Work Plan

for the establishment of the Agroforestry Industry Development Initiative research and demonstration truffières in British Columbia

Truffle Association of British Columbia

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Version 1

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Introduction

Truffles are the fruiting bodies of a fungus that forms an ectomycorrhizal (ECM) association with tree roots. Truffles are extremely valuable, garnering as much as $500-1500(US)/kg. The Périgord black truffle (*Tuber melanosporum*) was originally produced in France and Italy in truffle and oak orchards on calcareous soil. Techniques for successful Périgord black truffle production have been developed, tested and shown to be successful in New Zealand, Australia, and parts of the US.

Truffle orchards have not previously been attempted or established in BC within a research context, although anecdotal evidence indicates that some individuals have already inoculated and planted host trees. Our analysis of the climatic and soil conditions in all of the areas where Périgord truffle is successfully grown indicates that parts of southern interior and south-western BC should be suitable for truffle production.

Since the initial inputs are relatively high when establishing a truffle orchard, and the returns are delayed by up to10 years, it is essential that *T. melanosporum* mycorrhizas remain dominant on the host. It is therefore critical that experienced and trained personnel examine fruiting bodies, mycorrhizas, and DNA sequences of the fungus before inoculated seedlings are planted and while the mycorrhizas develop in the orchard. This will ensure that the fungus is correctly identified and that it dominates on root systems at time of establishment and throughout the orchard over time. It is also essential that soil characteristics are monitored for pH, organic matter, major and minor nutrients, especially calcium, nitrogen and phosphorus, key elements that are critical for mycorrhiza development.

Because we are proposing to introduce a non-native species into BC (*Tuber melanosporum*; the non-native host hazels and oaks have already been introduced into BC), we are working with CFIA to ensure that we do not inadvertently introduce pests or diseases. By investigating the methods used in New Zealand and Australia to introduce *T. melanosporum* into truffle orchards, we have determined that it is unlikely that *T. melanosporum* will escape from BC's truffle orchards. BC has hundreds of native species of ectomycorrhizal fungi that are threats to invade our truffle orchards and displace the *Tuber* species. To give the *Tuber* species a competitive edge against native ectomycorrhizal fungi in the truffle orchard, we must raise the soil pH to about 7.9; soil pH in native BC soils tends to be acidic to neutral and alkaline soils are not common.

With funding from the Agroforestry Development Initiative Fund, we will establish a demonstration and research truffiére at UBC South Farm and 3 other demonstration and research truffiéres with producers in the 3 regions of the province likely to produce truffles (south Vancouver Island, the Lower Fraser Valley, and the Okanagan Valley). Because more control can be exercised at the UBC demonstration truffiére than at industry cooperator truffières in terms of establishment, treatments, and monitoring, and because the location of the

demonstration truffiére at UBC will permit future workshops and tours to access both meeting rooms and a field site, we believe that the UBC truffiére will make a very important contribution to the success of this project. In addition, professors, researchers, undergraduate and graduate students who may participate in the project as it develops will have easy access to this site.

This work plan details the steps necessary to install, maintain and monitor these Périgord black truffle orchards for demonstration and research purposes.

Materials and Methods

Inoculated stock

The truffières will be planted with 1-2 year old hazelnut (mostly *Corylus avellana*) and oak (*Quercus robur* and *Q. ilex*) seedlings inoculated with *Tuber melanosporum.* Currently, TABC is working with Quentin and Kathy Wyne, Okanagan Truffle Trees in Lake Country, BC, and Dr. Charles Lefevre, New World Truffières, Inc., Eugene Oregon, to acquire this stock. The goals is to plant 250 trees per hectare.

The seedlings must appear healthy (no wounds or malformations, good apical buds, single stem, no root malformation, good root development). To be acceptable, inoculated seedlings must have 50% of root tips colonized by *Tuber melanosporum*, less than 30% of root tips colonized by other mycorrhizal fungi, and 0% colonization by other *Tuber* species.

Mycorrhizal colonization

Mycorrhizal colonization will be assessed 1) just before outplanting, and 2) after outplanting. We will assess the fine roots using morphological and molecular approaches to determine the species of ECM fungi and their relative abundance.

Before outplanting, we will destructively sample 20 seedlings per lot (lot = specific host + fungus + inoculation event + growth conditions combination) to determine ECM colonization. All seedlings to be planted will be non-destructively assessed; these seedlings must all meet all of the criteria listed above.

The number of post-outplanting plants to be sampled will be 5 per truffiére. Field sampling will occur at the end of the growing season but before winter. For the first year, we will sample by carefully excavating 3 main roots emanating from the stem in different directions and removing 15 root tips per main root. These samples will be kept cooled, transported to the lab and assessed within days.

Dr. Dan Durall, UBC-O, will carry out the assessment of the roots for mycorrhizal colonization. The roots will be gently washed free of potting medium or soil, placed into a try in water, cut into 2 cm lengths, thoroughly mixed, and sub sampled. At total of 200 root tips will be evaluated per seedlings or 225 root tips per truffiére for mycorrhizal status and ECM species under 400X or 1000X magnification either as the whole root tip or fungus only (done by peeling the mantle from the root tip) and classified according to the detailed procedure described by Goodman et al. (1996). Representative tips of each morphotype will be used for molecular analysis of fungal species. Molecular analysis of *Tuber* ECM from each truffiére will be

carried out. DNA will be extracted from ECM, amplified with fungal-specific primers, and sequenced.

To determine whether *Tuber melanosporum* is present, the mycorrhizas will be compared morphologically with published descriptions of mycorrhizas (Agerer 1987-2002, Rauscher et al. 1995). DNA sequences will be compared with sequences of correctly identified *Tuber melanosporum* sequences in Genbank (http://www.ncbi.nlm.nih.gov/) and with the in-house sequence database that we create from purchased or donated specimens.

Installation Timing

Seedlings can be planted in the fall or spring depending on when the site and stock are ready. The UBC truffiére will be planted starting in the fall, 2007. The truffière on south Vancouver Island (Dave Lestock-Kaye, near Cobble Hill) is partially installed, but further planting will occur in 2007. The truffières in the lower Fraser Valley (Sharmin Gamiet, Olera Farms, Abbotsford) and the Okanagan Valley (Quentin and Cathy Wyne, Lake Country) will be planted starting in the fall, 2007.

Determination of soil pH and soil fertility

The area that will be used to grow the inoculated seedlings should be marked and the rows where the inoculated seedlings will be planted should be identified. A map should be generated clearly showing this grid. Soil samples (0-20 cm) should be taken 75 cm from all stems in both directions along the row (see Figure 1). The exact location where the soil sample will be taken should be clearly marked on the soil and on the map. All sampling after liming must be taken from these exact locations, so that pH adjustments can be monitored. For this intensive pre-liming soil sampling, samples from 5 trees (10 samples) should be bulked to permit a detailed mapping of inherent conditions and characterization of the site. Subsequently, the intensity of sampling will be determined partly based on the results of this detailed characterization (e.g. taking into account variability).

In addition, around 4 L of soil should be collected, sifted to remove debris and dried. This soil should be set aside and will be used as a control for all future analysis.

Soils will be analyzed for pH (in water), total C, N, P, available N, P, and available cations (Ca, Mg, K, Fe, Al and Mn), soil texture and coarse fragment content.

A soil pit will be dug to determine soil depth and find compacted horizons.

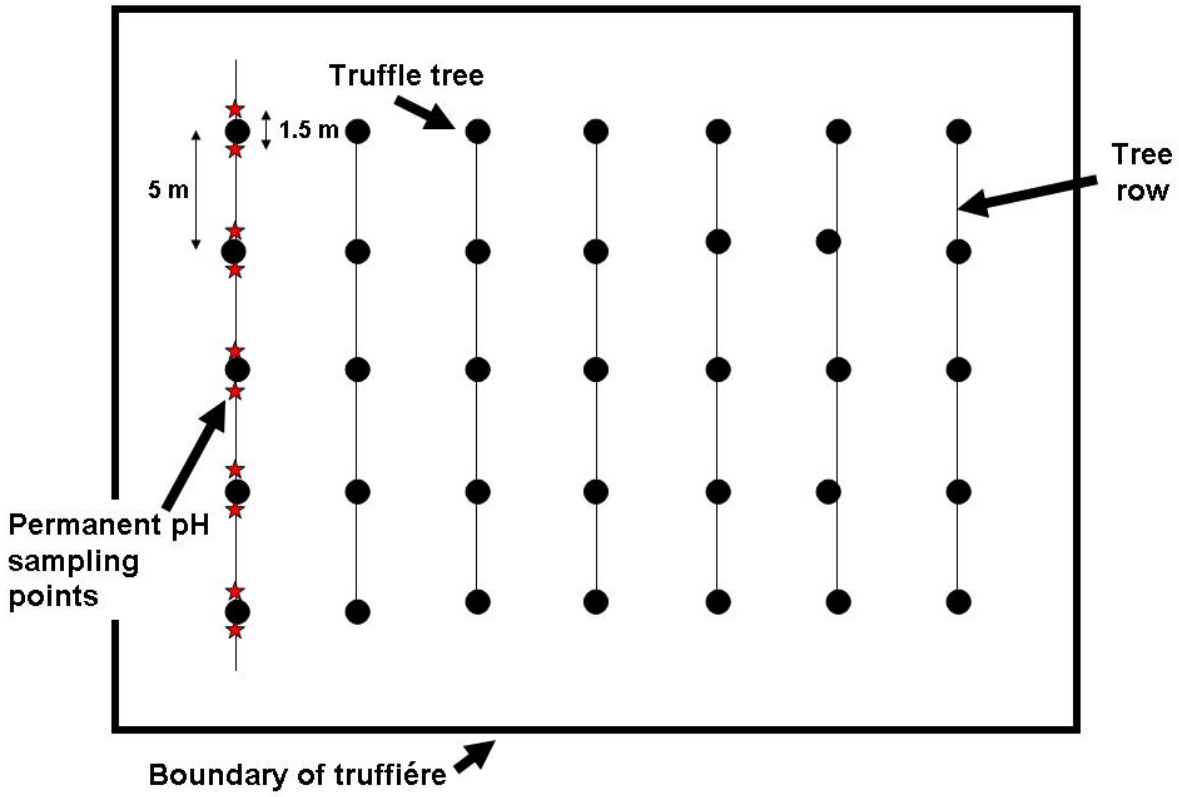

Figure 1. Field layout for assessing soil pH.

Lime requirement

The method to be used by the analytical lab consists of adding a known amount of base to a soil sample and measuring its affect on the pH of the given sample. By adding progressively larger volumes of the base to individual portions of the soil sample and by measuring the resulting pH a graph can be plotted. This graph can then be used to determine how much base should be added in order to raise the soil pH to 7.8.

The base additions are carried out on soil in a buffered (pH 7.0) calcium chloride solution as opposed to distilled water. The calcium chloride solution is used to moderate any pH changes caused by variable soluble salt concentrations in the soil samples.

For more information on how lime requirement will be determined, see *Soil, pH, lime requirement, and liming for Truffiéres producing the Périgord Black and Burgundy truffles* and *Determination of Soil Lime Requirement For Future Truffle Sites*.

Lime incorporation

For each truffiére site, we will determine the particle size and neutralizing value of the lime to be applied. To do this, we will collect 250 ml of lime from each of 3 bags of the lime to be used.

Lime particles should range from very fine (talcum powder) to larger coarse sizes. Soils will react quickly with the fine particles, so the pH will be raised quickly. Larger coarse particles will degrade slowly, allowing the soil pH to remain high. The recommended type of lime is calcitic (not dolomitic) limestone with 90% CaCO3 content or greater, which has been crushed and sieved prior to application.

Large amounts of lime should not be applied all at the same time. Lime should be split into at least 2 and preferably more applications if large amounts are to be applied to a field. On the coast lime can be applied from early spring to late fall, if the fields are not too wet.

Maintaining a Specific soil pH

Soil should be sampled and pH taken at least once in spring and then in the fall. If the soil pH begins to drop below the target pH, lime should be applied, especially in the root zone.

Drainage

Preferred truffiére sites have soils with free drainage, loamy, granular texture, and good aeration. If drainage is not free, then drainage need must be assessed and addressed, e.g. by installing drainage tile, by deep ripping of pan, etc.

Soil and ground preparation

The site must be free of ectomycorrhizal hosts for a minimum of 2-3 years before planting, including many native BC conifers.

If the site needs deep ripping, a deep rooted crop, e.g. alfalfa, fall rye, can be planted to assist in establishing better aeration and drainage. This crop would eventually be either tilled under or mowed aggressively.

Establishment Spacing

Spacing will be 5 x 5 m. If higher densities are used, the trees must be vigorously pruned to ensure that sunlight still reaches the ground to heat the soil. Various patterns of spacing of the oak and hazelnut trees are permitted although the hazelnut should be dominant (e.g. 5:1+).

Ground Cover

The between-row area not covered by trees should be covered by a mowable cover crop. Annual ryegrass tolerates pH from 5 to 8, with the optimum between 6.0 and 7.0 (http://extension.oregonstate.edu/catalog/html/em/em8691/). Austrian winter pea prefers a soil pH of 5.5 to 7.5 and is widely adapted; black medic prefers a soil pH of 7.0 to 7.5 but will grow in most soil types.

Fertilization

Generally, surface soil organic matter OM content should be maintained at approx. 8%. Plant available Ca should be high, P should be moderate, and N low.

Generally, the fertility of the soil should not be high as this could discourage mycorrhiza formation. No fertilizer will be applied in the first year. Fertilizer application in years 2+ will have to be determined based on soil and tissue analyses and recommendations in the literature.

Compost or manure applications to the site should be limited because the soil pH may drop and the site may become too fertile.

Irrigation

Through the first growing season, it will be critical to ensure that the seedlings are appropriately watered. The goal is to ensure good growth of the seedling shoot, roots and associated ECM fungus.

The recommended irrigation method is a micro-sprinkler system with 2 heads per tree pointed away from the tree trunk. Guidance in the design of the irrigation system can be found in the *Sprinkler Irrigation Design Information* irrigation fact sheet and the *Sprinkler Irrigation Manual*.

Weed and competing species management

With time, the fungus should develop a brulé in the root zone – an area which the fungus maintains free of competing vegetation. In the meantime, it will be necessary to control weeds by some other means.

The weed management strategy should involve tillage by hand or machine. In the first few years after planting, machine tillage in the root zone should be avoided. Weeds can also be controlled by mowing. Application of herbicide (e.g. Roundup), desiccant (e.g. Ignite), blowtorch, and steam are other option but should be used judiciously so as not to compromise the roots and ECM fungus.

Disease and Insects

Sites should be monitored carefully for insects that could damage the roots of the trees, including wire worm, Crane fly larva, and June beetle. They can be reduced by breaking the site out of sod one year prior to planting and by trapping them with bait objects such as apples or bait plants grown between rows.

Another major concern is hazelnut diseases, particularly the Bacterial Blight *Xanthomonas arboricola pv. corylina* and the Eastern Filbert Blight (*Anisogramma anomala*). The blights will be avoided by ensuring that planting stock is healthy and by monitoring established truffiéres.

The use of broad based systematic fungicides to control fungal diseases will destroy the ECM fungus, so its use is not recommended. However, contact foliar fungicides should not impact the ECM fungus.

Tree Protection

Many animals eat truffles and may compete with the orchardist for the truffle crop: slugs, squirrels, deer, pigs, voles etc. To minimize risks, immediately after planting, the seedlings must be protected against damage by grazers. Plastic collars will be used for vole and mouse protection. Ultra sonic emitters may help protect against these and moles.

If large animals (deer, pigs, humans) are a threat, fences will be needed.

Pruning

It is important to keep the truffiére open so that sun can penetrate to the ground.

Harvesting Truffles

Truffle harvesting is expected to be possible approximately 5 years after planting for Hazelnuts and approximately 7 years after planting for Oaks.

Finding mature truffles will require that trained dogs are available to search the truffières; only fully mature truffles produce the pungent aromas that characterize the truffle and make it possible for trained dogs to find them.

References

Agerer R, ed (1987-2002) Colour Atlas of Ectomycorrhizae, 1st-12th del., Einhorn-Verlag, Schwäbisch Gmünd

BC Ministry of Agriculture, Food and Fisheries. 1988. Sprinkler Irrigation Design Information. Order No. 552.000-3, Agdex: 753.
http://www.agf.gov.bc.ca/resmgmt/publist/500series/552000-3.pdf

BC Ministry of Agriculture and Food. 1995. B.C. Sprinkler Irrigation Manual. Order No. 552.000-1, Agdex: 753
http://www.agf.gov.bc.ca/resmgmt/publist/500series/552000-1.pdf

Gamiet, S., A Bomke, S Berch. 2007. Soil, pH, lime requirement, and liming for Truffiéres producing the Périgord Black and Burgundy truffles. February 2007, Truffle Association of British Columbia

Goodman, D.M., Durall, D.M. and Trofymow, J.A. 1996. Describing ectomycorrhizae. In Concise descriptions of North American ectomycorrhizae. Edited by D.M. Goodman, D.M. Durall, J.A. Trofymow and S.M. Berch. Mycologue Publications, Sidney, BC.
http://www.pfc.forestry.ca/biodiversity/bcern/cde/index_e.html

Milligan, E. 2006. Determination of Soil Lime Requirement For Future Truffle Sites. Prepared for the Truffle Association of British Columbia by Erica Milligan, November 17 2006

Rauscher, T., R. Agerer, and G. Chevalier. 1995. Nova Hedwigia 61: 283-296.