfy the assumptions of normality with any transformation. All means presented are non-transformed, and means were separated using Tukey's honestly significant difference at a significance level of  $\alpha < 0.05$ .

## **Results.**

**Expt. 1.** Survival of cuttings differed due to collection date at 1, 2, 3 and 4 months after collection ( $P < 0.0001$  for all) and differed with section of the shoot at 3 and 4 months after collection ( $P \leq 0.04$ ) (Table 1). There was no interaction between shoot collection date and section of the shoot for any months ( $P \geq 0.07$ ). Shoots collected from 21 Sep through 8 Dec 2022 had the highest survival rate: 99.8% on average at 1, 2 and 3 months after collection, and 99.5% on average 4 months after collection. Cuttings from 8 Sep 2022 had the lowest survival rate overall: 87%, 86%, 81% and 77% at 1, 2, 3 and 4 months after collection, respectively.

Plant height differed due to cutting collection date at 3 and 4 months after collection ( $P < 0.002$  for all) and differed with section of the shoot at 1, 2, 3 and 4 months after collection ( $P < 0.0001$  for all) (Table 2). There was an interaction between shoot collection date and section of the shoot at 4 months after collection ( $P = 0.01$ ). At 3 months after collection, cuttings collected on 21 Sep, 24 Oct, and 8 Feb had the greatest plant height (4.9 cm on average) and at 4 months after collection, cuttings collected on 8 Feb had the greatest plant height (6.6 cm). Overall, cuttings from the mid and bottom section had the greatest plant height at all times (average 5.0 cm at 1 month, average 5.1 cm at 2 months, average 5.2 cm at 3 months, and average 5.6 cm at 4 months).

Number of new leaves measured in Jun 2023 did not differ due to cutting collection date ( $P = 0.21$ ) but did differ for section of the shoot ( $P = 0.007$ ) (Table 3). There was no interaction between shoot collection date and section of the shoot ( $P = 0.67$ ). The top and mid-section of the shoot had the highest number of new leaves (3.8 leaves on average) while the bottom section had the lowest number of new leaves (2.8 leaves on average).

Overall plant health differed due to both collection date ( $P < 0.0001$  for all) and section of the shoot at 1, 2, 3 and 4 months after collection ( $P \leq 0.0008$ ) (Table 4). There was no interaction between shoot collection date and section of the shoot at any months ( $P \geq 0.08$ ). Cuttings collected on 6 and 24 Oct, and 8 Dec 2022 maintained the highest rating for overall health until 4 months of collection (more than 4.5 on average for all months). Cuttings collected on 8 Sep 2022 had the lowest but above-average rating in all months. The top section of the shoot had the highest rating for overall health in all months ( $\geq 4.5$ ).

**Expt. 2.** All the treatments had 100% plant survival (Table 5). Plant height differed with treatments at all data collection dates ( $P < 0.0001$  for all) (Table 6). The 3-node cuttings had the greatest plant height: 5.5 cm in March (4 months after collection), 5.9 cm in April (5 months after collection), 6.6 cm in May (6 months after collection), and 7.6 cm in June (7 months after collection). Number of new leaves did not

differ with treatments ( $P = 0.71$ ) and all treatments had 3 new leaves on average by the end of the experiment, 7 months after cutting collection (Table 7). Overall plant health did not differ with treatments ( $P \geq 0.38$ ), and was greater than 4.0 rating throughout the experiment and ranged from 4.3 to 4.6 on the last measurement date, which was 7 months after cutting collection (Table 8).

**Objective 2.** Establish a tea planting at WSU Mount Vernon NWREC with domestically sourced planting stock. Tea seedlings (unknown cultivar, referred to hereafter as ‘Fairhope’) were procured from a commercial tea farm in Alabama (Fairhope Tea Plantation) in 2019 and were planted in a field at WSU NWREC. Due to unfavorable field soil pH (6.26 in 2022 soil sampling) for tea plants, the plants did not show good growth. Hence, tea plants were removed from the field on 22 July 2022, planted in pots filled with peatmoss, vermiculite, and perlite in 5:3:2 ratio (4.5 pH), and maintained in the screen house with about 30% shade until 15 Sep 2022 when the plants were moved to a hardening-off tunnel.

We are carrying out a new field experiment to determine if transplanting time of year impacts plant establishment for ‘Fairhope’ and ‘Minto Pacific’ cultivars. The field experiment has a split plot design with 3 replications. The main plot treatments are fall and spring transplanting, and the subplot treatments are ‘Fairhope’ and ‘Minto Pacific’ cultivars (Fig. 6). Each subplot has 3 plants. Fall planting was done on 14 Oct 2022 and spring planting was on 8 Jun 2023. Beds were spaced 2.5 m center-to-center, mulched with a woven polyethylene mulch (Extenday, Yakima, WA, 85 g·m<sup>-2</sup> density), and plants were spaced 60 cm in a single row.

We planted 75 ‘Minto Pacific’ tea plants on 8 Jun 2023 next to the field experiment to establish a tea planting where future field production research can be carried out (Fig. 7).

**Results.** At this time, only data is available for fall-planted tea plants as plants that were placed in the field June 2023 have not yet become established. On 15 Jun 2023, fall-planted ‘Fairhope’ tea plants had better overall health status compared to ‘Minto Pacific’ tea plants (4 and 2.5, respectively) (Fig. 8). ‘Minto Pacific’ plants had defoliation over the 2022-23 winter, causing plant health ratings to be low. By the end of June 2023, all ‘Minto Pacific’ tea plants have new sprouts emerging from the base of the plant and leaves appear healthy (Fig. 9).

**Objective 3.** Develop a tea propagation guide for the nursery industry to support sustainable sales of tea nursery stock. We have developed a tea plant propagation guide (Fig. 10) based on our experimental results to date and this is available on our website <https://vegetables.wsu.edu/tea/>, freely accessible for nursery growers. We have also developed a short guide for tea plant transplanting (Fig. 11), so that nurseries can provide these instructions to their customers. This guide is also available on our website <https://vegetables.wsu.edu/tea/>.

## References

- Kumar, M.G.N. 2016. Propagating shrubs, vines, and trees from stem cuttings. A Pacific Northwest Extension Publication. PNW152.
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Table 1. Survival of tea cuttings collected on 8 Sep 2022 through 8 Feb 2023 from the top, mid, and bottom section of the shoots, at 1, 2, 3 and 4 months after collection at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection			
	1	2	3	4
Collection date				
8 Sep 2022	86.7 c	85.6 c	81.1 b	76.7 d
21 Sep 2022	100.0 a	100.0 a	100.0 a	98.9 ab
6 Oct 2022	100.0 a	100.0 a	100.0 a	100.0 a
24 Oct 2022	100.0 a	100.0 a	100.0 a	100.0 a
3 Nov 2022	100.0 a	100.0 a	100.0 a	100.0 a
21 Nov 2022	98.9 ab	98.9 ab	98.9 a	97.8 abc
8 Dec 2022	100.0 a	100.0 a	100.0 a	100.0 a
18 Jan 2023	100.0 a	93.3 abc	91.1 b	88.9 cd
8 Feb 2023	94.4 bc	92.2 bc	91.1 b	90.0 bcd
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Shoot section				
Top	98.9	97.0	96.3 ab	95.2 ab
Mid	98.1	98.1	97.0 a	96.3 a
Bottom	96.3	94.8	94.1 b	92.6 b
<i>P</i> value	0.12	0.08	0.02	0.04
<i>P</i> value date x shoot	0.80	0.74	0.07	0.68

Table 2. Plant height of tea cuttings collected on 8 Sep 2022 through 8 Feb 2023 from the top, mid, and bottom section of the shoots, at 1, 2, 3 and 4 months after collection at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection			
	1	2	3	4
Collection date				
8 Sep 2022	4.3	4.3	4.3 b	4.3 b
21 Sep 2022	4.6	4.6	4.6 ab	4.6 b
6 Oct 2022	4.1	4.1	4.1 b	4.1 b
24 Oct 2022	4.6	4.6	4.6 ab	4.7 b
3 Nov 2022	4.0	4.0	4.1 b	4.4 b
21 Nov 2022	3.9	3.9	4.0 b	4.1 b
8 Dec 2022	3.9	4.0	4.2 b	4.8 b
18 Jan 2023	4.1	4.1	4.4 b	4.8 b
8 Feb 2023	4.2	4.7	5.6 a	6.6 a
<i>P</i> value	0.15	0.05	0.002	0.0001
Shoot section				
Top	2.5 b	2.6 b	2.9 b	3.1 b
Mid	5.1 a	5.2 a	5.3 a	5.7 a
Bottom	4.9 a	5.0 a	5.1 a	5.4 a
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
<i>P</i> value date x shoot	0.08	0.25	0.12	0.01

Table 3. Number of new leaves developed on tea cuttings collected on 8 Sep 2022 through 8 Feb 2023 from the top, mid, and bottom section of the shoots, in Jun 2023 at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Jun 2023
Collection date	
8 Sep 2022	3.0
21 Sep 2022	2.0
6 Oct 2022	3.0
24 Oct 2022	5.0
3 Nov 2022	4.0
21 Nov 2022	4.0
8 Dec 2022	4.0
18 Jan 2023	2.0
8 Feb 2023	4.0
<i>P</i> value	0.21
Shoot section	
Top	3.5 ab
Mid	4.0 a
Bottom	2.8 b
<i>P</i> value	0.007
<i>P</i> value date x shoot	0.67

Table 4. Overall plant health of tea cuttings collected on 8 Sep 2022 through 8 Feb 2023 from the top, mid, and bottom section of the shoots, at 1, 2, 3 and 4 months after collection at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection			
	1	2	3	4
Collection date				
8 Sep 2022	3.8 d	3.7 d	3.5 c	3.3 e
21 Sep 2022	4.7 abc	4.6 bc	4.4 b	4.1 cd
6 Oct 2022	4.9 ab	4.8 ab	4.7 ab	4.6 ab
24 Oct 2022	5.0 a	5.0 a	4.9 a	4.9 a
3 Nov 2022	4.8 ab	4.6 ab	4.4 b	4.3 bc
21 Nov 2022	4.7 abc	4.6 bc	4.6 b	4.5 bc
8 Dec 2022	4.9 ab	4.8 ab	4.7 ab	4.6 ab
18 Jan 2023	4.7 bc	4.7 bc	4.6 b	4.5 bc
8 Feb 2023	4.3 c	4.1 c	3.9 c	3.8 de
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001
Shoot section				
Top	4.8 a	4.7 a	4.6 a	4.5 a
Mid	4.6 b	4.5 b	4.4 b	4.2 b
Bottom	4.6 b	4.5 b	4.3 b	4.1 b
<i>P</i> value	0.0008	0.0008	<0.0001	<0.0001
<i>P</i> value date x shoot	0.58	0.67	0.08	0.61



Table 5. Survival of 1-node, 2-node, and 3-node tea cuttings collected on 1 Nov 2022 at 1, 2, 3, 4, 5, 6, and 7 months after collection, at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection						
	1	2	3	4	5	6	7
1-node	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2-node	100.0	100.0	100.0	100.0	100.0	100.0	100.0
3-node	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 6. Plant height of 1-node, 2-node, and 3-node tea cuttings collected on 1 Nov 2022 at 4, 5, 6, and 7 months after collection, at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection			
	4	5	6	7
1-node	1.7 b	2.3 b	3.3 b	5.4 b
2-node	1.4 b	2.2 b	3.0 b	4.2 b
3-node	5.5 a	5.9 a	6.6 a	7.6 a
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001	0.0001

Table 7. Number of new leaves developed on 1-node, 2-node, and 3-node tea cuttings collected on 1 Nov 2022 at 7 months after collection (Jun 2023), at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Jun 2023
1-node	4.0
2-node	3.0
3-node	3.0
<i>P</i> value	0.71

Table 8. Overall plant health of 1-node, 2-node, and 3-node tea cuttings collected on 1 Nov 2022 at 1, 2, 3, 4, 5, 6, and 7 months after collection, at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA.

Treatment	Months After Collection						
	1	2	3	4	5	6	7
1-node	5.0	5.0	5.0	4.8	4.7	4.6	4.6
2-node	5.0	5.0	5.0	4.9	4.8	4.6	4.6
3-node	5.0	4.9	4.9	4.8	4.8	4.4	4.3
<i>P</i> value		0.38	0.48	0.46	0.97	0.70	0.47



Fig. 1. 'Minto Pacific' tea plants grown by John Vendeland in Burlington, WA.



Fig. 2. Tea shoot cuttings placed in water from time of collection until time of processing.

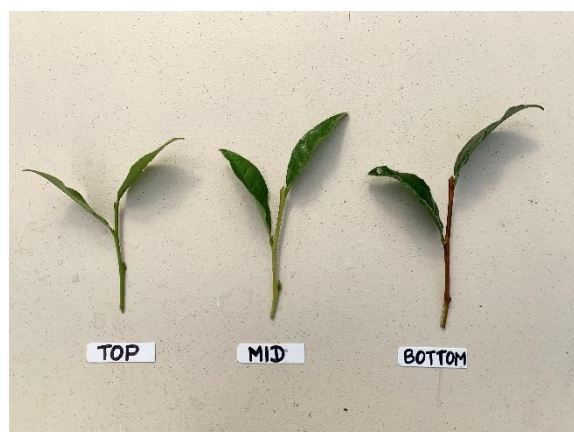


Fig. 3. Top, mid, and bottom section of tea shoots, collected on 21 Sep 2022.

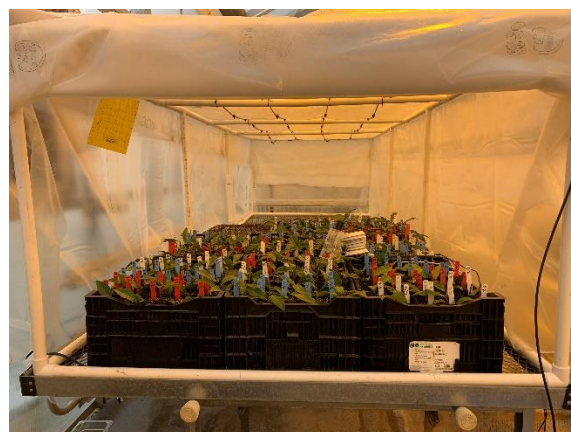


Fig. 4. Tea cuttings in the mist chamber.



Fig. 5. Single-node, two-node, and three-node tea cuttings, collected on 1 Nov 2022.



Fig. 6. Fairhope tea cultivar (left) and Minto Pacific tea cultivar (right) at time of field transplanting at WSU NWREC on 14 Oct 2022.



Fig. 7. Planting 'Minto Pacific' tea plants at WSU NWREC on 8 Jun 2023.



Fig. 8. 'Fairhope' (left) and 'Minto Pacific' (right) tea plants at WSU NWREC on 10 Jul 2023.





Fig. 9. 'Minto Pacific' tea plants sprouting after defoliation in winter at WSU NWREC.

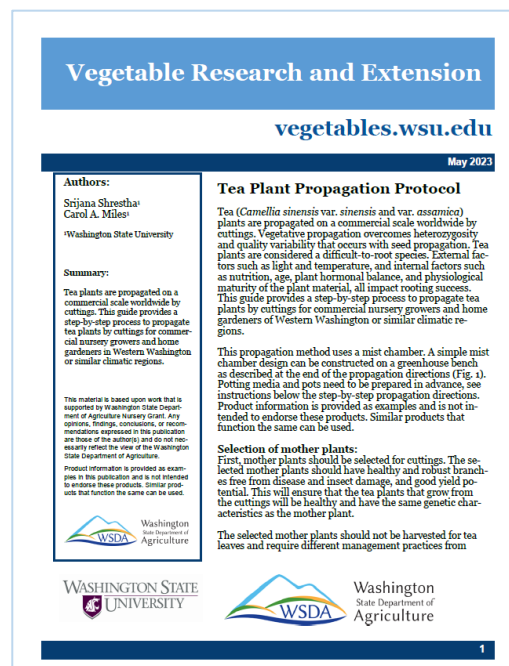


Fig. 10. Tea plant propagation guide available on <https://vegetables.wsu.edu/tea/>.

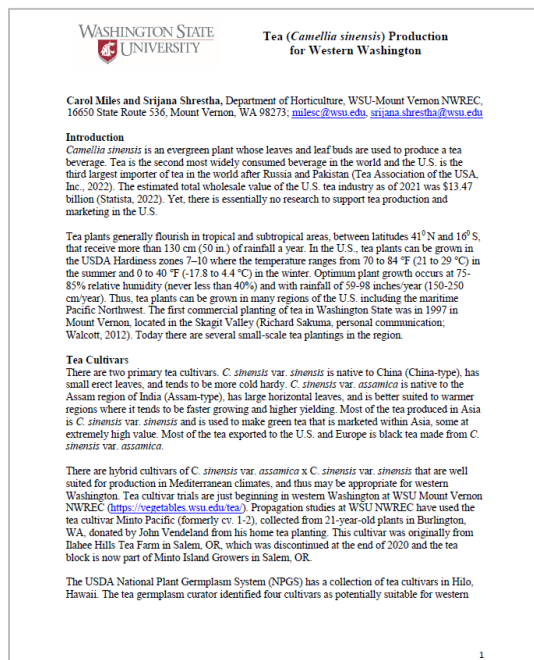


Fig. 11. Tea planting guide available on <https://vegetables.wsu.edu/tea/>.