Strategy and Guidance for Minimizing Hybridization Risk of *Castilleja levisecta* (CALE) with *Castilleja hispida* (CAHI) While Advancing Conservation of CALE and Taylor's Checkerspot Butterfly (*Euphydryas editha taylori*; TCB)

U.S. Fish and Wildlife Service, Washington Department of Fish and Wildlife, and Washington Department of Natural Resources (Natural Heritage and Natural Areas Programs)

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I. PURPOSE: This document's purpose is to 1) Communicate the issue of hybridization as a threat to the genetic integrity of CALE, currently listed as a federally Threatened species, 2) Outline solutions necessary for long-term protection of CALE viability and successful use of CAHI as forage for Taylor's checkerspot butterfly (TCB) recovery in proximity to CALE recovery sites, and 3) Provide specific information on sites, geographic areas, and proximity analysis guidance to successfully implement outlined solutions.

II. ISSUE: Rangewide, USFWS and partners have been successful at establishing populations of CALE at prairie sites, however, we are confronted with the impacts of CAHI hybridization on CALE populations in a few locations, especially in the South Puget Lowlands region. If hybridization issues increase they could hinder our ability to conserve CALE populations through time and have potential consequences for TCB restoration site selection (CALE and TCB+CAHI are in competition for limited recovery sites).

III. BACKGROUND: Hybridization can occur when populations of CALE and CAHI occupy the same site or are within a distance where cross-pollination may occur, resulting in the loss of the pure CALE genotype (Kaye and Blakely-Smith 2008, Clark 2015, entire). CALE is diploid (2n = 12) and CAHI may be diploid (2n = 12) or tetraploid (2n = 24). First generation hybrids between CALE and diploid CAHI can result in diploid hybrids. Although hybrids may have reduced pollen fertility, they are still capable of crossing with each other or back-crossing with either parental species. In a few years time, populations of CALE and CAHI that were genetically distinct can become a hybrid swarm of mixed genetic heritage. Hybridization between CALE and tetraploid populations of CAHI result in first generation progeny that are triploid (2n = 18). These hybrids cannot backcross with their parents, but could potentially cross with each other. Tetraploid populations of CAHI have been documented from Yellow Island in the North Puget Lowlands and on Joint Base Lewis McChord in the South Puget Lowlands. Other CAHI populations in the South Puget Lowlands are diploid, and diploid populations may also be present in the North Puget Lowlands based on the discovery of a single triploid CAHI on Yellow Island (Kaye and Blakely-Smith 2008). Mixed populations of CALE and tetraploid CAHI represent a much lower hybridization and back-crossing risk than those of CALE and diploid CAHI (Sandlin 2018; Fisher et al. 2015; Kaye and Blakely-Smith 2008), but any hybridization still represents an opportunity cost to CALE populations through the waste of ovules that might have produced viable CALE seed. Because CALE has a relatively short-lived seed bank, reduced

fecundity due to back-crossing can have an adverse impact on long-term persistence at a site (Sandlin 2018, p. 91; Sandlin, I. *in litt*. 2019).

Several mechanisms of hybridization between CALE and CAHI have been observed or suspected, including seeding or planting of both species in close proximity, seeding of both species due to accidental contamination of seed lots during seed processing or mixing, seeding of both species due to accidental contamination of field equipment or field work clothing, and seeding or planting of hybrids due to hybridization occurring at seed production facilities. The degree to which hybridization creates a conservation risk for CALE depends on the fitness and fertility of crossed progeny and the likelihood that inter-specific hybridizations will occur. Additionally, if CAHI is present in areas adjacent to, or within a pollinator-flight distance of sites that contain CALE, cross-fertilization can occur via pollination (Clark 2015, pp. 94-95). This can occur on field sites and with nursery-grown seed.

Hybridization has occurred at several sites in the South Puget Lowlands (Table 1). At some sites (e.g., West Rocky Prairie Wildlife Area, Tenalquot Preserve, Glacial Heritage Preserve) introduced populations of CALE were impacted by subsequent or simultaneous planting of large numbers of CAHI. The appearance of hybrid plants became so ubiquitous at two of these sites (West Rocky, Tenalquot) that efforts to manage hybridization were unsuccessful and CALE reintroduction was abandoned. This reduced the number of newly established populations eligible to be counted toward CALE recovery because hybridized populations do not contribute to CALE recovery. Additionally, accidental seeding of CAHI occurred in one CALE outplanting site in Southwest Washington (Steigerwald Lake National Wildlife Refuge), which has required site eradication and will require restoration of CALE plants on site using uncontaminated seed sources in the future.

CAHI is also a native plant, extant across much of the same range as TCB and CALE. It is promoted as a primary host plant for TCB recovery and as a species in general grassland restoration within portions of the same landscape where CALE recovery efforts are underway in Washington. TCB requires open grassland habitat dominated by short-statured grasses, with abundant forbs to serve as larval host plants and nectar sources. These habitats are found on prairies, shallow-soil balds (Chappell 2006, p. 1), grassland bluffs, and grassy openings within a forested matrix on south Vancouver Island, British Columbia[1]; the north Olympic Peninsula; south Puget Lowlands, Washington; and the Willamette Valley, Oregon. Appropriate habitat for TCB exhibits similar characteristics and some geographic overlap with CALE, although there are some notable differences.

In Washington and Oregon, TCB larvae feed on plants in the Broomrape (Orobanchaceae), Plantain (Plantaginaceae) and Honeysuckle (Caprifoliaceae) families containing iridoid glycosides. In Washington larvae feed primarily on narrowleaf plantain (*Plantago lanceolata*)

[1] There are currently three extant CALE sites on islands/islets off southeast Vancouver Island, British Columbia and one extant TCB site on Denman Island off east central Vancouver Island. Sites within the British Columbia region will not be addressed further in this document due to the three agencies' lack of jurisdiction within Canada. However, we strongly recommend similar strategies regarding the use of the two *Castilleja* species be taken by our British Columbia counterparts to avoid the risk of hybridization.

and CAHI, and may also feed on blue-eyed Mary (*Collinsia grandiflora* and *C. parviflora)* and sea blush (*Plectritis congesta*), and have been reported feeding on CALE in a few instances (Evans et al. 1984, unpub. data as cited in Dunwiddie et al. 2016; Kaye 2013, *in litt*.). In Oregon, they utilize *P. lanceolata*, CALE (Kaye 2013, *in litt*.), and *P. congesta*; however, CAHI is not known to occur on or near occupied TCB sites in Oregon. Larvae may also use different host species at different developmental stages (Kuussaari et al. 2004, p. 142). Ultimately, TCB exhibit complex patterns of geographic variation in diet and preference with negative effects on larval survival related to temporal variation in the quality and availability of host plants, which is influenced by microclimate and other factors (i.e., it is a localized problem).

Checkerspot larvae are known to feed on a wider range of host plants than females select for egg-laying (oviposition) (Kuussaari et al. 2004, p. 142). In Washington, TCB oviposits on two primary plants: CAHI and the non-native *P. lanceolata*, although oviposition on other plants has occasionally been observed (Stinson 2005; Linders 2006). TCBs in Oregon use *P. lanceolata* and CALE for egg laying. Variation in oviposition preference is genetically based with individual females differing within and among populations (Singer et al. 1988; Singer 2004, p. 126). In addition, 1) some individuals are less choosy than others, 2) some utilize more than one host species, and 3) preference for a particular host declines as time since oviposition increases (Haan et al. 2021). Female oviposition choice testing in checkerspots is an intensively studied technique; it is complicated and has many potential pitfalls (Singer 2004, entire).

Laboratory studies of oviposition preference in Washington found mixed results. A 2013 study reported TCB did not distinguish between CAHI and CALE, preferring both to *P. lanceolata* (Aubrey 2013; Aubrey Buckingham et al. 2016). More recent work by Haan et al. (2021) using an alternate technique found significant differences in the number of eggs laid on *P. lanceolata* (44 percent), CAHI (31 percent) and CALE (24 percent). In addition, some butterflies oviposited strongly on *P. lanceolata* and others strongly on CAHI; some used both or all three species. No individuals allocated more than half their eggs to CALE; eight allocated more than half to CAHI, thirteen allocated more than half to *P. lanceolata*, and the remaining eight apportioned their eggs more evenly among the three species. Both studies utilized the same South Puget Lowlands source population so differences could be the result of host plant variation, the smaller number of lineages used in the previous study (5 vs. 29), or methodology (Haan et al. 2021). It is well-established that frequency of use and order of oviposition preference vary between populations with the same potential host resources available and that patterns of larval survival follow female choice (Kuussaari et al. 2004, p. 142-143). However, larval survival may differ from female choice in populations undergoing rapid change as a result of anthropogenic influence.

Another recent body of work utilizing the same South Puget Lowlands population source evaluated several multi-trophic and host-parasite questions related to TCB, *P. lanceolata*, CAHI, CALE, and other plants in hemiparasitic relationships with those plants (Haan 2017). Haan (2017, p. 5-6) found that the host plant used, and the plants in hemiparasitic relationships with that host plant, had a strong influence on larval mass, growth rate, survival, and secondary

chemical sequestration. Haan's (2017, p. 140-141) work also suggested that CALE may have disadvantages as a larval host plant compared to CAHI. Thus, while the value of CALE in efforts to reintroduce and recover TCB on prairie landscapes in Washington remains uncertain, there is substantial evidence for the importance of CAHI and *P. lanceolata*. There is a greater need for field studies on the efficacy of all three species and on CALE/CAHI hybrids as hosts for Taylor's checkerspot to better understand their fitness contribution.

IV. SOLUTIONS: Presented below is a landscape-level, long-term strategy for conservation of prairie ecosystems with specific guidance for preserving CALE recovery sites and a decision guidance/framework for future prairie conservation actions, including the use of CAHI as a host plant for TCB recovery and general prairie restoration.

To eliminate or greatly minimize the potential for hybridization the U.S. Fish and Wildlife Service (USFWS), Washington Department of Fish and Wildlife (WDFW), and Washington Department of Natural Resources (WDNR) as public natural resource agencies advise the following actions:

a. <u>Prevent Hybridization in Other Geographic Areas:</u> To prevent hybridization in other geographies, we recommend maintaining adequate distance between planted patches of each species by following the guidance laid out below and in maps (Figures 3-7) and decision-making flow charts (Figures 8-10). *Castilleja* planting should adhere to the priority set for the appropriate region, cluster, or site. In areas with CALE priority, only CALE should be planted. In areas with TCB priority, only CAHI should be planted. This strategy works to minimize the *Castilleja* hybridization risk while advancing conservation of CALE and TCB. Due to the need to separate patches of CALE and CAHI, planting decisions involving either species affect more than their potential for hybridization; they affect conservation potential for CALE and TCB. Once a *Castilleja* species is planted in a place (e.g., CAHI for TCB), it likely precludes that place, and surrounding areas identified to minimize hybridization risk, for recovery of the other (e.g., CALE). However, exceptions may include TCB conservation at sites where TCB are shown to utilize CALE, or the polyploid CAHI line (assuming hybridization risk with CALE is determined to be acceptable).

In part because planting decisions have this broader effect on endangered species conservation potential, we strongly suggest that partners follow this landscape level design that outlines priority regions (Figure 1 and 2), sites[2], and site clusters[3] for planting of CALE and CAHI. This guidance is based on current understanding of landscape occupancy, habitat suitability and best available science. This guidance may be updated by the resource agencies as necessary upon protection of new lands for future conservation efforts, significant changes to existing site conditions, or if new science emerges; updated guidance will supersede any prior versions.

[2] A site is defined as an open grassland area of a single ownership that is or could be occupied by either CALE or CAHI.
[3] A cluster is defined as a group of sites linked by the potential for reproductive exchange.

i. North Puget Lowlands: The North Puget Lowlands is a priority recovery region for CALE and planting it should always take priority in suitable habitats (i.e. prairie soils, as opposed to shallow soil balds) (Figure 3). However, because there are extant CAHI locales within the region, particularly the San Juan islands (e.g., on shallow soil balds), *Castilleja* plantings should be assessed using the North Puget Lowlands decision-making flowchart (Figure 9). In addition, some specific areas within the region may be identified as priorities for future TCB conservation efforts.

ii. North Olympic Peninsula: The North Olympic Peninsula has been identified as a priority recovery region for TCB (Figure 4). Therefore, CALE should not be planted in this region. *Note: there are two small extant reintroduced populations of CALE in this region that are isolated from any known CAHI patches.*

iii. South Puget Lowlands: The South Puget Lowlands is a priority recovery region for both CALE and TCB. Many plantings of CALE and CAHI and their hybrids exist in this landscape. Because of this complexity, in this region recovery priority is assigned by site and site cluster (Figure 5 and Table 1).

iv. Southwest Washington: This region has not yet been comprehensively evaluated for recovery priorities. In most cases, conservation priorities have not yet been assigned at the site or cluster level with the exception of Steigerwald Lake National Wildlife Refuge, which has been assigned as a CALE priority site (Figure 6).

v. Willamette Valley: The Willamette Valley has been identified as a priority recovery region for CALE (Figure 7). Therefore, to prevent hybridization CAHI should not be planted in this region. The recovery of TCB is also a high priority for this region, however, CAHI is not used in TCB conservation efforts within the Willamette Valley. *Note: there are native populations of CAHI in this region, but they are not present around or near CALE or TCB sites*.

Table 1. South Puget Lowlands prairie conservation sites and their assigned CALE and TCB priority and hybrid status. Alternate shading indicates site clusters[4], which are sites [5]in close proximity to each other, refer to Figure 5.

Site Name	Ownership	Recovery priority	*Castilleja* occurrence	Hybrid Status
Mima Mounds Natural Area Preserve	WDNR	CALE	CALE, Hybrids	Low level of hybridization
Glacial Heritage Preserve	Thurston County, WDFW inholding	CALE	CALE, CAHI, Hybrids	Low level of hybridization
Scatter Creek Wildlife Area	WDFW	TCB	CAHI, Hybrids	Hybridized
Violet Prairie Seed Farm and Preserve	CNLM	TCB	CAHI	Not Hybridized
Fisher Ranch	Private, NRCS Easement	TCB	None	Not Hybridized
Mazama Meadows	CNLM	TCB	None	Not Hybridized
Cavness	TNC, CNLM	CALE	CALE, Hybrids	Low level of hybridization
Colvin	Private, NRCS Easement	Unresolved	CALE	Not hybridized
West Rocky Prairie Wildlife Area	WDFW	TCB	CAHI, Hybrids	Hybridized
Rocky Prairie Natural Area Preserve	WDNR	CALE	CALE	Not Hybridized
Wolf Haven International	Private	CALE	CALE, non-spreading tetraploid CAHI	Not Hybridized
Tenalquot Preserve	CNLM, WDFW Easement	TCB	CAHI, Hybrids	Hybridized
Deschutes River Preserve	CNLM	TCB	CALE	Not Hybridized
Bald Hill Cluster	WDNR, Weyerhaeuser	TCB	CAHI	Not Hybridized

b. <u>Implement Decision-Making Framework for New Sites Under Consideration for CALE or CAHI plantings:</u> To minimize hybridization risk at new sites within regions where conservation of both species (CALE and TCB) have been prioritized (e.g., South Puget Lowlands), we promote the implementation of the following decision-making framework and decision flow charts (Figures 8-10):

[4] A cluster is defined as a group of sites linked by the potential for reproductive exchange

[5] A site is defined as an open grassland area of a single ownership that is or could be occupied by either CALE or CAHI

- Do not plant the congener of CAHI or CALE within 1 km of existing sites.
- For plantings at new sites situated between 1 km and 2 km from existing sites where hybridization risk is elevated, site-based recommendations should be made by USFWS/WDFW/WDNR (see Appendix 1 for proposal submission instructions).
- Generally, hybridization risk is low when congeners are planted outside 2 km from existing sites. New plantings of either *Castilleja* should be proposed to USFWS/WDFW/WDNR so that plantings can be tracked and monitored for risk (see Appendix 1).

For potential sites in new geographic areas (e.g., in Southwest Washington), recovery priority should be discussed and assigned prior to any outplantings moving forward.

It is strongly recommended that conservation partners involve the resource agencies whenever new outplantings of either species of *Castilleja* are being considered. This involvement will 1) help ensure conservation objectives of both golden paintbrush and Taylor's checkerspot butterfly are sustained or not impaired, and 2) assist the resource agencies in inventorying and tracking the status of these plantings. For plantings that need to be reviewed (see flow charts; Figures 8-10), resource agencies agree to implement a process that reaches consensus in a timely manner, uses the best available information and/or appropriate experts, assesses a range of possible alternatives, and results in a decision that is scientifically sound and best achieves the conservation objectives entrusted to the three resource agencies. In addition, the resource agencies will review the decision over time to confirm that it is achieving those original objectives, and if not, revisit that decision. When a proposal is received from a conservation partner or project proponent (see Appendix 1 for proposal review form), the resource agencies will also ensure that objectives of that partner are also considered in the decision process.

The resource agencies invite submission of information for all plantings, including those that do not need to be reviewed by the resource agencies based upon the priority regions and decision flow charts (Figures 8-10)(e.g. planting CAHI in the North Olympic Peninsula). Planting information can be submitted in the same way as the proposals (see Appendix 1) and will be used to inventory the locations of CAHI and CALE plantings to inform future projects.

c. <u>Actively Manage Sites that are Hybridized and Site Clusters that have Both CAHI and CALE Priorities:</u> Some sites have high levels of hybridization. If feasible, eradicating hybrids on site and returning to a pure CAHI or CALE site is recommended.

There are existing clusters of sites that currently contain both CALE and CAHI sites and are priority to maintain both species within the cluster. In these clusters, managers should invest in efforts to maintain adequate buffers between sites. Ideally, where feasible and appropriate, there should be at least a 1 km distance between sites of these two species. Managers are refining strategies to ensure this situation is not repeated.

d. **<u>Map CALE and CAHI Distribution and Refine Active Inventory:</u>** To inform the adaptive management cycle, managers need an accurate understanding of CALE and CAHI historical and current distribution. Sites that contribute to CALE recovery and areas that have used CAHI in prairie restoration should be identified, data compiled and made available in a spatial dataset that can be used for future delineation of CAHI or CALE sites. CAHI ploidy level should be included in these datasets wherever possible. This spatial dataset should build upon existing information and be stewarded by WDNR Natural Heritage Program (NHP). Maps of current known CALE and TCB in North Puget Sound, North Olympic Peninsula, South Puget Sound, and Southwest Washington, Washington, and Willamette Valley, Oregon, are found in Figures 3-7.

e. **<u>Adopt Decontamination Procedures for Multi-Site Prairie Restoration Activity:</u>** All site stewardship and management entities sharing equipment across both CALE and CAHI sites should have active standard operating procedures (SOPs) that address the potential for seed material cross-contamination. In addition, SOPs should address the potential for cross-contamination from general visitation of prairie sites through the transfer of seeds from field gear, shoes, sample collection, etc.

f. **<u>Formalize Seed Production Risk Management:</u>** Seed-producing partners should produce and follow SOP for how seed contamination will be eliminated, if a partner produces both CALE and CAHI seed. The SOP will need to address growing, production, processing, and distribution of seed consistent with the guidance in this document, to minimize the risks of genetic mixing and of accidental seed mixing or contamination. The SOP should include review and input from interested seed producers, as well as from funders and end users of the seed, including USFWS, WDNR and WDFW.

g. **<u>Disseminate Information:</u>** It is important that restoration partners throughout the range of CALE and CAHI are aware of the hybridization threat and are able to implement the necessary precautions to avoid hybridization. This should be done by disseminating this document and any other related agency guidance to all prairie conservation/restoration partners. Preferred means for dissemination should be determined by management agencies and should include a signed MOU among key prairie partners. The final form of this strategy guidance document should be made readily available, for example: distributed throughout an organization's individual programs/divisions, posted on each partner's websites, included in grant/agreement funding award notices, etc. Partners will also need to ensure any new prairie conservation practitioners are made aware of this document.

h. **<u>Improve Our Understanding of Hybridization Risk and Its Impacts to Species:</u>**

i. <u>Address Risk at CALE and CAHI Sites in Near Proximity and with Expansion Potential</u>: Develop and formalize strategies that can be promoted to mitigate hybridization risk in areas where CAHI and CALE already occur in near proximity. These might include mismatched ploidy levels, forests as barriers, *P. lanceolata* use in conjunction with CALE

for TCB recovery, etc. Managers should also identify strategies to track population growth and expansion of CALE and CAHI outplantings and determine how that may impact implementation of this hybridization minimization strategy.

ii. Support Additional Pollinator Foraging Distance Research: Research concerning the pollinators of both paintbrush species is necessary to identify the foraging distance traveled by local shared pollinators of CALE and CAHI and assess the risk of contamination between CALE and/or CAHI sites. In lieu of specific research on the foraging distance of *Bombus californicus* (California bumble bee), the main shared pollinator of CALE and CAHI, we use best available science on other *Bombus* spp. In general, *Bombus* spp. can travel the farthest distance of known CALE and CAHI shared insect pollinators. Charman and others (2010) found 95% of foraging activity by *Bombus* spp. was completed within 0.96 km of nest locations.

iii. Support Research on TCB Fitness Relative to Host Plant Usage (Hybridized CAHI, polyploid CAHI, and CALE): CAHI is a primary oviposition plant for TCB, and prior to hybridization with CALE, the diploid form was the most commonly occurring variety, at least in the South Puget Lowlands. As a result of hybridization there has been a move to propagate the polyploid form of CAHI for use in habitat restoration on TCB sites as an alternative to the diploid form to mitigate the effects of hybridization, but without a solid understanding of use by and fitness for TCB. Likewise, little is known about TCB usage of CALE on sites where both species are present. Research is needed to evaluate the likelihood that TCB will oviposit on CALE or the various forms of CAHI (hybrids or polyploid) and estimate the rates of larval survival associated with each to understand their relative contribution to recovery.

iv. Support Research on Long-term Interactions Between CALE and Polyploid CAHI: Research indicates that the threat to CALE from hybridization is greatly reduced when polyploid CAHI seed stock is utilized as compared to diploid CAHI (Sandlin 2018). While gene flow is reduced in crosses between diploid CALE and polyploid CAHI, there is still potential for some genetic exchange to occur. Experiments focusing on quantifying the levels of hybridization between CALE and polyploid CAHI over multiple generations would inform how hybrid ploidy is manifested in interploidy crosses and potential impacts on future recovery efforts for both CALE and TCB.

V. CONCLUSION: The commitment of the USFWS, WDFW, and WDNR is to collaboratively work with our partners to successfully secure the conservation of both golden paintbrush and Taylor's checkerspot butterfly, while confronting challenges posed by potential hybridization between *Castilleja* species. This is a difficult task given the limited amount of remaining and potential habitat for these and other prairie species from the Puget Trough to the Willamette Valley. This strategy and guidance attempts to strike a balance for both species and is intended to provide additional transparency to our partners about where conservation for these two species is expected to take place. Our goal is to provide partners with added clarity to improve

planning and implementation efficacy of conservation and restoration activities across these prairie/grassland landscapes.

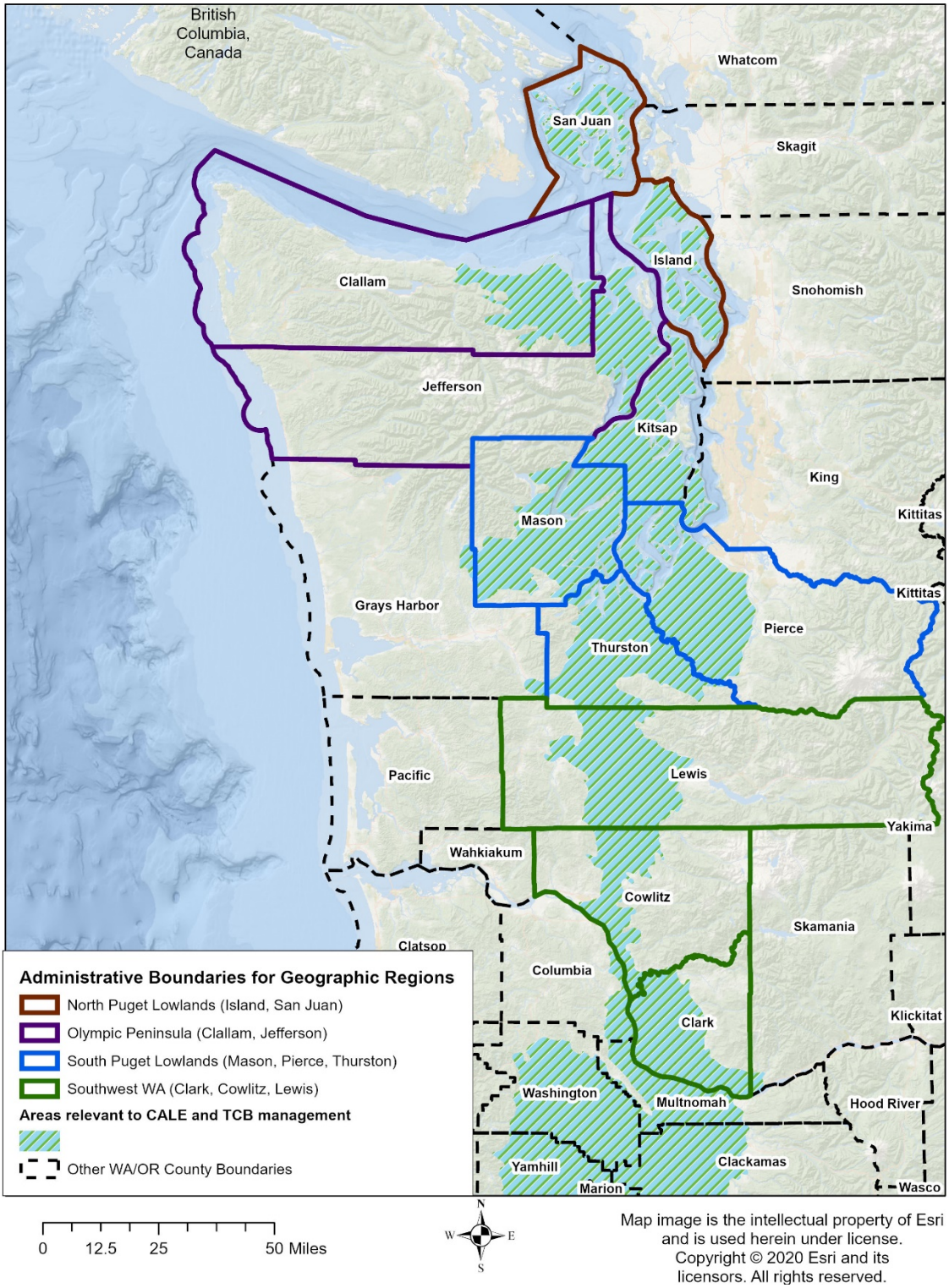


Figure 1. Geographic Regions in Washington and associated administrative boundaries used for guiding the prioritization of CALE and TCB conservation efforts.

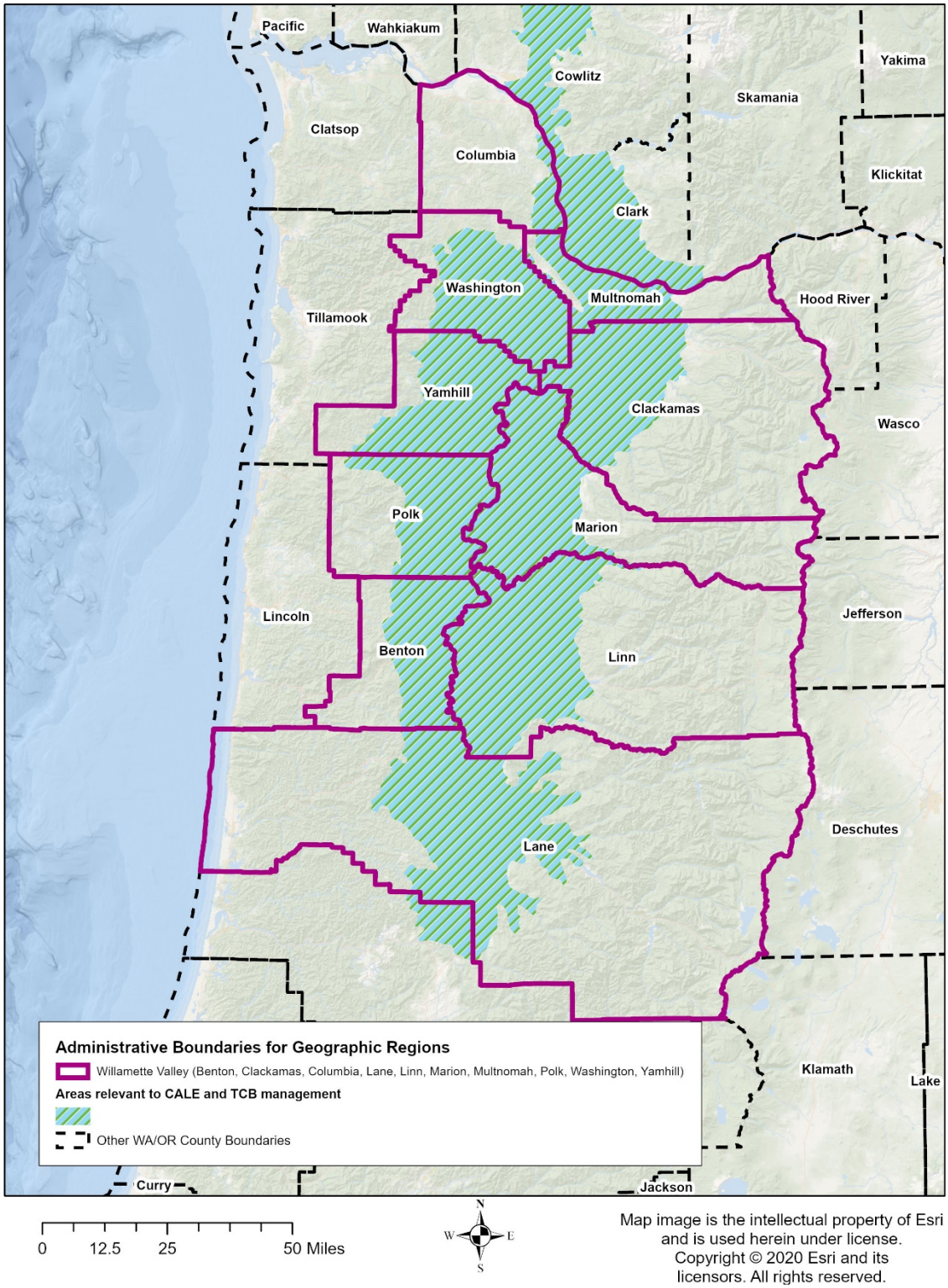


Figure 2. Geographic Regions in Oregon and associated administrative boundaries used for guiding the prioritization of CALE and TCB conservation efforts.

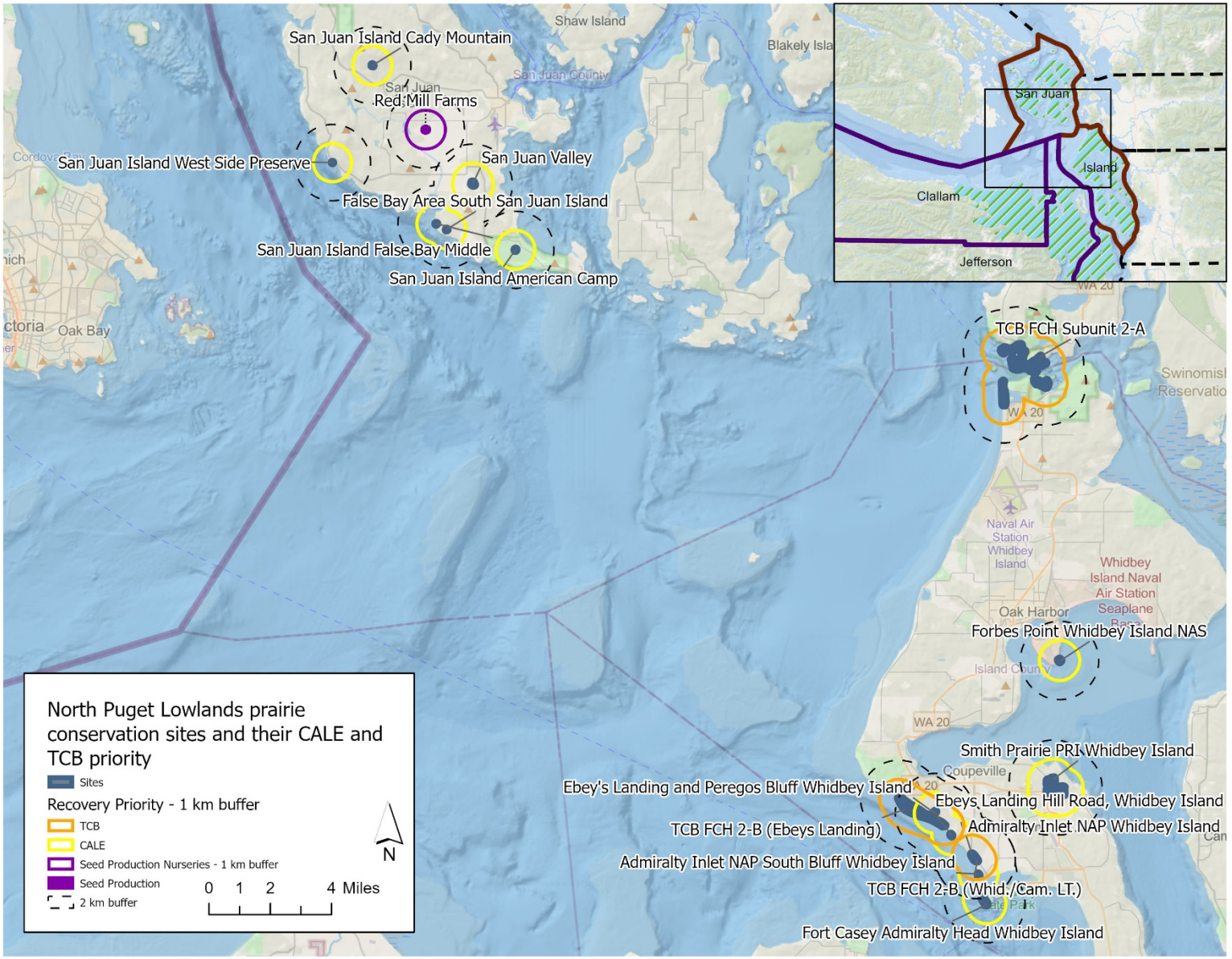


Figure 3. CALE sites and TCB critical habitat in the North Puget Lowlands Region in Washington. CALE conservation is prioritized in the North Puget Lowlands Region, except for in bald habitats.

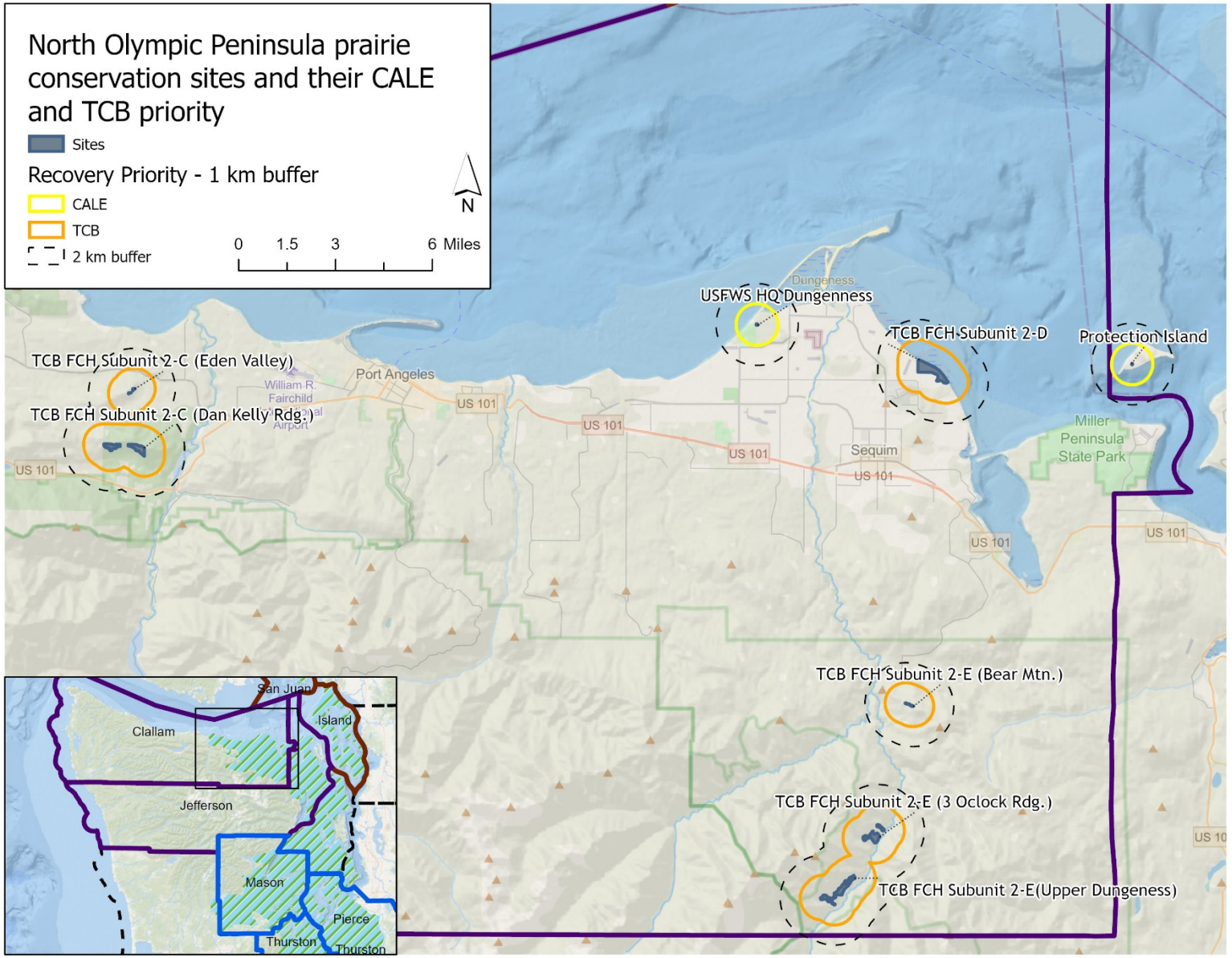
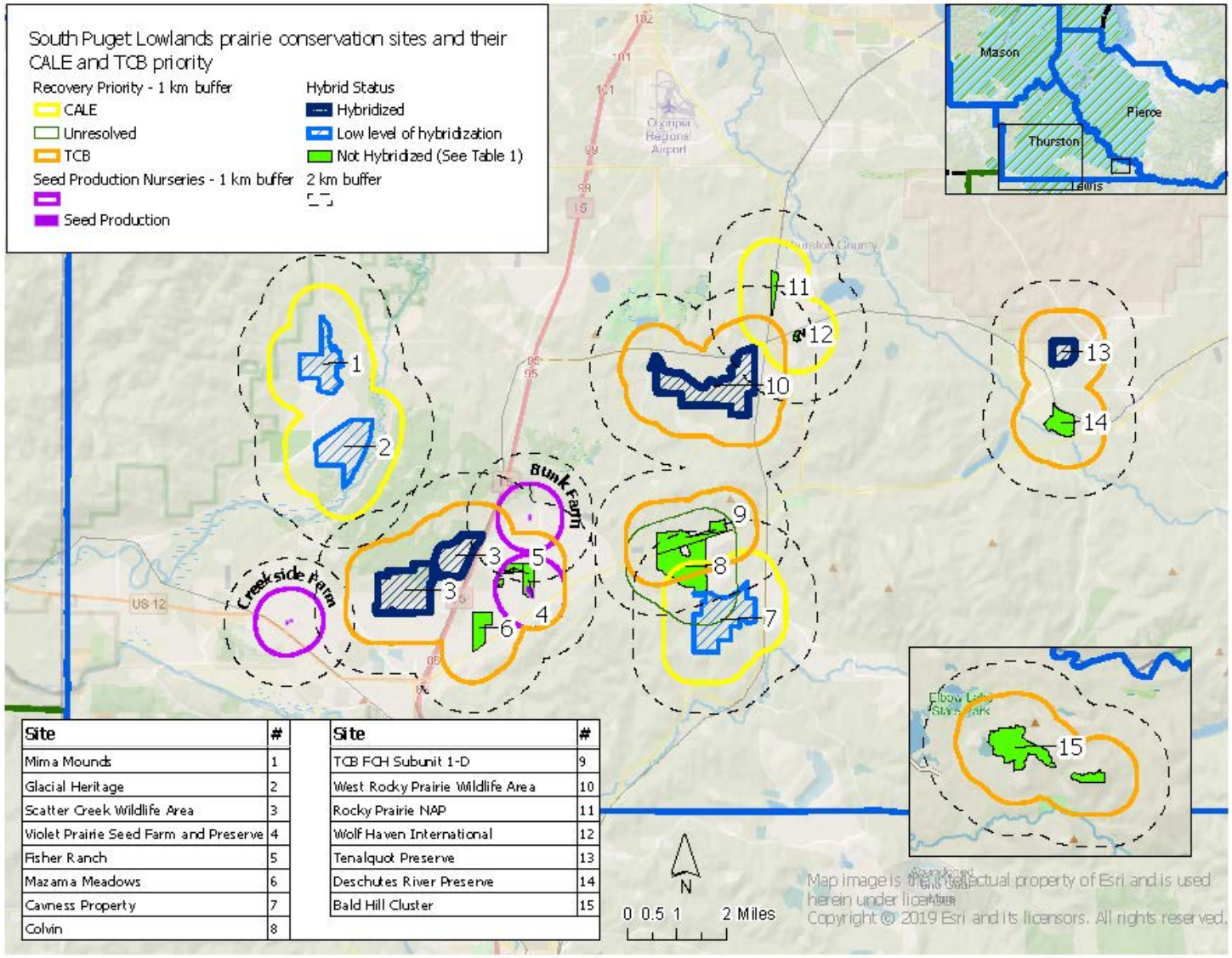


Figure 4. CALE sites and TCB critical habitat in the North Olympic Peninsula Region in Washington. TCB conservation is prioritized in the North Olympic Peninsula Region.



Site	#
Mima Mounds	1
Glacial Heritage	2
Scatter Creek Wildlife Area	3
Violet Prairie Seed Farm and Preserve	4
Fisher Ranch	5
Mazama Meadows	6
Cavness Property	7
Colvin	8

Site	#
TCB FCH Subunit 1-D	9
West Rocky Prairie Wildlife Area	10
Rocky Prairie NAP	11
Wolf Haven International	12
Tenalquot Preserve	13
Deschutes River Preserve	14
Bald Hill Cluster	15

Figure 5. CALE and TCB sites and seed production facilities in the South Puget Lowlands Region in Washington. Both CALE and TCB conservation is prioritized in the South Puget Lowlands Region, and has been more specifically divided by cluster and/or by site.

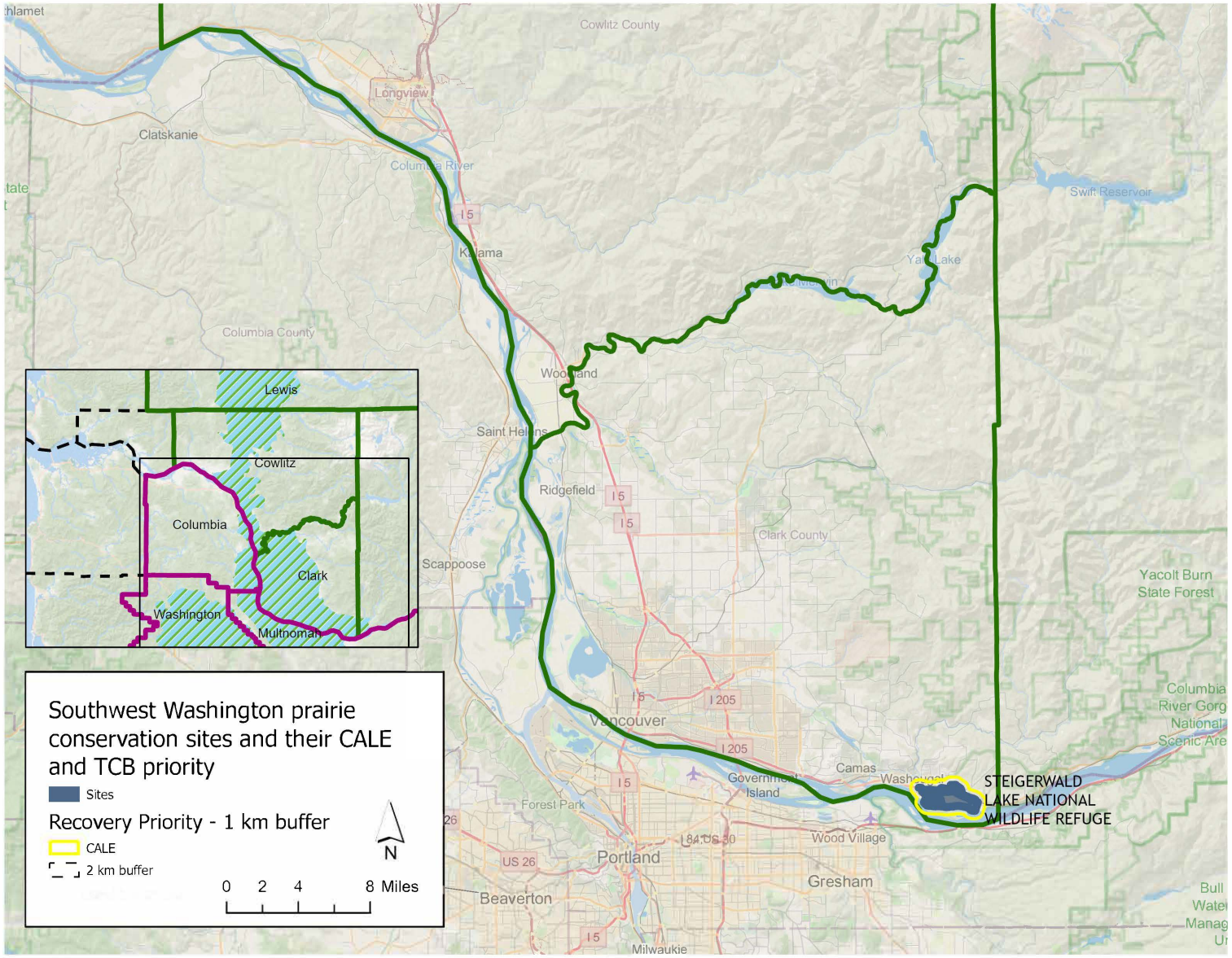


Figure 6. CALE and TCB sites in the Southwest Washington Region in Washington. Currently, no specific prioritization for either CALE or TCB conservation has been assigned for the Southwest Washington Region.

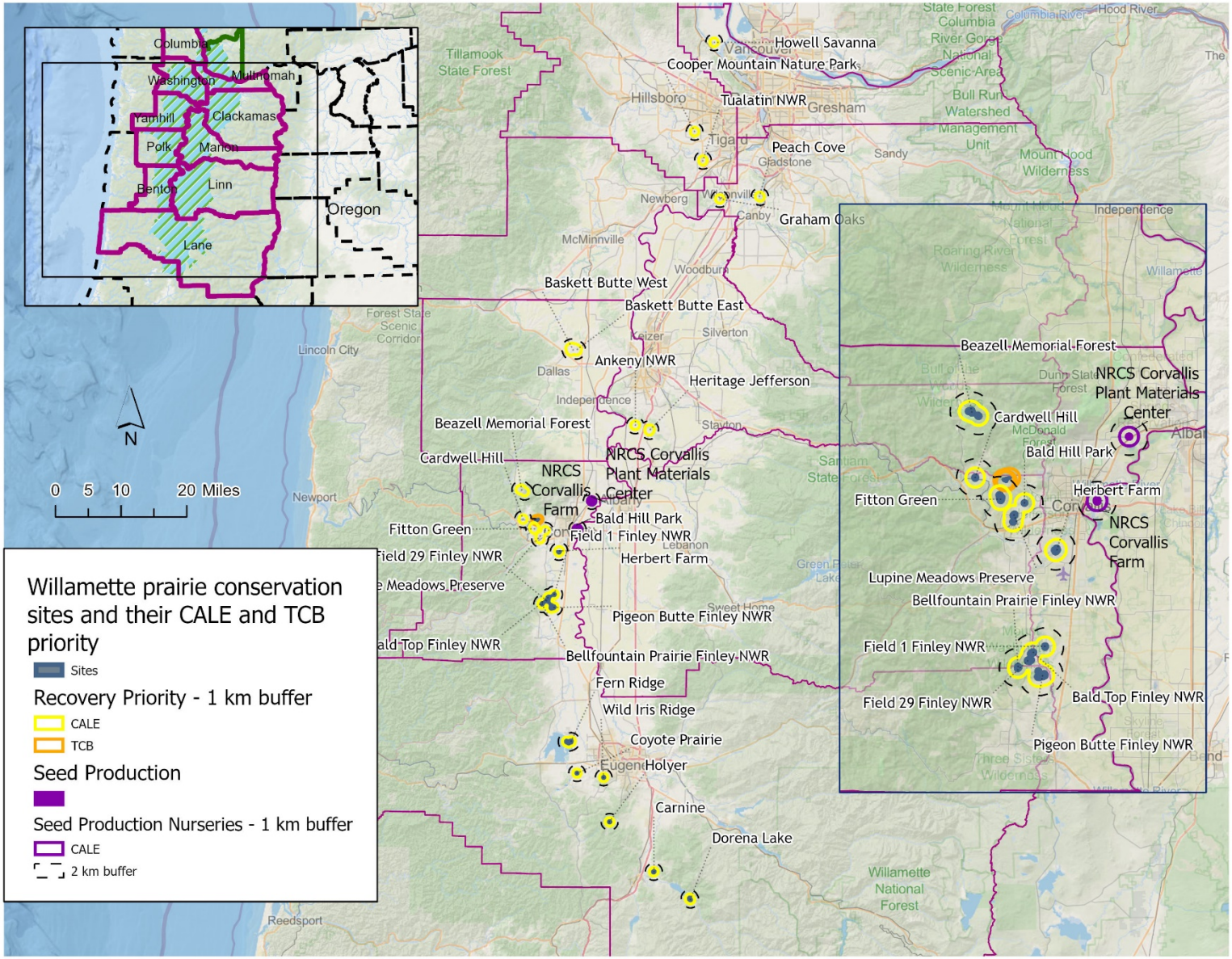


Figure 7. CALE sites, TCB critical habitat, and seed production facilities in the Willamette Valley Region in Oregon. CALE conservation is prioritized in the Willamette Valley Region. TCB conservation is also a high priority within this region, but it is conducted only with CALE when *Castilleja* is used.

Figure 8. Decision Flow Chart for Planting *Castilleja* Species:

Guide to Regions (please refer to planting priority maps, Figures 1 and 2)

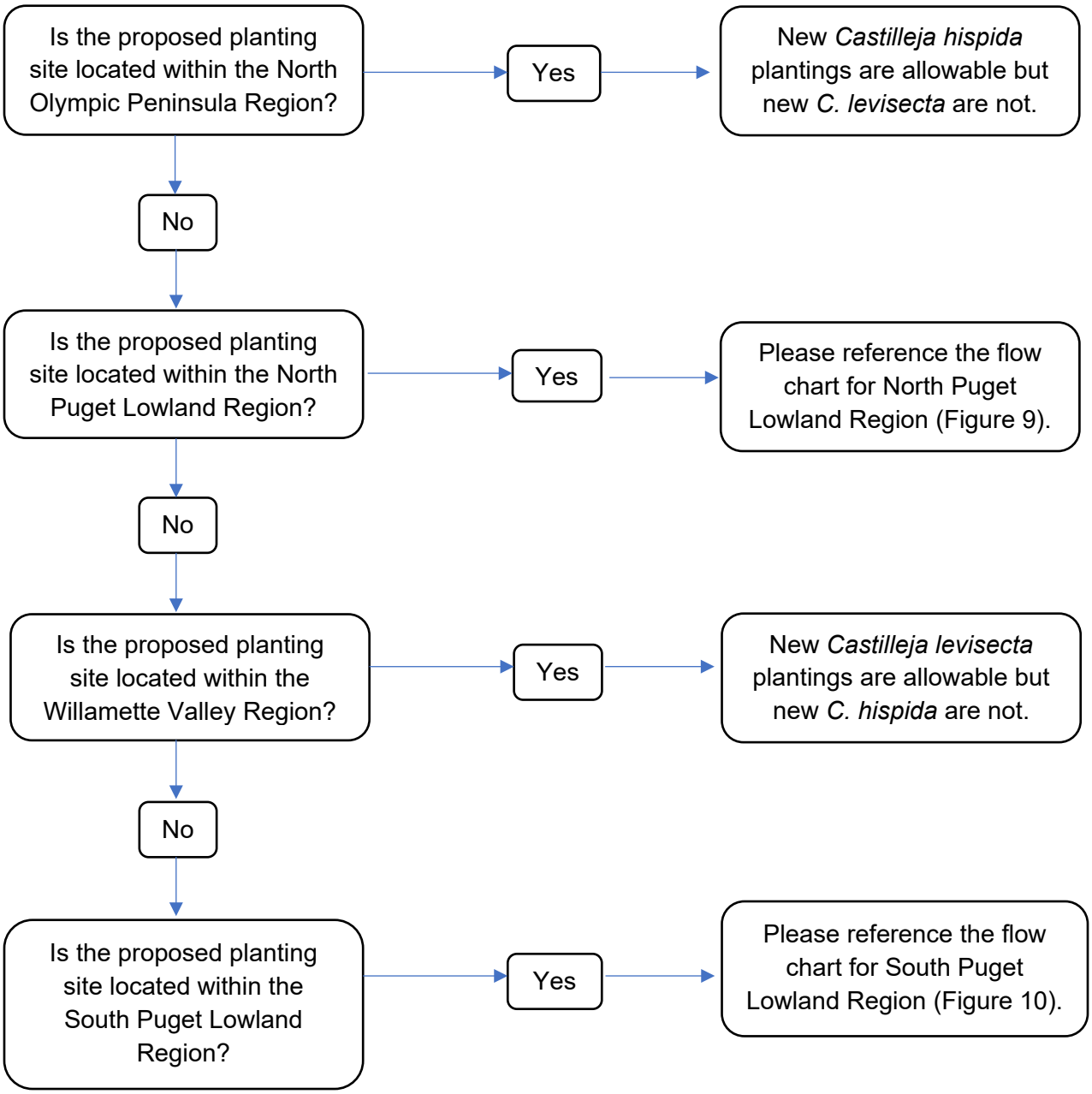


Figure 9. North Puget Lowland Planting Decision Flow Chart

Castilleja levisecta is the priority in this region and takes precedence, except when CAHI exists in close proximity; and/or soils are not suitable (e.g. shallow bald soils); or a specific area within the region has been identified as a priority for Taylor's checkerspot butterfly conservation, in which case CAHI is the priority.

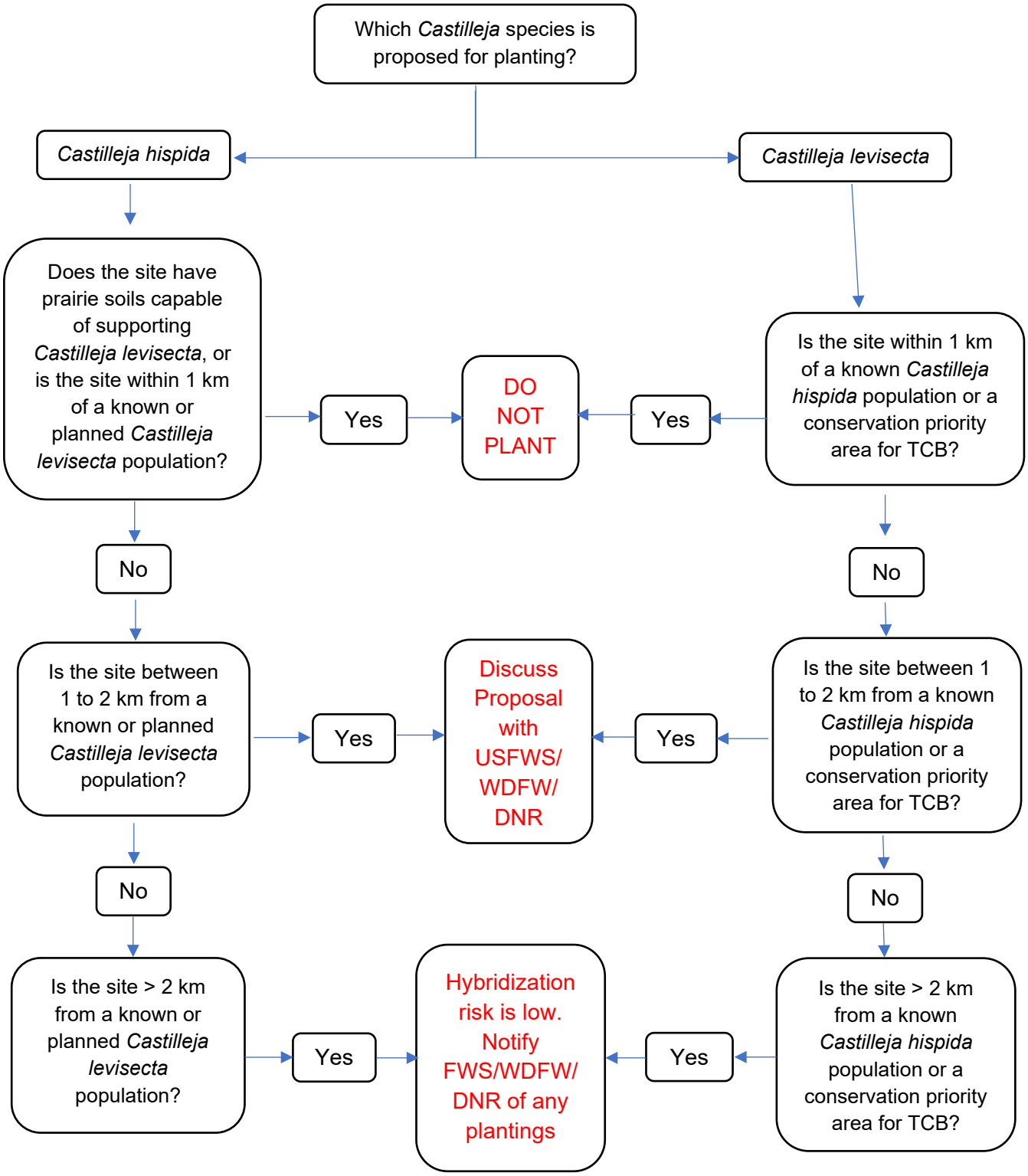
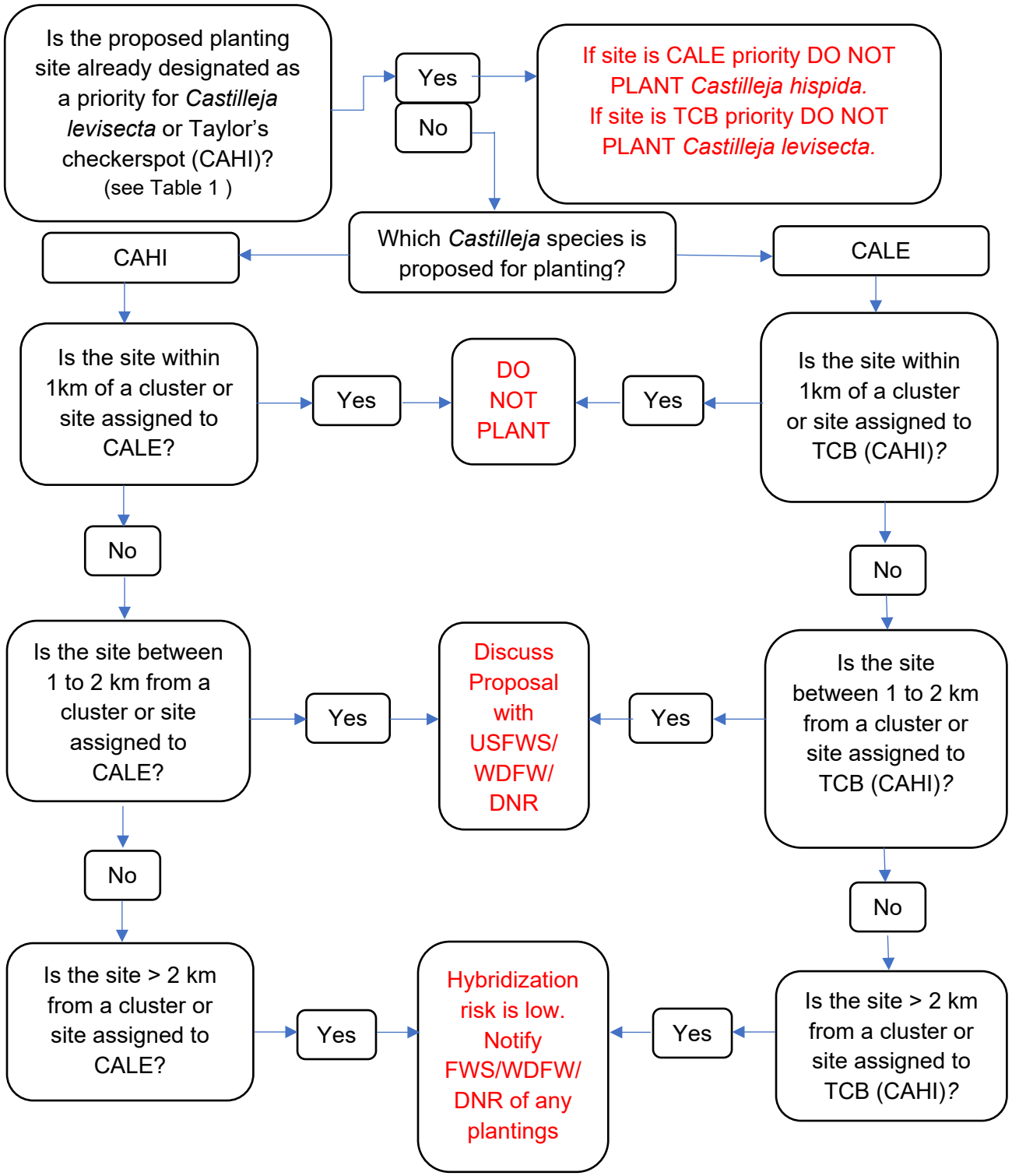


Figure 10. South Puget Lowlands Planting Decision Flow Chart



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Appendix 1. Golden (CALE) and Harsh (CAHI) Paintbrush Outplanting Proposal Review Form

Project Name/Title:	
Date:	
CONTACT INFO	
Project Proponent Name	
Mailing Address:	
Telephone:	
Email:	
PROJECT INFO	
Project Site Name:	
County & Nearest Town:	
Property Owner:	
Site Coordinates (Lat/Long or UTM)	
Maps and Photos:	(*Please attach maps and photos of the project location*)
Approximate Project Start Date:	
Project Description/Summary: (*Please identify which species of Castilleja is being proposed for planting and what mitigation measures are being proposed if the outplanting is known to be within 2 km of a location with the other Castilleja species*)	

Steps to submitting a proposal for review:

1. Complete the Proposal Review Form above, providing a contact person that is knowledgeable of the project and can answer any questions the Agency Review Team may have.

2. To expedite the process, please submit the proposal as far in advance of the proposed planting date as possible; proposals can take 30-90 days to review.

3. Please ensure project locations and maps are as exact as possible to help facilitate assessment of the project being proposed. If possible please include coordinates for the center of the site (Latitude/Longitude or UTM).

4. Submit your completed Review Form and any maps or photos to **FW1_paintbrush_hybridization@fws.gov**.

5. Upon receipt of your Review Form, you will receive an email confirmation from someone on the agency review team. If you do not receive an email confirmation within one week, please contact FW1_paintbrush_hybridization@fws.gov to confirm your Proposal Review Form was received.

Any questions about submitting this Proposal Review Form can be directed to FW1_paintbrush_hybridization@fws.gov.

What happens after the review?

Depending on the project proposal, expected review and response time is between 30-90 days.

Once our review is completed, the Agency Review Team will contact the project proponent to either 1) endorse implementing the project, 2) endorse implementing the project, but with recommended modifications, or 3) advise against proceeding with the project due to an elevated risk of hybridization that is unlikely to be mitigated. A record of the Agency Review Team's recommendation with associated rationale will be provided to the project proponent.

Should the Agency Review Team reach a decision that advises against implementing a project, the review team is agreeable to meet with a project proponent to explore alternative locations or alternative designs for a project that will reduce or avoid the risk of hybridization.