PROJECT SUMMARY

Instructions:
The summary is limited to 250 words. The names and affiliated organizations of all Project Directors/Principal Investigators (PD/PI) should be listed in addition to the title of the project. The summary should be a self-contained, specific description of the activity to be undertaken and should focus on: overall project goal(s) and supporting objectives; plans to accomplish project goal(s); and relevance of the project to the goals of the program. The importance of a concise, informative Project Summary cannot be overemphasized.

Title: Trapi - Truffle Research And Production Initiative

PD: Coleman, Mark, D.	**Institution:** University Of Idaho
CO-PD: PD/PI 2 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 3 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 4 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 5 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 6 Name (Last, First, MI)	**Institution:**
CO-PD: PD/PI 7 Name (Last, First, MI)	**Institution:**

North American commercial truffle production is far below its potential. Information about truffle cultural practices is available from Europe, Australia, New Zealand and South America, yet there is an important need to synthesize available information and translate it into pragmatic terms. Growers need to fill information gaps, especially in terms of adapting the culture of premium European truffles to North American climate and soils. The Truffle Research and Production Initiative (TRAPI) planning project will strengthen existing collaborations and build new partnerships among scientists and growers. The long-term goal is for the North American market to equal or exceed the levels of other truffle producing nations. The increase in truffle production will proportionally increase demand for support services such as truffle seedlings, agronomic supplies, truffle dog services, culinary arts, and marketing and distribution. Following identification of priority topics based on grower input, TRAPI research and extension scientists will synthesize existing knowledge on those topics from Europe and other nations that have successfully introduced truffle industries. TRAPI scientists will present findings to growers and prepare a review article that will form the basis of a comprehensive research program. This review will consider global and regional truffle markets and define information needs for a NA market analysis. Another important objective of this collaborative review process will be to develop and grow a network of research and extension scientists that can secure funding to support studies on production research, and develop outreach programs that supply pragmatic agronomic information on truffle culture.

TRAPI - Truffle Research and Production Initiative

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Executive Summary

1. Project Title: TRAPI - Truffle Research and Production Initiative

2. Project Type: Planning

3.Requesting consideration for Center of Excellence? (~~Yes or~~ No)

4. Legislatively Mandated Focus Areas

Focus area	Percentage addressed in this proposal
Plant Breeding and Genetics	25%
Pest Management	0%
Plant Production and Production Efficiency	50%
Technology	25%
Food Safety	0%

5. Program Staff

Role	Name	Title	Institution	City, State	Email
Project Director	Mark Coleman	Professor	University of Idaho	Moscow, ID	mcoleman@uidaho.edu

6. Critical stakeholder needs and long-term goals

Truffle industry stakeholders primarily need to improve yields and to understand the position and potential of the North American (NA) truffle market. Improved yields will not only increase profitability of growers but will also involve a whole system of truffière service providers, consumers, marketing and distribution, and expand the economic impact well beyond the market value of fresh truffles. While we understand the importance of these other primary sectors of the truffle industry, here we focus on four areas of critical need: 1) agronomic practices, 2) genetic monitoring tools, 3) economic decisions support information, and 4) quality assurance standards.

Agronomic practices are largely influenced by the truffière location. Growers need realistic soil and climate requirements for locating optimal sites and to evaluate the best truffle species for prospective truffières. Some truffle species have narrow site specification, while others have more robust requirements. Translation of these requirements into optimal ranges would support grower decisions on which species to manage for their location.

Irrigation and soil amendments will expand viable areas for truficulture. Irrigation will be necessary to produce truffles in many NA locations. Various irrigation scheduling approaches need to be evaluated based on reliability and effectiveness. Optimal soil moisture to maximize truffle yields should be developed for each truffle species. Research is also needed on effective amendments to create alkaline soil reactions and increase calcium. Alternatives to agricultural lime (e.g., calcareous shells, biochar, etc.) should be evaluated for effects on long-term stability of soil reaction, fungal mycelium and ascocarp formation.

Agronomic information is needed on maintaining the tree structure through pruning. Pruning is known to help maintain truffière production. Practical French truffière management prescriptions describe the need to suppress the trees. Management of natural truffle forests suggest that thinning restores ascocarp formation. Studies are needed to determine the effects of pruning intensity and timing on fungal hyphae, host roots, and ascocarp formation.

Spore inoculation is an agronomic practice unique to trufficulture. Spore inoculations can enhance truffle production by assuring paternal mating types are present to fertilize maternal mating types on mycorrhizal root tips and initiate ascocarp formation. This practice has demonstrated potential to improve yields, but rigorous controlled trials are needed to understand how, where and when to apply spores for greatest effect.

Growers need genetic tools to monitor mycorrhizal colonization and symbiont responses to management interventions. Traditional approaches for tracking progression of the ectomycorrhizal (ECM) symbiosis involves sampling roots and counting ECM root tips. However, these approaches are costly and imprecise. Modern DNA tools have revolutionized mycology and are now routinely available for monitoring truffle abundance in soil. Growers have strong interest in the use and development of such approaches. These methods need to be further developed to create affordable commercially available analytical services with consistent standards for interpreting results.

Economic information to support business decisions is needed by investors and growers. The NA truffle industry needs practical, empirical, fact-based, information on trufficulture that is up-to-date, available, and easily understood by growers and investors. Fundamental economic information is needed to support investment decisions including domestic and international commodity supply, prices, growth potential, and risks. Much of the truffle economic information is available for Europe and there have been global overviews, but similar analysis still needs to be adapted for NA market analysis.

Quality assurance standards must be established for seedlings and spore amendments to minimize risk. Seedling quality standards assure growers that planting stock has optimum opportunity for success through abundant mycorrhization with target species and no unwanted, low-value truffles. Post-planting spore amendments must also consist purely of the target truffle without any contaminants. Thus, standardized assessment approaches must be developed to not only protect individual growers, but also the regional industry.

The primary goals of the TRAPI project are to enhance production of truffles in NA and develop domestic and international markets for fresh NA truffles and truffle products. An ambitious, yet realistic long-term goal is to double yields of *T. melanosporum* in NA every year for 10 years. This exponential increase is consistent with projections for other non-European nations that have supported the development of *T. melanosporum* truffières and largely represents expansion of acreage. Reaching such a goal would bring NA production on par with other non-European nations. The increase in truffle production will proportionally increase demand for support services such as truffle seedlings, agronomic supplies, truffle hunting dogs, food services, etc. This industry expansion will result in not just substantial economic impact but also social enrichment by increasing appreciation and availability of truffles. The TRAPI project intends to review current knowledge of global and regional truffle markets and apply that knowledge to information needs for analysis of NA market growth within an international context.

7. Outreach plan summary

During the TRAPI planning project we will engage our main stakeholder group – truffle growers – in the development of an SCRI proposal and improve the knowledge base to support their demand for information on how to establish and manage truffières to maximize yields. We will gauge growers research priorities by conducting a member survey of North American Truffle Growers Association (NATGA). Our objective for the fall 2022-member survey is to receive 75 responses, which would almost double the number of respondents from fall 2021. TRAPI research and extension scientists will review current knowledge on priority research topics and present online seminars to NATGA members. Our objective for TRAPI online seminar series is to generate over 400 contact hours. This target is based on past NATGA webinars that typically receive over 40 participants and we expect to make eight one-hour presentations for the series. These seminars and a review manuscript on the current knowledge of trufficulture as it applies to NA will also provide information for preparing extension programs and bulletins. Preparation of those material will be included as objectives in the SCRI proposal planned for submission in 2024.

8. Potential benefits

The TRAPI project is intended to have both long term and immediate benefits on the NA truffle industry. A doubling of annual production for 10 years would also benefit the other main sectors of the truffle industry; namely, the processing and distribution system, and consumer and marketing system. Currently, NA growers are distributing their own products through personal connections or online. More available truffles would motivate establishment of distribution centers concentrating truffles from many truffières. Greater truffle availability will also allow promotion and marketing efforts and encourage restaurants and consumers to explore culinary possibilities. To achieve these long-term, system-wide goals, the immediate focus of TRAPI is on truffle production and market analysis of the NA truffle industry.

One immediate benefit of the TRAPI planning project will be to strengthen ties between truffle growers, researchers and extension specialists. These ties are needed because now growers are individually seeking pragmatic information on how to establish truffières and increase truffle yields by reading sparse European management guidelines, interpreting scientific literature, and contacting the few known individual research and extension scientists. Research scientist cannot always assist growers due to proximity and availability. Few extension specialists are familiar with trufficulture information. Therefore, an immediate benefit of the TRAPI planning project is the building of a network of scientists who will collaborate on synthesizing available information and sharing comprehensive and practical insights with growers. Developing a collaborative research network will ultimately create more rapid scientific advances that are relevant to NA truffle production. We are especially focused on collaborating with extension scientists to advance communication between scientists and practitioners. Inclusion of extension scientists in information synthesis will facilitate technology transfer during the planning process and assure integration of outreach in the resulting SCRI proposal. We will involve and develop research and extension scientists with essential levels of expertise that are geographically distributed across North America. Our intention is to forge lasting ties among research and extension scientists to contribute to long-term profitability and sustainability of the truffle industry. Once established, the TRAPI network is expected to expand collaboration by encouraging other colleagues and training students.

Another immediate benefit of the TRAPI planning project are products spawned from literature research on priority topics. TRAPI scientists will research, synthesize and present current knowledge about priority truffle production topics to growers and focus on practical applications. Scientists will also collaborate to prepare a manuscript reviewing current knowledge on priority research topics. The review manuscript will be shared with and evaluated by growers.

The final benefit of the TRAPI planning project will be the development of an SCRI proposal to request support for top priority research. TRAPI scientists will develop the proposal in collaboration NATGA. Research questions will address approaches to increase yields while outreach objectives will develop programs and extension bulletins describing trufficulture best practices.

Logic Model

SITUATION	INPUTS	ACTIVITIES	OUTPUTS	OUTCOMES		
				Knowledge	Actions	Conditions
Challenge: Success in truffle cultivation elsewhere provides general guidance but conditions are different in North America. Guidance based on research tailored to North America is needed. **Opportunity**: Creation of a network of collaborators who will work together to prioritize and plan applied research that will guide the industry to success.	**Resources**: Research scientists, social scientists, economists, extension experts, grower associations, truffle harvesters, truffle processors, truffle marketers, seedling producers **Contributions**: All participants except PD and assistant, contribute time and expertise to planning project. **Investments**: Years of experience; existing truffiéres as research sites.	PD and assistant manage this planning project. TRAPI scientists review research from elsewhere. TRAPI scientists present key findings via webinar. Survey NATGA growers to refine their research priorities Collaborators meet to plan key research needs. Team works together to write grants proposals.	Document synthesizing existing research in key areas of truffle cultivation from elsewhere. NATGA-hosted webinars on priority research areas NATGA AGM to facilitate dissemination and discussion of planning project. Survey results from NATGA growers to refine key challenges. Meeting of collaborators to begin drafting of funding proposals. Beginning of grant writing.	Growers learn from experts what is known/not known about key challenges encountered in truffle cultivation Truffle associations in North America will see how economic and socio-political issues faced by truffle industry elsewhere are overcome. Experts in many related and required fields will become deeply aware of the issues and opportunities in truffle cultivation research.	Collaboration in this planning project will bring together individuals, associations, enterprises, scientists, extension experts, seedling growers and clarify many opportunities that continued cooperation and information sharing will develop. Through this collaboration and cooperation, the North American truffle industry will be lifted up and put on the road to success.	Cooperation in research and extension side of a successful NA truffle industry will lead to the writing and submission of proposal(s) to tackle key research priorities. Success will build the foundation of a science-based North American truffle industry. Addressing key hurdles and providing sound guidance will lead to best agronomic practices, economic gain, and a full, vibrant truffle economy.

ASSUMPTIONS
Participants will actively participate in this planning project, including analyzing and synthesizing scientific information, and disseminating it to growers and the industry in useful, accessible means.

EXTERNAL FACTORS
Some key questions identified by growers may not have substantial published literature for researchers to review.

Project Narrative

Introduction

TRUFFLE RESEARCH AND PRODUCTION INITIATIVE

Truffles are subterranean ascocarps, or fruiting bodies, of ectomycorrhizal (ECM) fungi appreciated by gourmets and prized by chefs for their unique taste and complex aromatic qualities (Allen and Bennett 2021; Lee 2019). The highest valued truffles are native to Europe where their gastronomic appeal has been highly regarded for centuries creating folklore, romanticism, and intrigue (Hall et al. 2007; Jacobs 2019; Jacobsen 2021). The Italian white truffle (*Tuber magnatum*) is the highest priced truffle due not only to its unique gastronomic appeal but also because the market is exclusively sourced through wild collection. Production of truffles in artificial plantings was demonstrated in the late 20th century which has created new opportunities for truffière production (Bach et al. 2021). The Périgord black truffle (*T. melanosporum*) is the highest valued truffle that has been domesticated. High demand for *T. melanosporum* has spawned research on the species so that it is now better characterized than any other truffle species (Kues and Martin 2011). Through fundamental and applied research *T. melanosporum* is now widely cultivated in its native range in France, Spain and Italy, and plantations have been established in temperate regions across the globe (Berch and Bonito 2014; Reyna and Garcia-Barreda 2014; Zambonelli et al. 2015). Other high-value truffles include the burgundy truffle (*T. aestivum*) and bianchetto truffle (*T. borchii).* These are also successfully used to establish truffières. They tolerate a broader range of environmental conditions than *T. magnatum* and *T. melanosporum* and fruit in association with a greater number of host species (Berch and Bonito 2014; Zambonelli et al. 2015). Hence, truffières of these other high-value truffles have potential to succeed with native NA tree species and thrive across the range of NA soil and climatic conditions.

Truffles have significant world-wide economic impact because they are a highly valued and rare commodity. Natural production of truffles has declined over the last few decades in Europe, which is attributed to shifts in post-war demographics and land use change (Hall et al. 2007; Sourzat 2009). Consequently, consumer demand now exceeds supply, which results in prices reaching €4000/kg ($2000/lb) (Bach et al. 2021). The European national total markets range in value from 4 to 20 million euros (Reyna and Garcia-Barreda 2014), which by one estimate has a total economic importance in Europe of €3.5 billion (Lovrić et al. 2020). Artificial production is beginning to compensate for declining natural production, but prices are still volatile and fluctuate year-to-year depending on the weather conditions (Buntgen et al. 2019; Le Tacon et al. 2014).

Working in conjunction with the North American Truffle Growers Association (NATGA), researchers, academics, cooperative extension, and vendors we have developed an interdisciplinary team that is aware of the specialty crop challenges faced throughout North America. This group of stakeholder experts complement one another's knowledge and will work together to find solutions to the goal of increased truffle production within three primary systems of Production, Consumer & Markets, and Distribution & Processing as defined by the Sustainability Outcomes and Impacts.

Preliminary grower survey

A NATGA member survey was one way that we used to obtained stakeholder input during development of this planning proposal. Surveying growers about their priority management questions helped to identify applied research needs for increasing truffle yields. A membership survey was conducted during the October 2021 NATGA Fall Congress in Santa Rosa. The results provide a preliminary idea of what topics are of most interest to truffle growers. We will use this approach to involve growers in prioritizing research topics for the TRAPI planning project. Among the 44 growers that participated, *T. melanosporum* (89%) was overwhelmingly selected as a species of interest for cultivation, while *T. borchii* (27%), *T. aestivum* (23%) and native truffles (14%) are also of some interest. No other species gained more than one vote (2%). Over 58% of respondents considered irrigation and moisture management to be of high or very high importance as a technique for improving production, while soil amendments (52%) and pruning techniques (43%) are also of interest. Over 88% are interested in learning more about available tools to help track truffle production. When asked about having research conducted at their truffières, a large majority (86%) expressed an interest. These results helped us to prioritize the research needs for this planning proposal and will guide selection of topics for the initial online seminars during the TRAPI planning project. Stakeholders will continue to collaborate on TRAPI planning activities and in development of the resulting SCRI proposal.

Long-term goals

The primary goals of the Truffle Research and Production Initiative (TRAPI) are to enhance production of truffles in NA and develop domestic and international markets for fresh NA truffles and truffle products. An ambitious, yet realistic long-term goal is to double yields of *T. melanosporum* in NA every year for 10 years. This exponential increase is consistent with projections for other non-European nations that have supported the development of *T. melanosporum* truffières and largely represents expansion of truffière surface (Cejka et al. 2022). Reaching this goal would bring NA production on par with other non-European nations (Fig. 1).

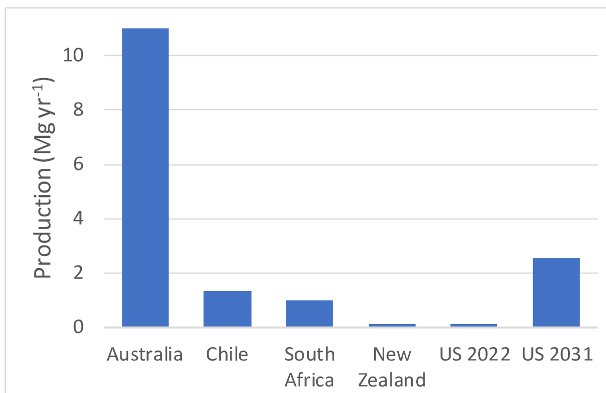

Fig. 1. Annual *Tuber melanosporum* production for non-European nations that are developing trufficulture industries (Cejka et al. 2022). A US 2031 projection denotes a doubled of annual production each year over ten years.

We intend to accomplish these long-term goals through TRAPI by organizing a collaboration among growers and the scientific community. We will strengthen existing collaborations and build new partnerships among scientists and between scientists and growers. To strengthen and build partnerships we will bring together research and extension scientists to examine priority production approaches and address significant socioeconomic questions. We will support the development of grower knowledge on these topics through presentations, synthesis reporting, and publication. The technical information acquired will also be translated for practical application through integrated extension programs and publications. Programs will be developed to enlighten and engage the public and thereby promote appreciation of their gastronomic qualities and interest in establishing truffières. A main intent of TRAPI is to freely share and widely distribute both available and acquired information on approaches to enhance production and in so doing we expect to build upon scientific and applied knowledge.

State of knowledge and demonstration of need

Scientific English-language literature on truffle biology and trufficulture has proliferated since the turn of the century. In the 20th century, best-practices recommendations were based on traditional and empirical knowledge and documented by European agricultural researchers (e.g. Chevalier and Grente 1979; Delmas and Poitou 1973; Mannozzi-Torini 1976; Sourzat 1997). During the 1990s the technical rigor and availability of peer-reviewed reports significantly increased (e.g. Mello et al. 1999; Paolocci et al. 1999). Considerable confidentiality surrounded early technology. While confidentiality still exists, there is now abundant public reporting of technical knowledge to establish trufficulture programs. North American researchers are mainly limited by available resources to conduct the comprehensive and technically rigorous trials that are necessary to achieve and optimize potential yields.

Truffière production depends on numerous cultural methods, or trufficulture practices including site location, preparing quality planting stock, proper truffière management, monitoring status of fungal colonization, and use of spore amendments. In the following sections we provide background on biology and ecology for these practices and describe research needs that are expected to advance yields.

Site selection

The highest valued truffles have specific environmental site requirements, which likely limits supply. Soil and climate conditions for effective truffle production of European species are well known (Garcia-Barreda et al. 2019; Garcia-Montero et al. 2008; Hall et al. 2017). These include alkaline soils (pH 7.5 -8.0) of moderate texture, limited nitrogen availability and otherwise balanced nutrition (Bonet et al. 2009; Chevalier and Sourzat 2012). In Europe this includes soils developed on limestone parent material. Indeed, extractable calcium carbonate is more strongly related to *T. melanosporum* ascocarp production than other soil quality variables (Garcia-Montero et al. 2006). Calcium carbonate not only provides high levels of available Ca but also maintains alkaline soil reaction. Loamy soil texture is necessary, but it can range from sandy-loam to clay-loam (Chevalier and Sourzat 2012) so that there is a balance between aeration and water retention (Ponce et al. 2014). Soils do not need to be rich in nutrients (Fischer et al. 2017) otherwise the tree will not require mycorrhizal symbioses. Rather, sufficient organic matter for the truffle fungus to mineralize nutrients and transport to the host will assure maintenance of the symbiotic association (Stuart and Plett 2020). *T. melanosporum* does best in Mediterranean climates i.e., cool moist winter, hot dry summers with sufficient but not excessive soil moisture (Colinas et al. 2007; Hall et al. 2017). Other truffle species such as *T. borchii* and *T. aestivum* have much more robust site requirement (Gryndler et al. 2017; Hall et al. 2007; Wedén et al. 2009) than *T. melanosporum.* For instance, *T. borchii* prefers neutral soil reaction (pH 7.0-7.5) and both can tolerate cold conditions (Gryndler et al. 2017; Wedén et al. 2009). This knowledge suggests that it will be appropriate to consider these more robust truffle species for broader distribution across NA than *T. melanosporum*. Known ranges of soil and climate specifications should be applied when considering establishment of NA truffières for each of the commercially viable truffle species similar to that done in Spain (Ponce et al. 2010; Sanchez et al. 2016; Serrano-Notivoli et al. 2015). Selecting sites that are free from other ECM species is necessary to avoid competition with truffle crop species. Previous cropping with grasses, legumes, vineyards, or fruit orchards are favored (Bonet et al. 2009). Applying molecular genetic approaches to profile abundance of all ECM fungi would aid site selection and post-establishment monitoring but those approaches need to be developed for application to trufficulture and growers need service labs that can analyze and interpret results.

Quality planting stock

In Europe, seedlings must meet rigorous quality standards of the nursery industry including morphological and physiological traits (Duryea 1984; Zambonelli et al. 2010). Nursery capacity for producing high-quality seedlings is an essential component of truffière establishment and productivity. High-quality seedlings are characterized by healthy, vigorous stock that contains more than 30% ECM tips colonized by target truffle species and little to no competing ECM species (Bonet et al. 2009; Donnini et al. 2014). Contamination between *T. melanosporum* and lower value truffles due to poor screening of inoculum is a problem with substantial economic impact on truffière establishment (Mamoun and Olivier 1993; Zambonelli et al. 2010). Murat (2015) recommends genetic assessment of spore inoculum and seedling root systems to avoid concerns that morphological characteristics fail to distinguish low value contaminants from target species. Truffle seedling quality standards have long been in place in European countries (Andres-Alpuente et al. 2014) and more recently in Australia (ATIA 2021) but no such standards exist in NA. North Carolina Extension does offer morphological and genetic testing services (NC State 2022); however, the lack of NA quality standards is often raised as a concern by growers. Developing standards and approving third parties to assist growers by quantifying mycorrhization amount and species occurring in planting stock is a necessary step to assure security and reputation of the NA truffle industry.

Agronomic practices

Proper truffière management includes irrigation and soil moisture monitoring, pruning, soil analysis and soil amendments. Irrigation is the main management factor affecting yields. Truffle yields for *T. melanosporum* are optimum at moderate levels of soil moisture (Buntgen et al. 2015) and the DNA quantity of *T. aestivum* increases with increasing water stress (Todesco et al. 2019). However, we expect optimum moisture levels for white truffles to be higher because of their native habitat. For instance, the Alba white truffle, *T. magnatum,* occurs naturally only in moist alluvial soils (Todesco et al. 2019) indicating that it favors higher soil moisture than *T. melanosporum*. Indeed, our current truffière irrigation study with the white truffle *T. borchii* shows more soil mycelial DNA occurs at the highest irrigation level (Fig. 2). Knowing optimal moisture levels for prospective host species is useful for evaluating native site moisture availability and for scheduling irrigation.

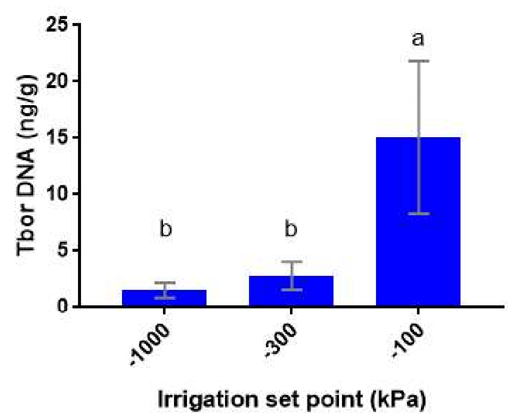

Fig. 2. Concentration of *T. borchii* DNA in response to three soil water potential set points that triggered irrigation.

Le Tacon (1982) evaluated water requirements for *T. melanosporum* truffières and recommends regulating truffière soil moisture based on soil water potential. Both Le Tacon (1982)band Todesco et al (2019) favor use of water potential over volumetrric soil moisture because water potential is comparable among all soils and precisely relates to water stress of host and symbiont (Kramer 1983). Still, soil moisture sensors have been unreliable for irrigation scheduling, regardless of the variable measured and their use must be practically evaluated in comparison to other effective irrigation scheduling approaches such as water balance (Peters et al.), use of crop coefficients to condition potential evapotranspiration (USBR 2017), approaches involving meteorological measures such as surface renewal (Hu et al. 2018), or numerical modeling (Dabach et al. 2013).

Pruning is an essential truffière management activity. Sourzat (2012) describes the importance of maintaining an equilibrium between host tree and symbiont so that the tree is suppressed and the symbiont is more vigorous. The vigor of the symbiont is monitored with the 'brûlé" surrounding the host trees. The brûlé is a unique allelopathic zone with suppressed weed growth that extends over time as the symbiosis expands (Chevalier and Sourzat 2012). Ascocarp production primarily occurs within the brûlé (Menta et al. 2014). The equilibrium between host and symbiont is maintained by pruning the tree so that crown dimensions are smaller than that of the brûlé (Sourzat 2012). Sourzat (2012) also describes similar criteria regarding tree spacing. Once adjacent brûlés meet, trees should be removed to increase spacing for continued brûlé expansion by retained trees. Unfortunately, we are aware of no English-language scientific reports supporting the empirical knowledge regarding tree crown relative to brûlé expansion. Garcia-Barreda et al. (2013) report the results of thinning a natural truffle forest to restore truffle production. They show that decreasing canopy cover by more than 60% increases brûlé surface area and the number of truffles harvested compared with removing less or none of the canopy, which suggests that recommended pruning and thinning techniques that open the canopy will result in positive truffle yields. Research needs include reviewing non-English trufficulture literature to determine the scientific basis for pruning and thinning recommendations and conducting controlled truffière experiments to evaluate assumptions upon which those recommendations are based.

Native *T. melanosporum* soils in Europe develop on limestone or dolomite which causes them to have alkaline soil reaction (pH 7.7-8.4), high portions of exchangeable calcium and extractable carbonate (Chevalier and Sourzat 2012; Jaillard et al. 2014). It is possible to raise pH and exchangeable cations into the appropriate range through lime or other amendments, which has resulted in successful truffle production on soil with more acidic parent material (Hall et al. 2007). Unfortunately, calculations of lime requirements based on buffer tests are calibrated to bring acid soils to little more that neutral (Wolf et al. 2008) and do not typically include targets above pH 7.5. Truffle growers need buffer test calibrations extended. Calcium carbonate derived from crushed limestone is frequently used to raise soil pH, which adds essential calcium ions and active carbonate which are known to improve truffle production (Garcia-Montero et al. 2008). Other materials besides agricultural lime can also be used to alter pH including wood ash, oyster shells or CO2-sorbent biochar (Chintala et al. 2014; Lee et al. 2008; Li et al. 2016). These materials should be evaluated for short- and long-term effectiveness at maintaining alkalinity.

ECM Monitoring status of fungal colonization

Assessment of the mycorrhizal status of truffières is used to confirm colonization during the pre-production stage of trufficulture. It can be accomplished by observing brûlé progression, evaluating ECM root tips, or using genetic tools (Meadows et al. 2020; Sanchez et al. 2014; Taschen et al. 2020). While the appearance of the brûlé is a good indicator of advancement of truffle colonization, it is not always well defined, and when it is observed, it does not guarantee ascocarp formation (Streiblová et al. 2012). High spatial variation within truffières may also cause decreased yields due to inherently slow fungal growth relative to tree roots (Sourzat 2005), patchy colonization, (Chen et al. 2021; De la Varga et al. 2017; Napoli et al. 2010) and fungal life cycle traits that require sexual recombination for fruiting (Zambonelli et al. 2015). Evaluating mycorrhization with morphological assessment of truffle ECM is expensive because it involve manual observations, and high variability requires numerous samples. Morphological assessment can also be inaccurate because many truffle species have similar anatomical appearance, so it is not possible to morphologically distinguish low- from high-value species (Agerer 1991).

Increasingly, truffière assessment involves genetic analysis of soil samples. Genetic assessment of *Tuber* species is far ahead of most fungal species due to their high commercial value and ecological function as mycorrhizal symbionts (Kues and Martin 2011; Mello et al. 2006). Numerous genetic primers are available for identifying truffle species (Parlade et al. 2016). Use of these primers in quantitative polymerase chain reaction (qPCR) assays of soil samples measures the concentrations of target and contaminating truffles using either single species or multiplexed assays (Amicucci et al. 2000; Rubini et al. 1998; Zampieri et al. 2009). Quantitative-PCR assays can be used to monitor truffière colonization and responses to agronomic treatments (Fig. 2, Todesco et al. 2019). In support of this approach, Chen et al (2021) demonstrated a correlation between high soil DNA concentration and greater truffle yields. This positive correlation between soil DNA and fruiting must be verified in trials with various agronomic treatments designed to improve truffle yields. Such validations are necessary to raise confidence in qPCR assessment and provide more rapid feedback of treatment effectiveness than possible with monitoring ascocarp development.

Next generation sequencing technology also allows assessment of the relative abundance of all ECM fungi (De Miguel et al. 2016; Sanchez et al. 2014; Taschen et al. 2015). These approaches allow assessment of the impacts of management treatments on the predominance of truffle crop species in comparison to other ECM (Salerni et al. 2014; Sanchez et al. 2014). Advanced sequencing platforms (e.g. Illumina, PacBio) are available at most university service labs. Research and extension effort requires further development to determine the value of these sequencing approaches and potentially make them available to truffle growers.

Spore amendments

The use of spore amendments is one unique aspect of trufficulture. While the above establishment and management principles are consistent with many best agricultural and tree orchard practices, the use of spore inocula arose from empirical observations and knowledge of the two mating types in truffles. Truffle mycelia are haploid and truffle individuals have one or other of the mating types (Le Tacon et al. 2016). The presence of two mating types is required for sexual reproduction and thus the production of truffle fruiting bodies. The truffle fungus individual that occupies the soil, forms mycorrhizal root tips on trees and gives rise to the truffle is called “maternal” (De la Varga et al. 2017). It is still unclear how the “paternal” individual exists in the soil. In natural truffle ecosystems and producing truffières, spores from unharvested truffles or in feces of dispersal agents such as wild boar may act as the “paternal” agent. Recent evidence suggests that inoculation with truffle spores can lead to truffle formation (Murat et al.

2016). In advance of definitive research, current practice seems to be moving toward routine inoculation of truffières with spores to incite truffle production, but challenges exist with purity of the inoculum, optimal spore dosage rates, methods of delivery and cost/benefit analysis.

Critical needs of the NA truffle industry

Agronomic practices

Growers need realistic soil and climate requirements to evaluate the best truffle species and practices for prospective and existing truffières. Some truffle species such as *T. magnatum* and *T. melanosporum* have narrow site specification, while others like *T. borchii* and *T. aestivum* have more robust requirements. Translation of these requirements into optimal ranges and farming protocols would support grower decisions about which species should be managed in specific regions. Furthermore, the range of locations might be expanded with research into management practices and controlled experiments that could justify or nullify the necessity of significant investment.

Irrigation will be necessary to produce truffles in many NA locations. Irrigation scheduling for truffières needs further evaluation. Various irrigation scheduling approaches need to be considered based on cost, reliability and effectiveness. Growers need to be informed in the practical applications of available tools to maintain soil moisture within optimal ranges. Furthermore, optimal soil water potential targets, and timing to maximize truffle yields need to be scrutinized individually for each truffle species to set targets for growers and determine appropriate growing regions and whether irrigation is necessary.

Host pruning is known to maintain production, but the processes involved are uncertain. Practical French truffière management reports describe the need to suppress the trees while forest management of natural truffle forests suggest that thinning to invigorate retained trees is effective. Controlled experiments are needed to determine the effects of pruning intensity and timing on fungal hyphae, host roots, their symbiotic interaction within the brûlé, and ascocarp formation.

Spore inoculations can enhance truffle production by assuring paternal mating types are present to fertilize maternal mating types on mycorrhizal root tips and initiate ascocarp formation. Spore inoculations have proven potential to improve yields, but rigorous controlled trials are needed to understand how, where and when to apply spores for greatest effect.

Research is needed on effective long-term amendments to create alkaline soil reactions, increase calcium, and add active carbonate. Alkaline soil targets challenge conventional calculations for lime requirement, which typically focus on raising acidic soils to near neutrality. Research is needed to extend lime requirement calculation above neutrality. Alternative agricultural lime, including dolomite, calcareous shells, wood ash and biochar should also be evaluated for any positive and lasting effects on symbiont concentrations, brûlé and ascocarp formation.

Molecular tools to monitor symbiont

Growers need tools to monitor fungal colonization and responses to management interventions. Traditional approaches of tracking progression of the ECM symbiosis by sampling roots and counting ECM root tips is costly and imprecise. Modern DNA tools are revolutionizing truffle monitoring in the soil. Growers have strong interest in the use and development of such approaches, but they need affordable commercial labs to supply the analytical services, and standards to interpret results.

Economic information to support business decisions

The NA truffle industry needs practical, empirical, fact-based, information on trufficulture that is up-to-date, available, and easily understood by growers and investors. Fundamental economic information is needed to support investment decisions including domestic and international commodity supply, prices, growth potential and risks. Much of the truffle economic information is available for Europe and there have been global overviews, but similar analysis still needs to be adapted for NA market analysis.

Quality assurance

Third-party assessment services for seedlings and spore amendments are crucial for minimizing risk. Seedling assessment services assure growers that planting stock has optimum opportunity for success through abundant mycorrhization with target species and no unwanted, low-value truffles. Post-planting spore amendments must also consist purely of the target truffle without any contaminants. Thus, standardized assessment approaches must be developed to not only protect individual growers, but also the regional industry.

Objectives

The ultimate objective of the TRAPI planning project is to assemble a network of truffle scientists and stakeholders to collaborate in the preparation of a future SCRI proposal to fund truffle productivity research. Developing this planning proposal has already established a network of scientists eager to address practical questions of truffle production. Their letters of support demonstrate a willingness to participate in the TRAPI planning process, to research literature, and synthesize it for both scientific and applied purposes. This review and synthesis will lead to developing an SCRI proposal with the overarching objective of expanding the NA truffle industry by improving truffle productivity.

For the proposed planning project, our research objectives emphasize review and synthesis of available literature with special concentration on the critical needs of NA trufficulture. We expect to prepare a comprehensive truffle literature database with the objective of including more than 500 articles in numerous languages that we will share among TRAPI collaborators and available on the NATGA website. For each article in the database, we intend to include English abstracts for each reference, with any necessary translations. We also expect to prepare a collaborative review manuscript on the potential for trufficulture in NA. This article will be comprehensive and technically detailed, and it will be written for a global scientific audience. Our intention is for this document to be used by TRAPI research and extension scientists to prepare the 2024 SCRI proposal.

There are several outreach objectives for the TRAPI planning project that are designed to support growers' demand for information on how to maximize truffle yields. The Fall 2022 NATGA Member Survey will seek growers' input on research needs. Our objective is to receive 75 responses, more than doubling the number of respondents from Fall 2021. The TRAPI online seminar series will provide practical truffière management information. Our objective is to generate over 320 contact hours based on eight one-hour presentations. Past NATGA webinars typically involve over 40 participants. These seminars and the review manuscript will also provide information for preparing extension programs and bulletins.

Rationale and Significance

The TRAPI project is designed to assemble a network of truffle scientists to specifically address the critical needs of the NA truffle industry. With collaborators representing ten states within the US, two Canadian provinces and Mexico, this scientific network fully encompasses national and regional questions and is globally engaged with growers and scientists in other nations. By including the extent of possible NA latitudes, we can evaluate the available range of climate limitations to truffle production. TRAPI scientists fully encompass agricultural disciplines including biophysical and socioeconomic expertise and include both research and extension specialists. By engaging the few known NA truffle scientist with truffle growers in the TRAPI network we expect to develop long-term alliance among scientists and stakeholders across the NA truffle industry. TRAPI collaborations will be further reinforced by information sharing on priority research topics and the development of an SCRI proposal. Through these activities, we expect to develop an enduring partnership dedicated to the free flow of information. We are keen to develop early-career scientists and to train students in trufficulture science.

There is important potential to create a significant NA truffle market through farmer and consumer education. Several examples demonstrate the potential. Focused effort and government investment in Spain has transformed a degraded and abandoned agricultural landscape into a thriving rural economy by restoring woodland ecosystems through the planting of truffle inoculated seedlings (Bonet et al. 2006; Garcia-Barreda et al. 2018; Samils et al. 2008). The restoration effort was effective because, unlike the former management of high-demand grain crops, the planted host trees are well adapted to marginal sites and the mycorrhizal symbionts function to acquire limited growth resources through expansive hyphal networks. Farmer subsidies for conservation projects involving planting truffle inoculated oak trees and consumer interest is also realizing significant socioeconomic benefits in other European countries focused on *T. borchii* and *T. aestivum* (Rosa-Gruszecka et al. 2017; Vlahova 2021). Expanding trufficulture outside the native range of Périgord black truffle has also been successful (Hall et al. 2007; Zambonelli et al. 2015). The best example of this is Australia where concerted efforts through grower association strategic planning (Fitzpatrick 2021) and public investment in research and farmer education (Lee 2008) have established the fourth largest truffle producing nation globally (Cejka et al. 2022). A similar effort in Chile has established a significant export market through government supported academic research, nursery technology development, establishing best management practices and outreach (Reyna and Garcia-Barreda 2014). North American production lags other global regions (Cejka et al. 2022; Reyna and Garcia-Barreda 2014) due to difficulties in adapting introduced European truffles into novel soil and climates, lack of government support for an organized and comprehensive research effort, and non-cooperative tendencies amongst various stakeholders.

The limited success and unrealized potential of the emerging NA truffle industry causes growers to seek research and extension services from academic and government scientists and services from foreign consultants. Progress in NA trufficulture requires assessment of successful management practices in foreign truffières and to either adapt them to varied NA climate and soil conditions or innovate specific tools and approaches relevant to NA conditions. There is critical need for detailed problem analysis associated with unique NA conditions with focus on lowering cost and improving yields.

Relevant legislatively mandated focus areas

During the TRAPI planning project, we expect to address several of the priority focus areas mandated in the SCRI legislation. Although the planned grower survey will dictate the actual proportions, the letters of support (Appendix D) and 2021 Membership Survey described above provide preliminary views that we use to describe TRAPI focus area approximate proportions.

As our title suggests, Production and Production Efficiency (P & PE) are the motivations for the work and the main priority focus area that we will address (50%). Letters of support from growers overwhelmingly describe the need for research to increase truffle yields. US and Canadian grower associations and individual growers describe that productivity research is foundational to developing the NA market for fresh truffles. Consequently, most of our effort will be on P & PE research including establishment and management practices that shorten the time to production, and development of approaches to increase uniformity of production and maximize yields. The P & PE focus area includes achieving a better understanding of the microbiome of the soil rhizosphere. Of course, mycorrhizal fungi are components of the rhizosphere microbiome. A range of mycorrhizal fungi in the rhizosphere compete with the cultivated species for a finite number of root tips. Therefore, the soil microbiome is an inherent component of trufficulture and will contribute added attention to the P & PE focus area.

Technology (Tech) will also figure prominently in the TRAPI project (25%). The 2021 Membership Survey overwhelmingly selected irrigation and moisture management as the most effective technique for maximizing productivity, which agrees with literature reports as described above. Consequently, we expect that technological systems to monitor soil moisture and control irrigation will be a prominent research topic (see letters of support from Meter Group and Hunter Industries). Furthermore, 88% of survey respondents were very interested in knowing more about tools for tracing truffle production, which involves rapid scientific advances in applications of molecular genetic technology. Given the need for irrigation and moisture management and molecular genetics, Tech will be of critical importance to TRAPI research topics.

The genetic portion of the Plant Breeding, Genetics (PB & G) focus area will also play an important role in the TRAPI project (25%). Genetic tools will be applied to seedling quality control, monitoring truffière colonization, evaluating the abundance of competing ECM "pest" species, and screening spore inoculants for possible contamination of lower value truffle species such as *T. brumale* or *T. indicum*. As previously described above regarding rhizosphere microbiome under P & PE and regarding molecular biology under the Tech focus area, we expect that rapidly advancing field of genetic analysis will figure prominently in TRAPI research.

Approach

The TRAPI planning project connects truffle growers with truffle research and extension scientists across NA to answer questions associated with production limitations. TRAPI will connect growers with scientists through NATGA using both their annual meetings and their online seminar series. A core component of NATGA, the Research Task Force, has been instrumental in proposal preparation and will continue to coordinate and embody growers' perspective throughout the planning process. Two of three TRAPI meetings will be at NATGA Truffle Congresses. The first of which already occurred at the Fall 2021 Truffle Congress, Sonoma, CA where the Research Task Force conducted a grower survey (see above section on Preliminary grower survey) and plans were laid with the PD for submission of this SCRI planning proposal. The preliminary grower survey identified four priority research topic areas that we will use to initiate the TRAPI online seminar series at the beginning of the planning process and prior to the next Truffle Congress. These topics include irrigation and moisture management, soil amendments, truffle production tracking tools, and harvesting.

The second TRAPI meeting will occur at the Fall 2022 NATGA Truffle Congress in Roanoke, Virginia where the PD will explain the TRAPI planning process. He will emphasize that the main objective of the TRAPI planning process is to openly share trufficulture information and prepare a collaborative grant proposal to obtain research and extension funding on truffle production and marketing. Key TRAPI scientists will offer invited meeting presentations about their research on truffle productivity and relevant research as applied to truffle economics and marketing. Such applied science presentations will offer ideas about fruitful research topics. With the help of the Research Task Force and TRAPI socioeconomic scientists, we will conduct an in-depth Qualtrics survey of the growers for the purpose of gathering further insights into their ideas about priority research needs. We will announce the survey to the membership and other interested parties ahead of the Fall 2022 Congress. Task Force members will describe the process, explain the purpose, and announce an in-meeting listening session. We will use the listening session to gather greater detail on research needs than surveys can achieve. The survey will remain open for at least two weeks after the Congress.

The PD will distribute survey results to TRAPI scientists and growers and he will designate to TRAPI scientists the responsibility of reviewing available information on specific topics aligned with their expertise. Scientists agreeing to topic assignments will synthesize available literature and use that information to prepare and offer online presentations over the following six months. These seminars will complete the TRAPI online seminar series that was started with the initial topics identified as priorities in Fall 2021. We expect up to eight priority research topics to emerge with one or more scientists working on a topic. We will also attempt to pair research and extension scientists to work on topics together so they will review rigorous scientific content from both technical and applied perspectives. NATGA can offer the online seminar series to their membership through their webinar series, or we can offer them as a University of Idaho seminar series. We will record presentations and make them available through NATGA and UIdaho websites. TRAPI scientists will share among growers and scientists a listing of literature found and any translations produced (copyrights permitting). An important purpose is to engender free information flow and discourage confidentiality. TRAPI scientists taking responsibility for priority research topics will prepare a collaborative, synthesis review manuscript on available trufficulture knowledge as applied to NA. This collaborative review will be essential for designing an SCRI proposal addressing critical research needs of the NA truffle industry. In concert with preparing this manuscript, the PD will motivate online discussions among TRAPI collaborators on questions and approaches that can address identified research needs.

The third TRAPI meeting will be the collaborative grant proposal writing workshop during summer of 2023 to focus on preparing a grant proposal for the 2024 SCRI solicitation. The SCRI proposal is the ultimate output of the planning project. The workshop will be timed to follow the seminar series, drafting of the review manuscript, and discussions about addressing research needs. Contributing to the TRAPI Seminar Series and contributing to the synthesis review manuscript will be prerequisites for scientists receiving travel support to attend the TRAPI grant proposal writing workshop. Thus, pre-workshop activities allow TRAPI to build toward writing an SCRI grant proposal by gathering and synthesizing information, discussing potential project objectives, and identifying ardent and contributing collaborators.

The grant proposal writing workshop will collaboratively lay out a 2024 SCRI proposal. Workshop tasks will include settling on research and extension objectives, choosing grant type, outlining grant activities, assigning proposal writing responsibilities, presenting a work plan and timeline, and sketching resource requirements for budgeting purposes. PD will organize and manage the workshop to retain focus on research with the greatest potential to increase truffle yields and expand the NA truffle market. At first, key TRAPI scientists will highlight critical needs of the NA truffle industry and identify knowledge gaps. Then the NATGA Research Task Force will facilitate a discussion to select primary project objectives from highlighted critical needs by encouraging critiques, refining ideas, and building consensus. This is also a point to confirm consensus on project scope, or specifically, whether to select a SREP or CAP. Following consensus, attendees will divide into working groups based on settled objectives. Each working group will design grant activities related to their assigned objective, including experimental designs, outreach activities, and required resources. Previous, online discussion should expedite this process. Working-group leads will report back, and the Task Force will facilitate whole group discussion to identify natural linkages and efficiencies among objectives.

Given the extent of TRAPI activities, it will then be possible for the PD and Research Task Force to identify visionary and democratic leaders to assemble proposal text and budget, much of which will occur as an online collaborative (email, video chat, shared cloud drive). The PD and lead TRAPI scientists will prepare proposal text from the synthesis review manuscript, on-line discussions, and working group deliberations. Lead scientists will use resource requirements reported from working groups to advise PD on budget and cost share distributions. Finally, the workshop will conclude with the PD reviewing the work plan and timeline and explaining plans for document management and communications. A critical task in the timeline will be using the draft full proposal to prepare and submit an outstanding Stakeholder Relevance Statement. The Research Task Force will assist with obtaining letters of support from the full range of enterprises affiliated with the NA truffle industry.

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