Symbiotic of *Ventenata dubia*, *Bromus tectorum*, *Boechera stricta*, and *Phoenix dactylifera L*

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Authorization to Submit Dissertation

This dissertation of Maryam M. Alomran, submitted for the degree of Doctor of Philosophy with a Major in Plant Science and titled "Symbiotic of *Ventenata dubia*, *Bromus tectorum*, *Boechera stricta*, and *Phoenix dactylifera L*," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Symbiotic of *Ventenata dubia*, *Bromus tectorum*, *Boechera stricta*, and *Phoenix dactylifera* investigates the intricate nature of plant symbiosis as the relationship between specific plant species (four plant species *V. dubia*, *B. tectorum*, *B. stricta*, and *P. dactylifera*,) in specific contexts (northwest USA and Saudi Arabia), utilizing laboratory experimental design and literature review methodologies. Symbiosis is a relationship between organisms of different species, and the interaction may be beneficial or harmful. There are three types of symbiotic relationships: mutualism, commensalism, and parasitism. Mutualism is a relationship between organisms of different species with each benefiting. Commensalism is a relationship between organisms of different species without any harm but benefiting one organism and this relationship applies to chapter 3, A bottleneck for microbes in seeds of *Ventenata dubia*, *Bromus tectorum*, and *Boechera stricta*, as endophytes have benefit and no effects on the three-plant species *V. dubia*, *B. tectorum*, *B. stricta*. Endophytes are microorganisms that live in the interior of the plant and have no apparent of damage to their host. Endophytes play essential roles in plant protection, act against herbivores, insects, and pathogens of the host and may even increase plant resistance to pathogens, biotic, and abiotic stresses.

Lastly, parasitism involves two organisms, for one benefit and the other, which is considered a host is harmed. For example, plant pathogens cause disease to plants. For example, *P. dactylifera* (date palm) is parasitized by *Aspergillus tubingensis* (Chapter 4), and we are setting the groundwork to search for a biological control agent for *V. dubia* (Chapter 2).

The dissertation addresses four plant species that can be categorized as native, introduced, and invasive. *Boechera stricta* is native to North America and is widespread and has a great potential for studies involving symbiosis. The second category, introduced, is represented by date palm (*Phoenix dactylifera L.*), intentionally introduced by humans to a new area where it was not previously found. Date palm is native to the arid Arabian Peninsula, North Africa, and the Middle East however to date palm has been introduced to many countries including Australia, India, Pakistan, Mexico, South Africa, and the United States. Dates have been a staple food in the Middle East region for years. Dates are the primary source of income and basic food for the local population in many countries where they are grown and have played essential roles in the economy, society, and environment of those countries.

The final relationship involves *Ventenata dubia* and *Bromus tectorum* and these two species are invasive. Invasive is defined as a non-native species that changes how an ecosystem functions. Invasive plant species are one of the main and most rapidly developing threats to food security, animal, human health, and biodiversity. The transportation of these invader plants can be through

global travel, transport, tourism, and trade, timber products, and ornamental flora. When a new and aggressive species is brought into an ecosystem, it might be in a stage of enemy release leaving all its enemies in their native range, and this helps the invader to spread quickly and to take over an area from the native plants. They can change the plant community by competing with native species in the light, water, or nutrient resources. Invasive plants are threats to native wildlife. Prevention, eradication, and management of invasive species are a costly challenge. For example, in this dissertation *V. dubia and B. tectorum* are choosen because they are especially damaging the native plants. These two are threatening many of the plant communities in the Pacific Northwest, and they are creating economic and ecosystem losses. The reason to be concerned about losing any native species is that loss of a single species can affect the interconnected in life on earth. If enough "living connections" are broken, entire ecosystems in the earth could fail, and balance of nature be forever changed. Additionally, the diversity of animals and plants could change as well, and when species were lost humans would lose the benefits of them as food and medicines forever.

European settlers introduced many plants to North America from their homelands, for food, medicinal, ornamental, and other purposes. Introductions of non-native plants continue today, and are even increasing due to elevated worldwide travel and expanded worldwide trade. Many introduced plants have become naturalized over the continent, and a few are replacing North American native plant species.

Parallel to overpopulation and global immigration, we also contend with impact from climate change on invasive plant species and an overall change of ecosystems. It is hard to know the immediate measurable effects of climate change because we are uncertain about how the invasive species will respond to diverse parameters of climate change, such as temperature and precipitation. However, there is more than one reason to believe that most climate change will have an impact by increasing the frequency and severity of invasive species in any given geographic region. Invasive species will increase in abundance and emergence because the changing climate becomes unsuitable for the native species. With high nutrition resources, invasive species are well suited to succeed in new environments. Climate change leads to warmer temperatures, and higher CO_2 concentration. Severe storms become more common due to climate change and may disperse invasive plant seeds more widely.

Presented are four chapters: all chapters are related to the concept of plant symbiosis. All of the four plant species, *V. dubia*, *B. tectorum*, *B. stricta*, and *P. dactylifera*, have symbiotic relationships with microorganisms. However, each chapter has a different experimental design, and the objectives for each are not the same. The first and second chapters focus on *V. dubia*, an invasive plant in PNW that is affecting ecosystems by reducing native species abundance and diversity and causing

an economic loss in the area infected by *V. dubia*. The species likely is in a state of enemy release, and thus a biological control would assist in its management. The native range of *V. dubia* had to be specified so that biological control discovery could be carried out within the native range, and we can look for a control agent.

The third and fourth chapters involve symbiosis of the four plants species mentioned above. The third chapter tests a hypothesis of a bottleneck and exclusionary interactions for microbes in seeds of invasive plant species *V. dubia*, *B. tectorum*, and native plant species *B. stricta* and builds on prior research. Experimental design in this chapter was different than Newcombe et al. (2018) to see if this could make any difference. In this experiment, different factors were used: varied age of seed, surface-sterilization protocol, and isolation medium, inoculated versus uninoculated during flowering stage and plant genotypes.

The fourth chapter is about date palm seeds and the hypothesis that *Aspergillus tubingensis* affects the emergence of date palm seedlings. In the beginning, we examined the seeds' endophytes, and we noted that there is an effect of *Aspergillus* to the seedlings of the date palm and no impact to the seedlings by the other endophytes that were found in the seeds of the date palm.

This dissertation's style is each chapter follow by its references, tables, and figures for better organization. Full credit is given to the dissertation author for tables in chapter 2 synthesizing literature review around *V. dubia* as all tables were compiled by her.

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Dedication

To my dear parents, Mohammed and Munira, my husband Abdullah, my beautiful daughters, Sarah, Sadeem, Judy and Lana. Finally, to my siblings.

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Chapter 1: Foundational Research & Literature Review on *Ventenata dubia*

Plant invasion threatens many of the world's plant communities (Dogra et al. 2010). Invasions are creating economic losses by reducing crop yields and forage. A subset, the invasive grasses, are increasing the frequency and severity of fires which affect wildlife, thus changing ecosystem structure and function (D'Antonio and Vitousek 1992). Invasive plants can change biological soil crusts by reducing soil nutrients, water, and cover (D'Antonio and Vitousek 1992). They reduce environmental function through competition for resources and change the natural regimes by disturbance. They are replacing local plant species, thereby decreasing forage and habitat for wildlife (Reynecke 2012). For example, cheatgrass (*Bromus tectorum*) increases fire frequency/intensity and reduces nitrogen levels in the soil (Link et al. 2006). *Ventenata dubia* is another example, like cheatgrass, of a winter annual grass that poses a serious threat in the western United States. Currently, it is considered less widespread than the other grasses (James 2008), but it is now beginning to receive a lot of attention due to its rate of recent spread and the difficulty of control (Scheinost et al. 2009).

Taxonomic placement, Biology and Background of Ventenata dubia

Ventenata dubia (Leers) Coss. & Durieuis is in the family Poaceae, one of the largest families of flowering plants in the world, with about 600 genera and 10,000 species. Altogether grasses represent about 20% of the world's vegetation coverage (Ocak et al. 2009).

The genus *Ventenata* honors the French botanist Pierre Etienne Ventenat, who lived from 1757 to 1805. The common names for *V. dubia* include ventenata, North Africa grass, wiregrass, softbearded oat grass and hairgrass which stem from its presumed native range or descriptions of the plant's form, respectively. Synonyms include *Avena dubia* Leers, and *Ventenata avenacea* Koeler (Martin 2000; Scheinost et al. 2009).

Ventenata dubia is a winter annual that germinates, emerges, and grows in the fall and produces flowers and matures seeds in the spring (Scheinost et al. 2009). Seeds germinate in the fall when the fall rain begins, and soil temperatures are between 23 and 29 C (optimal). Most seedlings appear within six weeks after germination starts (Wallace et al. 2015). Stems are erect and range from 1.5 to 7 dm. (approx. 6 to 28 inches). They look smooth, despite tiny hairs that are noticeable when magnified. The leaf ligule is about 1-6 mm long and 1-3 mm wide. Inflorescences are panicles, and they are about 3-10 cm long and open. Panicle branches are long and may droop. Spikelets are 10-15 mm long, stalked and near the branch tips. Glumes are lancelolate and ending in a sharp tip. The lemmas have bent awns arising from their backs (Figure 1.1) (Martin 2000).

Ventenata dubia in May and June produces ligules in lengths of up to 8.4 mm with reddish

black-colored nodes. In the middle to the end of summer, misidentification can occur with Aveneae species because of the tendency of *V. dubia* to produce twisted, bent awns upon senescence. Ventenata and cheatgrass flower in May and June (Wallace et al. 2015).

Ventenata dubia reproduces exclusively by seeds. Each plant produces from 15 to 35 seeds (Pavek et al. 2011). Seeds stay viable in the seed bank only a few years, and most germinate in the first year after dispersal. Some seeds may germinate even after three years at shallow soil depths (approx. 2 cm). Bent, twisted awns allow "self-burial" of seeds in the soil and attachment of seeds to vectors such as animals, equipment, and humans (Wallace et al. 2015).

Habitat

Ventenata dubia grows in a variety of dry, open habitats, often disturbed, but it tends to prefer sites which are flooded in early spring but dry by late spring. South-facing hillsides with shallow, rocky, clay soil are common habitats (Scheinost et al. 2009). In eastern Oregon, central and eastern Washington, and northern Idaho, *V. dubia* grows in areas that receive 35-112 cm of annual rainfall (Pavek et al. 2011). It grows in pastures, meadows, roadsides, open forests, rocky swales. *V. dubia* does overlap sites that support *B. tectorum*. Agricultural sites can also support *V. dubia* and include cereal grain and grass hay fields (Wallace et al. 2015). It also grows in native Palouse Prairie remnants (Nyamai et al. 2011).

Vectors and other mechanisms of spread

Ventenata dubia spreads in different ways, such as a contaminant of grass hay, Kentucky bluegrass seed, and other annual crops (Scheinost et al. 2009). Grass hay harvest in the Pacific Northwest occurs at the beginning of July when *V. dubia* seed are present at the same time (Wallace et al. 2015). *Ventenata dubia* grows along roadsides and is likely to be moved along such transport corridors where it may spread into the surrounding areas. Humans and animals can also spread it because the long awns adhere to clothing or fur (Scheinost et al. 2009).

A survey of landowners in areas infested by *V. dubia*, in Eastern Oregon, Central, and Eastern Washington, and Northern Idaho, indicated that *V. dubia* was present in 74% of the counties. It was primarily in the pastures, grass hay including Timothy hay.

Impacts

Invasions are now the second leading cause of endangerment and extinction of species in the United States and around the world (Simberloff 2001). About 42% of all the species listed in the Endangered Species Act are threatened partly or entirely by non-native species (Simberloff 2001). In the Pacific Northwest, *V. dubia* has spread in the following states: Oregon, Washington, Idaho, Montana, Nevada, Utah, and Wyoming. An annual rate of spread for the Pacific Northwest was reported only in 2001, and it is estimated at 3 million acres per year (Fryer 2017).

Invasive non-native species impose enormous economic costs not only on nature but agriculture, forestry, industry, and public health (Simberloff 2001). The most significant problems with non-natives, in terms of ecological damage, are usually caused by plant species that overgrow entire communities, replacing native plants (Simberloff 2001). The negative impact on native species can be direct or indirect. For example, an invasive weed which is undesirable as a food source may out-compete and displace local grasses. The native grasses and other plants replaced may be the primary forage for native animals, resulting in animals moving to a new location or have reduced population because of reduced availability of food. Invasive non-native species are harmful to the environments because they significantly decrease the native species which are food resources for animals in the same area, and this will affect the environmental food chain (Invasive Species Advisory Committee 2006).

Ventenata dubia is invasive and very competitive in several grass crops, wildland grazing areas, and pastures. It is undesirable because it replaces forbs and native perennial grasses and its shallow root system makes the soil more prone to erosion (Wolff 2013). *Ventenata dubia* negatively affects millions of hectares of grassland of the United States. It can reduce the richness of native species abundance, and livestock carrying capacity, and change microbial communities (Rinella et al. 2014). *Ventenata dubia* once established, may be more competitive than the annual bromes (some of which are desirable as forage, others less so) and even medusahead (*Taeniatherum caput-medusae*) (Wallace et al. 2015). *Ventenata dubia* impacts land use by increasing the frequency of forest fires (USDA 2012).

The expansion of *V. dubia* has been documented along with its economic impact. It has severely affected forage producers by reducing forage yield and quality in all parts of the Pacific Northwest (Prather and Steele 2009). *Ventenata dubia* has expanded rapidly in perennial grass systems, and it has affected managed areas in the past two decades in the Pacific Northwest (Pavek et al. 2011). It expanded in the Snake River and sagebrush steppe, where sites were previously dominated by *B. tectorum* and *T. caput-medusae* (Wallace et al. 2015). Agricultural producers, land managers, and researchers saw an increase in invasion by *V. dubia* in the Inland Northwest in the past decade (Prather 2012). Those invasions led to economic losses in the hay industry, especially in the Timothy hay market (Prather 2012). The result of that is having significant yield reductions of 50% or more within a few growing seasons (Prather 2012). Besides, hay has been rejected for export markets, and "wire -like" structure makes harvest difficult by binding up machines; moreover, it reduces hay stands longevity (Prather et al. 2017). There are also growing fears of habitat degradation in the Conservation Reserve Program (CRP) land (Prather et al. 2017). *Ventenata dubia* has severely affected forage producers, reducing forage yield and quality in all parts of the Pacific Northwest (Prather and

Steele 2009). Over time, *V. dubia* has a negative economic impact by reducing productivity and changed land conditions, which results in reduced land values.

Prevention and Management of V. dubia

Preventing the introduction of invasive species is an optimal defense against invasions. Early detection and rapid response (EDRR) can increase the chance of localizing invasiveness, with possible elimination before the invasive species become established on a large scale. EDRR addresses where invasive species are located and where they are likely to spread (Invasive Species Advisory Committee 2006). Knowing the associated plant species in both native and current ranges of *V. dubia* can help to find out where *V. dubia* is likely to spread in the invaded range in North America. *Ventenata dubia* has expanded south into sagebrush steppe, and such expansion is affected by composition of sagebrush steppe communities (Jones et al. 2018).

Ventenata dubia has been found associated with other plant species in the United Kingdom, Ukraine, Turkey, and Serbia (Copping 1987; Duran and Dural 2003; Didukh et al. 2004; Ocak et al. 2009; Panjković et al. 2012). As in Table 1.1, there is a list of major associated plant species, and these plant species have been found in the scientific literature during searching for records of *V. dubia*. As the table shows in the first column, *V. dubia* has been found around the world, including in the United States. It is important to have an idea about possible future areas where *V. dubia* could be found.

Table 1.1 provides major associated species for each geographic area. In this table all the species are present in the USA except *Genista vuralii, Trifolium grandiflorum*, *Aegilops margrafii*, and *Ranunculus polyphyllus*. *Bromus commutatus* is an associated species that has a broad native range, with latitudes ranging from 34° S to 54° N and it is introduced in most of USA and Canada with latitudes ≈ 31° S to 66 ° N. Another species associated in North America is *Bromus tectorum*, its native range encompassing latitudes ≈ 20° S to 60° N and its latitudes in the introduced range being ≈31° S to 66° N. *Avena sativa* has a native range of latitudes ≈ 20° S to 60° N and its latitudes in introduced range are ≈31° S to 64 ° N. For *Lolium perenne L*., its native range is latitudes ≈ 26° S to 60° N and in introduced range ≈31 ° S to 66° N. *Koeleria cristata* (L.) Pers. is native to the USA and Canada, and its range covers most of those countries. *Taeniatherum caput-medusae* is a highly invasive plant in the USA, and its introduced range is ≈26° S to 41° N. Study shows that *V. dubia* and *T. caput-medusae* cover increased together, however downy brome (*Bromus tectorum* L.) abundance decreased, which implies that *V. dubia* and *T. caput-medusae* are sharing the same niche, and this further implies that *V. dubia* abundance will be high when *T. caput-medusae* is present in the area (Jones et al. 2018).

Management of *V. dubia* after dispersal requires persistence. It is hard to control using common weed management strategies (Prather and Steele 2009). A recent study by Wallace et al.

(2016) found pre-emergence and post-emergence herbicides, including rimsulfuron, sulfosulfuron, and flufenacet plus metribuzin applied in the fall, are controlling *V. dubia* for one year across a range of perennial grass systems. Besides, spring N application is useful to control *V. dubia* because spring N increases in perennial grass cover and decreases in *V. dubia* cover and the application could be used as a long term of management plan (Wallace et al. 2016). However, herbicides application should be combined with other management techniques to control *V. dubia* (Washington State Noxious Weed Control Board 2016). Manual and mechanical control, for example, hand pulling plants when the soil is moist, but by these methods, only small areas can be treated. It may help to prevent the *V. dubia* seed production (Scheinost et al. 2009). Mowing is difficult because ventenata, when mowed during heading, stems tend to become tangled in the swather, and if it is mowed once before heading, the plants may produce a new flush of heads. Mowing can be successful if done several times during the growing season (Scheinost et al. 2009).

Other strategies are cultural controls, and they are temporarily used until biological control is found. Reduce *V. dubia* entry into fields: if working around *V. dubia* make sure to clean equipment and clothing to prevent dispersal of seeds to new locations. Because some *V. dubia* seeds can survive for at least up to 3 years in the seed bank, intensive integrated management methods should be used for at least 3-4 years to reduce the effects on grass systems (Wallace et al. 2015). Fire shows no promise as a means of control. Where forest fires or prescribed fires burned in Oregon, the *V. dubia* population has flourished (Scheinost et al. 2009). Biocontrol by microbial or invertebrate natural enemies has certainly been contemplated, but candidate organisms are not readily available or even documented (Scheinost et al. 2009). Biocontrol via grazing might be potentially reliable, but animals tend to avoid plants, especially when *V. dubia* matures (Prather and Steele 2009).

Natural Enemies of V. dubia in its Native Range

Plant natural enemies include invertebrates and herbivores, and fungal, bacterial and viral pathogens (Keane and Crawley 2002). Studies have shown that natural enemies are more abundant in the native range, compared with the introduced range (Widmer et al. 2007). It can be hypothesized that *V. dubia* in the United States has escaped from its enemies in its native range (Chapter 2) with an increase in its distribution and abundance. Unfortunately, little to nothing is known of its natural enemies in its native range.

Summary

Within the last 20 years, more knowledge of the biology and impacts of *V. dubia* has been discovered. Further understanding of the native range of *V. dubia* provides insights into its ability to persist in a wide range of plant communities and climatic regimes. A more robust understanding of symbiosis with *V. dubia* may lead to a greater understanding of how it became invasive and key to

potential management. Discovery of its limited abundance within the proposed native range suggests biological control has potential as a tool for integrated management.

Literature Cited

Copping A (1987) Aliens and adventives. *Ventenata dubia* (Leers) FW Schultz in Grays Chalk Quarry, Essex, 1986. BSBI News 45:24-25

D'Antonio C M, Vitousek P M (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual review of ecology and systematics 23:63-87

Didukh Y, Romo A, Boratyñski, A (2004) On five rare vascular plant species reported from Crimea, Ukraine. Willdenowia 34:407-410

Dogra K S, Sood S K, Dobhal P K, Sharma S (2010) Alien plant invasion and their impact on indigenous species diversity at global scale: A review. Journal of ecology and the natural environment 2:175-186

Duran A, Dural H (2003) *Genistavuralii* (Fabaceae), a new species from Turkey. In Annales Botanici Fennici. Finnish Zoological and Botanical Publishing Board 40:113-116

Fryer J L (2017) *Ventenata dubia*. — In: Fire effects information system.U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Missoula Fire Sciences Laboratory. https://www.fs.fed.us/database/feis/plants/graminoid/vendub/all.pdf. Accessed January 26, 2018

Gardens: Royal Botanic Garden Edinburgh (2015) Royal Botanic Garden Edinburgh. www.rbge.org.uk. Accessed: 17 February 2015

GBIF.org (2015) Global Biodiversity Information Facility. http://gbif.org . Accessed: 17 February 2015

GRIN (2015) National Germplasm Resources Laboratory, Beltsville, Maryland. Retrieved from http://www.ars-grin.gov/npgs. Accessed: 27 July 2015

Invasive Species Advisory Committee (2006) Invasive species definition clarification and guidance white paper. National Invasive Species Council. US Department of Agriculture, National

Agricultural Library.Washington, DC. https://www.doi.gov/sites/doi.gov/files/uploads/isac_definitions_white_paper_rev.pdf Accessed October 23, 2016

James J J (2008) Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. Journal of Arid Environments 72:1775-1784

Jones L C, Norton N, Prather T S (2018) Indicators of Ventenata (*Ventenata dubia*) Invasion in Sagebrush Steppe Rangelands. Invasive Plant Science and Management 11:1-9

Keane R. M, Crawley M J (2002) Exotic plant invasions and the enemy release hypothesis. Trends in ecology and evolution 17:164-170

Link S O, Keeler C W, Hill R W, Hagen E (2006) *Bromus tectorum* cover mapping and fire risk. International Journal of Wildland Fire 15:113-119

Martin, Tunyalee (2000) Weed Alert: *Ventenata dubia*. http://wiki.bugwood.org/index.php?title=Ventenata_dubia&oldid=49943 Accessed April 23, 2015

Nyamai P A, Prather T S, Wallace J M (2011) Evaluating restoration methods across a range of plant communities dominated by invasive annual grasses to native perennial grasses. Invasive Plant Science and Management. 4:306-316

Ocak A, Ture C, Senmerdan A B, Bocuk H (2009) An investigation of diversity, distribution and monitoring on Poaceae (Gramineae) species growing naturally in Bilecik province at the intersection of three Phytogeographical regions (Northwest Anatolia-Turkey). Pakistan Journal of Botany 41:1091-1106

Panjković B, Perić R, Stojšić V, Batanjski V (2012). New data on the distribution of *Ranunculus polyphyllus* Waldst. Kit. ex Willd. in Serbia. Archives of Biological Sciences 64:715-720

Pavek P, Wallace J. Prather T (2011) Ventenata biology and distribution in the Pacific Northwest. Proceedings of the Western Society of Weed Science. Spokane, WA: Western Society of Weed Science 64:107

Prather T (2012) Developing a decision support tool for ventenata IPM in the Inland Northwest. WSARE Annual Report. https://projects.sare.org/sare_project/sw10-103/?ar=2012.

Accessed October 23, 2016

Prather T, V Steele (2009) Ventenata Control Strategies Found for Forage Producers.UI Impact Statement

Prather T S, Mackey A, Wallace J (2017) Managing *Ventenata dubia* in Timothy Hay. 47th California Alfalfa and Grain Symposium

The United States Department of Agriculture (USDA) (2012) improved conservation outcomes on u.s. rangeland RANGELAND, U.S. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/16/stelprdb1049009.pdf. Accessed November 12, 2017

USDA (2015) Natural Resources Conservation Service. The Plant Database. http://plants.usda.gov. Accessed: 17 February 2015

Reynecke B K (2012) Plant community restoration on mima mounds at Turnbull National Wildlife Refuge, WA

Rinella M J, Bellows S E, Roth A D (2014) Aminopyralid constrains seed production of the invasive annual grasses medusahead and ventenata. Rangeland ecology and management 67:406-411

Scheinost P, Stannard M, Prather T (2009) USDA NRCS Plant Guide*: Ventenata dubia*. Pullman, WA: Pullman Plant Materials Center

Simberloff D (2001) Biological invasions—how are they affecting us, and what can we do about them?.Western North American Naturalist 61:7

Wallace J M, Pavek P L, Prather T S (2015) Ecological characteristics of *Ventenata dubia* in the Intermountain Pacific Northwest. Invasive Plant Science and Management 8:57-71

Wallace J M, Prather T S (2016) Herbicide control strategies for *Ventenata dubia* in the Intermountain Pacific Northwest. Invasive Plant Science and Management 9:128-137

Washington State Noxious Weed Control Board (2016) *Ventenata dubia*. Olympia, WA: Washington State Noxious Weed Control Board.

https://www.nwcb.wa.gov/images/weeds/Ventenata_dubia_written_findings_final.pdf Accessed December 17, 2017

Widmer T L, Guermache F, Dolgovskaia, M Y, Reznik, S Y (2007) Enhanced growth and seed properties in introduced vs. native populations of yellow starthistle (*Centaurea solstitialis*). Weed Science 55:465-473

Wolff, Benjamin (2013) North Africa grass. FIELD GUIDE

Table 1.1 Associated plant species: summary review of literature by author.

Area	Associated species	Associated species Range	Presence in USA	Author/Reference
Eastern Rhodope Mountains (Bulgaria)	*Taeniatherum caput-medusae*	Algeria, Egypt, Libya, Morocco, Tunisia, Afghanistan, Cyprus, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Russian Federation – Dagestan, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Pakistan – Balochistan, Hungary, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Macedonia, Romania, Serbia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
Grays Chalk Quarry, Essex, United Kingdom	1.*Bromus commutatus*	Tunisia, Cyprus, Iran, Iraq, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Dagestan, Ireland, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Estonia, Latvia, Lithuania, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy Romania, Serbia, Slovenia, France, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory; and Copping 1987)
	2. *B. tectorum*	Spain - Canary Islands, Algeria, Egypt, Morocco, Tunisia, Kuwait, Saudi Arabia, Afghanistan, Cyprus, Egypt – Sinai, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Dagestan, Western Siberia, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, China ,Pakistan, Denmark; Norway, Sweden, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Portugal.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	3. *Apera spica-*	Buryatia, Irkutsk, Kemerovo, Krasnoyarsk, Kurgan, Omsk, Tomsk, Tuva, Tyumen, Yakutia-Sakha, Kazakhstan, United Kingdom, Austria,	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National

	venti	Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Switzerland, Belarus, Estonia, Lithuania, Moldova, Russian Federation, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Spain.		Germplasm Resources Laboratory)
	4. *Apera interrupta*.	Egypt, Tunisia, Afghanistan, Iraq, Palestine/Israel, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Russian Federation - Ciscaucasia, Dagestan, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Norway, Sweden, Austria, Czech Republic, Germany, Hungary, Slovakia, Switzerland, Ukraine – Krym, Bulgaria, Croatia, Italy, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
Crimea, Ukraine	1.*Trifolium grandiflorum*	Iran, Iraq, Lebanon, Syria, Turkey, Albania, Bulgaria, Former Yugoslavia, Greece, Italy.	No	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory; Didukh et al. 2004)
Turkey	1. *Genista vuralii*	Turkey- Cankiri, Izmir, Kastamonu, Kastamonu, Gumushane, Nigde. Italy-Calábria.	No	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory; and Duran et al. 2003)
Bilecik Province, Turkey	1. *Brachyopodium sylvaticum*	Sweden, France, Germany.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory; and Ocak et al. 2009)
	2. *Hordeum geniculatum*	Turkey, Iraq, Armenia, Jordan, Iran, Egypt.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)

	3. *Aegilops triuncialis* L. subsp. *Triuncialis*	Algeria , Egypt , Libya , Morocco, Tunisia , Afghanistan, Cyprus, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Azerbaijan, Georgia, Ciscaucasia, Dagestan, Kazakhstan, Tajikistan, Turkmenistan, Uzbekistan, Pakistan, Austria, Czech Republic, Hungary, Slovakia, Moldova, Astrakhan, Volgograd, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Macedonia, Montenegro, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	4. *Aegilops geniculata*	Spain - Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Cyprus, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Azerbaijan, Georgia, Hungary, Switzerland, Ukraine – Krym, Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Italy, Macedonia, Malta, Montenegro, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	5. *Aegilops margrafii*	Greece, Australia, Syrian Arab Republic, Turkey.	No	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	6. *Triticum aestivum* L.	Spain, Germany, Sweden, Norway, Netherlands, Algeria, France, Finland, China, Japan.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	7. *Avena sativa* L.	France, Germany, Ireland, Sweden, Norway.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	8. *Arrhenatherum elatius Elatius*	Spain - Canary Islands, Algeria, Morocco, Cyprus, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Western Siberia, Turkmenistan, Denmark, Finland, Iceland,	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)

		Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Estonia, Latvia, Lithuania, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece; Italy, Romania; Serbia, Slovenia, France ,Portugal, Spain.		
	9. *Bromus rubens* L.	Spain - Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Afghanistan, Cyprus, Egypt – Sinai, Iran, Iraq, Palestine/Israel, Jordan, Syria, Turkey, Azerbaijan, Russian Federation - Dagestan, Tajikistan, Turkmenistan, Uzbekistan, China - Xizang, Greece, Italy, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	10. *Koeleria cristata*	United Kingdom, Jordan, Tunisia, Turkey, Egypt, Iran, Australia, Spain, Morocco, Iraq, Greece, Saudi.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	11. *Holcus lanatus* L.	Saudi Arabia, China, Nepal, United Arab Emirates, Portugal - Madeira Islands, Spain - Canary Islands, Algeria, Morocco, Tunisia, Lebanon, Turkey, Azerbaijan, Georgia, Ciscaucasia, Denmark, Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Estonia, Latvia, Lithuania, Ukraine, Albania, Bosnia, Herzegovina, Bulgaria, Croatia, Greece, Italy ,Romania, Serbia, Slovenia ,France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	12. *Apera spica-venti*	Russian Federation - Buryatia, Irkutsk, Kemerovo, Krasnoyarsk, Kurgan, Omsk, Tomsk, Tuva, Tyumen, Yakutia-Sakha , Kazakhstan ,Spain, Sweden, Bhutan, United Kingdom, Turkey, Algeria, Morocco, Bolivia, Argentina, St Helena, Bulgaria, Finland, Georgia, Australia, Portugal, Germany, Denmark, Hungary, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands; Poland, Switzerland, Belarus,	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)

		Estonia, Lithuania, Moldova, Ukraine, Albania, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France.		
	13. *Aleopecurus myosuroides*	Algeria, Egypt, Libya, Tunisia, Afghanistan, Cyprus, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Dagestan, Turkmenistan; Uzbekistan, India, Pakistan, United Kingdom, Belgium, Germany, Netherlands, Russian Federation, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	14. *Vulpia myuros*	Portugal - Madeira Islands, Spain - Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Eritrea, Kenya, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, ,Russian Federation - Ciscaucasia, Dagestan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Bhutan; India, Pakistan, Ireland, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	15. *Festuca callieri*	Australia, United Kingdom, Turkey, Tajikistan. Iran, Iraq, Georgia, Egypt, Afghanistan, Jordan, Palestine/Israel, Portugal, Lebanon, Syria, Georgia, Ukraine – Krym, Albania, Bulgaria, Greece, Romania.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	16. *Lolium perenne* L.	Portugal - Madeira Islands, Spain - Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Saudi Arabia, Afghanistan, Cyprus, Egypt – Sinai, Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Dagestan, India, Pakistan, Denmark, Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland,	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)

		Slovakia, Switzerland, Belarus, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France.		
	17. *Cynosorus cristatus* L.	Bhutan, Cyprus, Iraq, Jordan, Turkey, Palestine/Israel, Argentina, Egypt, Chile, Yemen, Nepal, St Helena, Lebanon, Australia, Portugal - Madeira Islands, Turkey, Armenia, Azerbaijan, Georgia, Ciscaucasia, Denmark, Finland, Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Estonia, Latvia, Lithuania, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	18. *Cynosorus echinatus* L	Portugal - Madeira Islands, Spain - Canary Islands, Algeria, Morocco, Tunisia, Cyprus, Iran, Iraq, Palestine/Israel, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Russian Federation – Dagestan, India, Slovakia, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Portugal, Spain, United Kingdom, New Zealand, Australia.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	19. *Briza media* L.	Cyprus, Turkey, Armenia, Azerbaijan, Georgia, Russian Federation - Ciscaucasia, Dagestan, India, Pakistan, Denmark, Finland, Ireland, Norway, Sweden, United Kingdom, Austria, Belgium, Czech Republic, Germany, Hungary, Netherlands, Poland, Slovakia, Switzerland, Belarus, Estonia, Latvia, Lithuania, Moldova, Russian Federation -Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Serbia, Slovenia, France, Portugal, Spain.	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)
	20. *Digitaria sanguinalis* (L.) Scop	Spain - Canary Islands, Algeria, Egypt, Libya, Morocco, Tunisia, Saudi Arabia, Afghanistan, Cyprus, Egypt ,Iran, Iraq, Palestine/Israel, Jordan, Lebanon, Syria, Turkey, Armenia,	Yes	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory)

		Azerbaijan, Georgia, Russian Federation - Ciscaucasia, Dagestan, Kazakhstan, Kyrgyzstan, Turkmenistan, Uzbekistan, China - Anhui, Gansu, Hebei, Henan, Shaanxi, Shandong, Sichuan, Xinjiang, Xizang , India, Nepal, Pakistan, Hungary, Slovakia, Belarus, Moldova, Ukraine, Albania, Bulgaria, Croatia, Greece, Italy, Romania, Slovenia. France, Portugal, Spain.		
Province of Vojvodina, Serbia	1. *Ranunculus polyphyllus*	Hungary, Romania.	No	(USDA, Natural Resources Conservation Service; Gardens: Royal Botanic Garden Edinburgh; GBIF. Global Biodiversity Information Facility; GRIN. National Germplasm Resources Laboratory; and Panjković et al. 2012)

Figure 1.1 *V. dubia* inflorescences and seeds from USA.

Chapter 2: *Ventenata dubia's* Native Range and Consideration of Plant Pathogens for Biological Control.

Abstract

Ventenata dubia (Leers) Cosson is an exotic, invasive grass in the inland Pacific Northwest (PNW) of the United States and Canada. It appears to be in a state of 'enemy release' relative to pathogens. Surveys in the PNW (Idaho, Montana, Oregon and Washington) were entirely negative for fungi on *V. dubia* in Europe (i.e., *Septoria ventenatae*, *Tilletia fusca*, and *Tilletia elisabethae*). Nor were pathogens that might be expected in grasses (e.g., rust, powdery mildew, choke) found on *V. dubia*. In its native range its natural enemies may limit its abundance. One group of natural enemies that may limit abundance of *V. dubia* are fungal pathogens. Pathogens of *V. dubia* from its native range may hold potential and if deemed safe, could be introduced into the PNW as classical, biological control agents. To ascertain the native range in the Mediterranean Basin we compiled data from herbarium specimens, consulted with herbarium curators in the region and searched relevant literature. We found that *V. dubia* primarily is reported in southern Europe and western Asia. *Ventenata dubia* has been reported only occasionally from North Africa in Algeria and Morocco. The common name 'North Africa grass' likely originated from references to *V. dubia* in the 19th-century, botanical explorations in Algeria of the French botanist, Ernest Cosson, who published the current scientific name based on a pre-existing name in 1854. Another finding of interest is that the latitudinal range of collections from Europe and North Africa of *V. dubia* spans Tunisia to Finland. The plant may thus be adapted to a range of environments and could become widely distributed in North America. Efforts to search its native range for pathogens should also consider the range of environmental conditions found within its native and introduced ranges.

Keywords: Invader, native range, distributions, enemy release, biological control.

Ventenata dubia is a relatively new invader in the inland Pacific Northwest (PNW) of North America. It was first reported in Northern Idaho (Kootenai County) in 1956 (Northam and Callihan 1994). Prior to the year 2000 it was not reported as weedy. In recent years it has become a serious invader of pastures, Conservation Reserve Program (CRP) fields, and hay fields (Mackey 2014; Wallace et al. 2015). Perennial grass foliar cover increased 10 to 20% when *V. dubia* was controlled suggesting the annual grass reduced productivity of perennial grasses (Wallace et al. 2015). Timothy hay production declined from 4540 kg/ha to 2270 kg/ha when *V. dubia* was not controlled (Mackey

2014). *Ventenata dubia* negative impacts are recognized in the west with addition to state noxious weed lists in Nevada, Oregon, Utah, Washington, and Wyoming.

Natural enemies likely limit the abundance and distribution of *V. dubia*, suggested by its listing as rare or endangered in some areas of Europe (Bicknell 1896; Frey and Paszko 1998; Merce et al. 2007). Arthropods may be involved in regulating abundance and distribution of *V. dubia* yet the species has relatively high silica content (DiTomaso et al. 2013) and silica can be a deterrent to herbivory (Keeping and Kvedaras 2008). While pathogens have been introduced for biological control (Trujillo 2005), a pathogen of grasses has yet to be introduced and would receive extra scrutiny since so many of our agricultural crops are grasses. Currently pathogen use against grass weeds in the United States has been limited to use of native fungi (Chandramohan and Charudattan 2001).

Biogeography is important to understanding risk of invasion (Pheloung et al. 1999). Gaining an understanding of the range in latitude of *V. dubia's* distribution in its native range would increase our understanding of risk to invasion within North America. In addition, collecting distributional data provides parameters for exploration for biological control agents. We investigated the distribution of *V. dubia* across its suspected native range to assist in discovery of biological control agents and to provide insight into risk of invasion. Further, support for biological control was investigated by searching for evidence of pathogenic expression in the Pacific Northwest and search of literature for fungi that occur on *V. dubia*.

Materials and Methods

Plants were collected to look for signs and symptoms of disease within the introduced range in the PNW. In 2014, samples of *V. dubia* were collected across a broad geographical area with a minimum of six individual plants per site from 41 sites ranging from Pend Oreille County, Washington, in the north and south to Grant County, Oregon, east to Elmore County, Idaho, and one sample from Gallatin County, Montana. The protocol for sampling was ten plants per site with each plant separately bagged. In addition, roughly 1000 *V. dubia* plants near the campus of the University of Idaho were observed for pathogens each year in early June from 2016 to 2018. *Septoria ventenatae* causes necrotic leaf lesions in *V. dubia* (Radulescu et al, 1973); these lesions are very similar to the lesions that other species of *Septoria* cause in other plant hosts and they were surveyed by visual inspection and then, if suspects had been found, sporulation would have been induced by moist-incubation. Spores would have been assessed morphologically and cultures sequenced. *Tilletia* species replace the seeds of their hosts with their own spores (Denchev and Denchev, 2018; Scholz and Scholz, 1988), and this disease, commonly called smut or bunt, was surveyed in the field, searching for dark seeds filled with a powdery mass. Field diagnosis would then have been followed by

morphological and sequence-based confirmation and species determination. We also looked for rust although no rust has ever been reported for *V. dubia*.

Location of geographic records was accomplished using the search engines Google Books and Google Scholar and key words *Ventenata dubia* and its synonyms *Avena dubia*, *Ventenata avenacea*, *Avena tenuis* and *Gaudinia tenuis*. Regional monographs containing *V. dubia* were obtained from libraries at University of Idaho, Washington State University, collections of USDA-ARS Plant Introduction in Pullman, and through Inter-Library Loan. People who have published materials that included *V. dubia* were contacted as were herbaria located in areas where *V. dubia* has been reported to occur.

Results and Discussion

Our disease surveys did not reveal any pathogens of *V. dubia* in the PNW. Globally, there are only two records of fungal pathogens of *V. dubia*. Both fungal pathogens are from Europe within *V. dubia*'s native range: *Septoria ventenatae* reported in Romania and *Tilletia fusca* in Germany (Farr and Rossman 2017). In addition *V. dubia* has been identified as a host for the *barley yellow dwarf virus* but without any expression of symptoms due to BYDV (Ingwell and Bosque-Pérez 2015).

Classical biological control of *V. dubia* might be attempted should a pathogen be found that limits plant abundance in its native range. Pathogens could contribute to its lack of abundance as suggested by the listing of *V. dubia* as rare or endangered in some areas of Europe. For example, it is rare in the following areas: between Coldirodi and San Romolo, Italy (Bicknell 1896), Poland (Frey and Paszko 1998), Codru-Moma Mountains in Romania (Merce et al. 2007), and Andalusia, Spain (MariateVizoso, personal communication). *Ventenata dubia* is on a red list and is an endangered species in the state of Hesse in Germany (Uebeler et al. 2008). As well it is an endangered species in Slovakia (P Eliášjun and Viera Feráková, personal communication, Slovakia; Turis et al. 2014; Dúbravková and Jaroslav 2012), In the Czech Republic, it is extremely rare and critically threatened (Kaplan Zdeněk, personal communication; Danihelka et al. 2012), and it is critically endangered in the Nature Park Papuk, Slavonia, eastern Croatia (Pandža 2010).

Pathogens have been utilized for biological control. Plant pathogens are increasing in importance in biocontrol of invasive plants (Morin et al, 2006). Species of rusts are highly specific to the plant species that they infect (Kolmer et al. 2009). That specificity makes it unlikely that non-target plants would be affected. One example of a rust used as a biological control agent is *Puccinia chondrillina* for control of rush skeletonweed (*Chondrilla juncea*), introduced to both North America and Australia (Lee1986). Several fungi have been reported for *V. dubia* that include *Septoria ventenatae* in Romania (Radulescu et al. 1973), *Tilletia fusca* in Germany (Scholz and Scholz 1988),

and finally *Tilletia elizabethae* in Slovakia and *Tilletia ventenatae* from Turkey (Denchev and Denchev 2018). The literature has presented a small list and no rusts reported for *V. dubia*, yet a focused effort may yield a host-specific fungus useful for biological control.

Records of *V. dubia*, according to the Global Biodiversity Information Facility (GBIF) (Figure 1), were entirely from Europe; none were from North Africa. Since *V. dubia* was collected in Algeria by Cosson in mid-19thcentury, it was clear that the GBIF map was missing key records. We thus sought other records from North Africa. Cosson had found the species in the Djebel Herfah and Djebel Tougour Mountains of Algeria, and based his new name for this species (*Ventenata dubia* rather than *Avena dubia*) on these collections. We found a second record: from Mont de Bellezm from Algeria more than a century later (Quezel and Santa 1962). Recently, *V. dubia* was also reported from Morocco (Timothy Seipel, Montana State University, personal communication). Apart from the three records from Algeria and Morocco, we could find no other records from North Africa. In fact, Salima Benhouhou (École Nationale Supérieure Agronomique (ENSA), Algeria) stated that *V. dubia* is currently rare in Algeria (Salima Benhouhou, Algeria, personal communication). We found no evidence of *V. dubia* in other countries of North Africa: Egypt (Ashraf Tawfiek Soliman, Kamal Shaltout, and Ahmed Elkordy, Egypt, personal communication), Libya (Hossain et al. 1988) or Tunisia (Maire 1952; Mounir Mekki, Tunisia, personal communication). Records of *V. dubia* in Europe were quite common as the GBIF map suggested (Figure 1 and Table 1). *Ventenata dubia* has been found in the following European countries: Austria, Bulgaria (Dimitar Dimitrov, Bulgaria, personal communication), Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Macedonia (Renata Arsovska, Macedonia, personal communication), Moldova, Montenegro, Netherlands, Poland, Romania, Serbia (Vera Batanjski, Serbia, personal communication), Slovakia, Spain, Ukraine, United Kingdom, and Finland. *Ventenata dubia* was especially common in Spain, France, and Germany (Table 1). Records from SW Asia may indicate that parts of the following countries are also in the native range of *V. dubia*: Azerbaijan, Iran, Russia, and Turkey (Yusuf Ziya KOCABAS, Turkey, personal communication). There are no records of *V. dubia* in Middle East countries: Saudi Arabia (Yahya Sulaiman Masrahi, Jacob Thomas Pandalayil, Saudi Arabia, personal communication), Kuwait, Iraq, Syria and Palestine/Israel (Table 1).

Ventenata dubia appears to be in a state of pathogen release in the PNW of North America since we searched in vain for any diseased plants. European literature and personal communications from Algeria suggest that there may be pathogens that limit *V. dubia*. Search for potential biological control agents should focus on these areas where it has been frequently reported but also considered limited in its distribution.

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Literature Cited

Al Rawi A (1987) Flora of Kuwait. Compositae and Monocotyledonae.Volume 2. Kuwait University

Anthos (2015) Information System of the plants of Spain. Real Jardín Botánico, CSIC - Fundación Biodiversidad. Electronic resource at www.anthos.es. Accessed: August 29, 2015

Arabaci T, Yildiz B (2004) A floristical study on Poaceae spp. growing naturally in Malatya Province. Turkish Journal of Botany 28: 361-368

Bauer N (2012) Vegetation of Mt Badacsony. Studia botanica 43:103-28

Bicknell C (1896) Flora of Bordighera and San Remo, Pietro Gibelli. Pp 305

BioCASE Data Portal (2008) Biological Collection Access Service for Europe. http://search.biocase.org/europe/index. Accessed September 29, 2014

Bor N L (1968) Flora of Iraq, Gramineae. Volume 9. Ministry of Agriculture, Baghdad

Boulos L (1988) The weed flora of Kuwait. Kuwait University

Chandramohan S, Charudattan R (2001) Control of seven grasses with a mixture of three fungal pathogens with restricted host ranges. Biological Control 22(3):246-255.

Conti F, Bonacquisti S, Abbate G (2005) An annotated checklist of the Italian vascular flora. Palombi

Copping A (1987) Aliens and adventives. *Ventenata dubia* (Leers) FW Schultz in Grays Chalk Quarry, Essex, 1986. BSBI News 45: 24-25

Cosson E, de Maisonneuve, M C D (1854). Introduction à la Flore d'Algérie: phanérogamie: groupe des Glumacées. Pp 103-105

Danihelka J, Chrtek Jr J, Kaplan Z (2012) Checklist of vascular plants of the Czech Republic. Preslia 84(3):647-811

Davis P (1985) Flora of Turkey and the east Aegean islands. Volume1-9. Edinburgh Univ. Pres. Edinburgh

Denchev, T. T. and C.M. Denchev. (2018) Two new smut fungi on *Ventenata* (Poaceae): *Tilletia elizabethae* from Slovakia and *T. ventenatae* from Turkey. Wildenowia 48(2):177-184

Didukh Y, Romo A, Boratyñski, A (2004) On five rare vascular plant species reported from Crimea, Ukraine. Willdenowia 34: 407-410

DiTomaso J, Kyser G, Oneto S, Wilson R, Orloff S, Anderson L, Wright S, Roncoroni J, Miller T, Prather T, Ransom C, Beck K, Duncan C, Wilson K, Mann J (2013) *Ventenata dubia* (Leers) Coss. & Dur.: North African wiregrass. In: Weed control in natural areas in the western United States. Davis, CA: University of California, Weed Research and Information Center: 417-418

Dúbravková D, Jaroslav K (2012) Acidophilous dry grasslands on the quartzite bedrock in western Slovakia. Hacquetia 11: 249-269

Duran A, Dural H (2003) *Genistavuralii* (Fabaceae), a new species from Turkey. In Annales Botanici Fennici. Finnish Zoological and Botanical Publishing Board 40:113-116

Edinburgh (2016) Royal Botanic Garden Edinburgh Herbarium (E). www.rbge.org.uk. Accessed: 20 March, 2016

Farr D F, Rossman A Y Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases. Accessed: October 20, 2017

Frey L, Paszko B (1998) *Ventenata dubia* [Poaceae] - a rare ephemerophyte in Poland. Fragm. Flor. Geobot. Polonica 5: 15-20

GBIF Austria (2015) Global Biodiversity Information Facility – Austria. http://www.gbif.at. Accessed: 17 February 2015

GBIF.org (2015) Global Biodiversity Information Facility. http://gbif.org . Accessed: 17 February 2015

GBIF Sweden (2016) Global Biodiversity Information Facility- Swedish Museum of Natural History. http://www.gbif.se. Accessed: 17 February 2016

Ghahremaninejad F, Bidarlord M, Atar, F (2012) Floristic study of steppe parts of Lissar protected area (N Iran). Botanical Journal of Iran 13: 164-188

Herbarium WU (2015) University of Vienna, Institute for Botany. http://herbarium.univie.ac.at . Accessed: 17 February 2015

Herbarium of the University and ETH Zurich (2015) Herbaria of the University and ETH Zürich (Z+ZT).http://www.herbarien.uzh.ch/static/database/details_en.php?&spTypFlg=&spBarCod=Z-000069025&spHer=Z. Accessed: 17 February 2015

Hossain A E, Siddiqi M, Sherif A, El-Gadi A (1988) Flora of Libya (No. 145). Al Faateh University, Faculty of Science, Department of Botany

Ingwell L L, Bosque-Pérez N A (2015) The invasive weed *Ventenata dubia* is a host of *Barley yellow dwarf virus* with implications for an endangered grassland habitat. Weed Research, 55: 62-70

Keeping M, Kvedaras, O (2008) Silicon as a plant defence against insect herbivory: response to Massey, Ennos and Hartley. Journal of Animal Ecology 77(3):631-633

Kolmer J A, Ordonez M E, Groth, J V (2009) The rust fungi. Encyclopedia of Life Sciences (ELS). Wiley, Chichester

Komarov V L (1963) Flora of the USSR. Volume 18. National Science Foundation, Washington, DC, and the Smithsonian Institution

Lee Gary A (1986) Integrated control of rush skeletonweed (*Chondrilla juncea*) in the western US. Weed Science 34: 2-6

Licim A, Kocabaş Y Z, Başaran H (2008) Şekeroba Çevresinin (Kahramanmaraş) Floristik Yönden İncelenmesi. KSÜ Fen ve Mühendislik Dergisi.11:13-22

López G B, Romero M C, Cabezudo B, Torres C M, Salazar C (Eds.) (2011) Claves de la flora vascular de Andalucía oriental. Volume 2. Universidad Almería. Pp 334–335

Mackey A M (2014) Developing a decision support tool for ventenata (*Ventenata dubia*) integrated pest management in the inland northwest. MS Thesis. Moscow, ID: University of Idaho. 11 p

Maire R (1952) Flore de l'Afrique du Nord (Maroc, Algérie, Tunisie, Tripolitaine, Cyrénaïque et Sahara). Volume 5. Paul Lechevaller. Paris

Mandaville J (1990) Flora of Eastern Saudi Arabia. London: Kegan Paul International

Mareković S, Hršak V, Jelavska SD, Nikolić T, Plazibat M (2009) The grasses (Poaceae) of Medvednica Nature Park, Croatia 18: 135–154

Merce O, Oarcea Z, Adrian S (2007) Studiu de fundamentare pentru constituirea Parcului Natural Codru Moma (Foundation study for the establishment of Codru Moma Natural Park). Revista de Silvicultură şi Cinegetică. Anul XII: 23:15

Migahid A M (1978) Flora of Saudi Arabia. Volume 2. Riyadh: Riyadh University Publications

MNHN - Museum national d'Histoire naturelle (2017) The vascular plants collection (P) at the Herbarium of the Muséum national d'HistoireNaturelle (MNHN - Paris). http://www.mnhn.fr/ Accessed: 17 September 2017

Morales R (2003) Catálogo de plantas vasculares de la Comunidad de Madrid (España). Botanica Complutensis 27:31-70

Morin, L, Evans, K J and Sheppard, AW (2006) Selection of pathogen agents in weed biological control: critical issues and peculiarities in relation to arthropod agents. Australian Journal of Entomology 45(4):349-365

Naturalis (2015) Naturalis Biodiversity Center- Netherlands. http://www.naturalis.nl/en

Accessed: 02 November 2017

Northam F E, Callihan R H (1994) New weedy grasses associated with downy brome. General Technical Report–Intermountain Research Station INT-313. USDA Forest Service, Ogden 211–212

Ocak A, Ture C, Senmerdan A B, Bocuk H (2009) An investigation of diversity, distribution and monitoring on Poaceae (Gramineae) species growing naturally in Bilecik province at the

intersection of three Phytogeographical regions (Northwest Anatolia-Turkey). Pakistan Journal of Botany 41:1091-1106

Pandža M (2010) Flora parka prirode papuk (Slavonija, Hrvatska). Šumarski list 134: 25-43

Panjković B, Perić R, Stojšić V, Batanjski V. (2012). New data on the distribution of Ranunculus polyphyllus Waldst. & Kit. ex Willd. in Serbia. Archives of Biological Sciences. 64; 715-720

Pavlova D, Kozuharova E, Dimitrov D (2003) A floristic catalogue of serpentine areas in the eastern Rhodope mountains (Bulgaria). Polish Botanical Journal 48: 21-41

Pheloung P, Williams P, Halloy S (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. Journal of Environmental Management 57:239-251

Pignatti S (1982) Flora d'Italia. Volume 3. Bologna: Edagricole

Post G E (1932) Flora of Syria, Palestine, and Sinai. Volume 1. РиполКлассик

Quezel P, Santa S (1962) Nouvelle flore de l'Algerie et des regions desertiques meridionales. Editions du Centre Nationale de Recherche Scientifique, Paris

Radulescu, E., A. Negru and E. Docea. 1973. Septoriozele din Romania. Ed. Acad Rep. Soc. Romania Bucarest 325 pp

Sârbu A, SârbuI, Mihai D (2009) Unprotected grassland areas from Dobrogea, of high botanical value. Contributii Botanice 44: 67-75

Scholz, H. and I. Scholz (1988) Die Brandpilze Deutschlands (Ustilaginales). Englera 8:1-691

Selvi F (2010) A critical checklist of the vascular flora of Tuscan Maremma (Grosseto province, Italy). Fl. Medit 20: 47-139

Trujillo E (2005) History and successes of plant pathogens for biological control of introduced weeds in Hawaii. Biological Control 33(1):113-122

Turis P, Kliment J, Ferakova V, Dite D, Elias P, Hrivnák R, Košťál J, Šuvada R, Mráz P, Bernátová D (2014) Red List of vascular plants of the Carpathian part of Slovakia. Thaiszia Journal of Botany 24(1):35-87

Tzvelev N (1976) Zlaki SSSR (grasses of the Soviet Union). Leningrad: Academiya Nauk SSSR. Pp176-189

Uebeler M, Ehmke W, Nawrath S, König A, Wittig R (2008) Ergebnisse der Floristischen Kartierung im Hohen Taunus. Geobot Kolloq 21: 23-42

Wallace J, Pavek P, Prather T (2015) Ecological characteristics of *Ventenata dubia* in the Intermountain Pacific Northwest. Invasive Plant Science and Management 8(1):57-71

Table 2.1 Summary of *V. dubia* locations.

Country	Earliest record	Record Category			Inst.[4]	References
		Lit.[1]	Spec.[2]	PC[3]		
Algeria	1854	2	-	rare	-	(Cosson and Maisonneuve 1854; Quezel and Santa 1962)
Austria	1859	-	33	-	A	(Herbarium WU; BioCASE; GBIF Austria; Naturalis)
Azerbaijan	1976	1	-	-	-	(Tzvelev 1976)
Belgium	1853	-	4	-	B	(Naturalis; BioCASE)
Bulgaria	1999	1	10	present	C	(BioCASE; Edinburgh; GBIF; Herbarium WU; Pavlova et al. 2003)
Croatia	2009	2	-	-	-	(Pandža 2010; Mareković et al. 2009)
Czech Republic	1906	1	7	rare	D	(Danihelka et al. 2012, GBIF; BioCASE; Naturalis)
Egypt	-	-	-	absent	-	-
Finland	2002	-	1	-	F	(BioCASE)
France	1830	-	66	-	H	(BioCASE; GBIF; MNHN; Naturalis)
Germany	1854	1	54	-	I	(BioCASE; GBIF – Austria; Herbarium WU; Herbaria of the University and ETH Zürich; GBIF; Naturalis)
Greece	1989	-	14	-	J	(BioCASE; GBIF; Herbarium WU; GBIF-Sweden)
Hungary	1899	2	14	-	K	(Herbarium WU; GBIF; BioCASE; Naturalis; Bauer 2012; GBIF - Austria)
Iran	2007	-	2	-	X	(Herbarium WU; Ghahremaninejad et al. 2012)
Iraq	1968	absent	-	-	-	(Bor 1968)

Italy	1919	11	5	-	L	(Bicknell 1896; Selvi 2010; Pignatti 1982; Conti et al. 2005; Herbarium WU; BioCASE)
Kuwait	1987	absent	-	-	-	(Al Rawi 1987; Boulos 1988)
Libya	1988	absent	-	-	-	(Hossain et al. 1988)
Macedonia	2015	-	-	present	-	-
Morocco	2018	absent	-	present	-	(Maire 1952)
Moldova	1966	1	1	-	M	(Tzvelev 1976; Naturalis)
Montenegro	2002	-	1	-	-	(Herbarium WU)
Netherlands	1936	-	1	-	N	(Naturalis)
Palestine/Israel	1932	absent	-	-	-	(Post 1932)
Poland	1998	1	-	-	-	(Frey and Paszko 1998)
Romania	1913	2	4	-	O	(Naturalis; BioCASE; Merce et al. 2007; Sârbu et al. 2009)
Russia	1963	2	2	-	P	(Tzvelev 1976; Komarov 1963; Olga Demina, and Alexey Shipunov, PC)
Saudi Arabia	1978	absent	-	absent	-	(Migahid 1978; Mandaville 1990)
Serbia	2012	1	-	present	-	(Panjković et al. 2012)
Slovakia	1906	1	8	rare	Q	(Naturalis; BioCASE; GBIF; Turis et al. 2014)
Spain	1861	41	91	-	R	(BioCASE; Anthos; Morales 2003; López et al. 2011; BioCASE; GBIF; Anthos)
Syria	-	absent	-	-	-	(Post 1932)
Tunisia	-	absent	-	absent	-	(Maire 1952)
Turkey	1856	13	16	present	S	(Edinburgh; GBIF; Naturalis, Davis 1965; Arabaci and Yildiz 2004; Licim et al. 2008; Duran and Dural 2003; Ocak et al. 2009)

Ukraine	1901	2	33	-	T	(Didukh et al. 2004; Tzvelev 1976; GBIF; Herbarium WU; BioCASE)
United Kingdom	1986	1	-	-	-	(Copping 1987)

Literature[1]: number of books and journals. Specimen[2]: number of herbarium specimens and /or human observation without collect samples, more details: **Austria (**33 herbarium specimens), **Belgium** (4 herbarium specimens), **Bulgaria** (10 herbarium specimens), **Czech Republic** (7 herbarium specimens), **Finland** (1 herbarium specimen), **France (**13 herbarium specimens and 53 human records of human observation without collect samples), **Germany** (49 herbarium specimens and 5 human records of human observation without collect samples), **Greece** (14 herbarium specimens), **Hungary** (14 herbarium specimens), **Iran** (2 herbarium specimens), **Italy** (5 herbarium specimens), **Macedonia** (1 human record of human observation without collect sample), **Moldova** (1 herbarium specimen), **Montenegro** (1 herbarium specimen), **Netherlands** (1 herbarium specimens), **Romania** (4 herbarium specimens), **Russia** (2 human records of human observation without collect samples**), Slovakia** (8 herbarium specimens), **Spain** (39 herbarium specimens and 52 human records of human observation without collect samples), **Turkey** (16 herbarium specimens), **Ukraine** (33 herbarium specimens). Personal communication (PC)[3]: personal communications with botanists and herbarium curators by email. Institution[4]: The locations of the records, more details: **A** (Herbarium GZU, Vascular Plant Herbarium Oslo, Naturalis Biodiversity Center, Herbarium WU, Biologiezentrum Linz), **B** (Naturalis Biodiversity Center, Museum National d'Histoire naturelle), **C** (Herbarium Berolinense, Universidad de Sevilla, Real Jardín Botánico, Naturhistorisches Museum Wien, Universidad de Santiago de Compostela, Vascular Plant Herbarium, , Museo de Ciencias Naturales de Alava, Universidad de Salamanca), **D** (Vascular Plant Herbarium, Biological Museum, Oskarshamn, Universitat de València, Institute of Botany, Academy of Sciences of the Czech Republic, Museum of Evolution, Naturalis Biodiversity Center), **F** (FMNH), **H** (Conservatoire botanique national du Bassin parisien, Museum national d'Histoire naturelle, Naturalis Biodiversity Center, Universidad de Salamanca, Universitat de València, Conservatoire Botanique National Alpin, Royal Botanic Garden Edinburgh), **I** ((Naturalis, , Natural History Museum, Herbarium, Naturhistorisches Museum Wien, Herbaria of the University and ETH Zürich, Senckenberg Gesellschaft für Naturforschung: Senckenberg Forschungsinstitut und Naturmuseum, Vascular Plant Herbarium Oslo, Flora exsiccata Bavarica, Biological Museum, Oskarshamn, Digitization of plant specimens from Rhoen and Vogelsberg, Museum of Natural History Mainz, Herbarium Berolinense, Inatura Dornbirn, Karl-Franzens-Universität Graz, Naturalis Biodiversity Center), **J** (Lund University, Herbarium Berolinense), **K** (Herbarium Karl-Franzens-Universität Graz, Vascular Plant Herbarium, Real Jardín Botánico, Naturalis Biodiversity Center, Museo Nacional de Historia Natural, Herbarium Universalmuseum Joanneum), **L** (Naturhistorisches Museum Wien, Museo Nacional de Historia Natural, **M** (Museo Nacional de Historia Natural), **N** (Herbarium Jansen and Wachter), **O** (Naturalis Biodiversity Center), **P** (Southern Federal University), **Q** (Herbarium Naturhistorisches Museum Wien, Vascular Plant Herbarium Oslo, National Museum of Natural History, Naturalis Biodiversity Center), **R** (Institut Menorquí d'Estudis, Conservatoire et Jardin botaniques de la Ville de Genève, Universidad de Salamanca, Universitat de València, Universidad de Santiago de Compostela, Real Jardín Botánico, Universidad de León, Museo de Ciencias Naturales de Alava, Universidad de Sevilla, Naturalis Biodiversity Center; Universidad de Navarra), **S** (Naturalis Biodiversity Center, Royal Botanic Garden Edinburgh Herbarium, National Museum of Natural History, Moscow Digital Herbarium - Moscow State University), **T** (Moscow State University Herbarium, Biological Museum herbarium, Oskarshamn, Herbarium of Universität Wien),**X** (Naturhistorisches Museum Wien). Within a categorya dash (–) means no information for presence or absence was found.

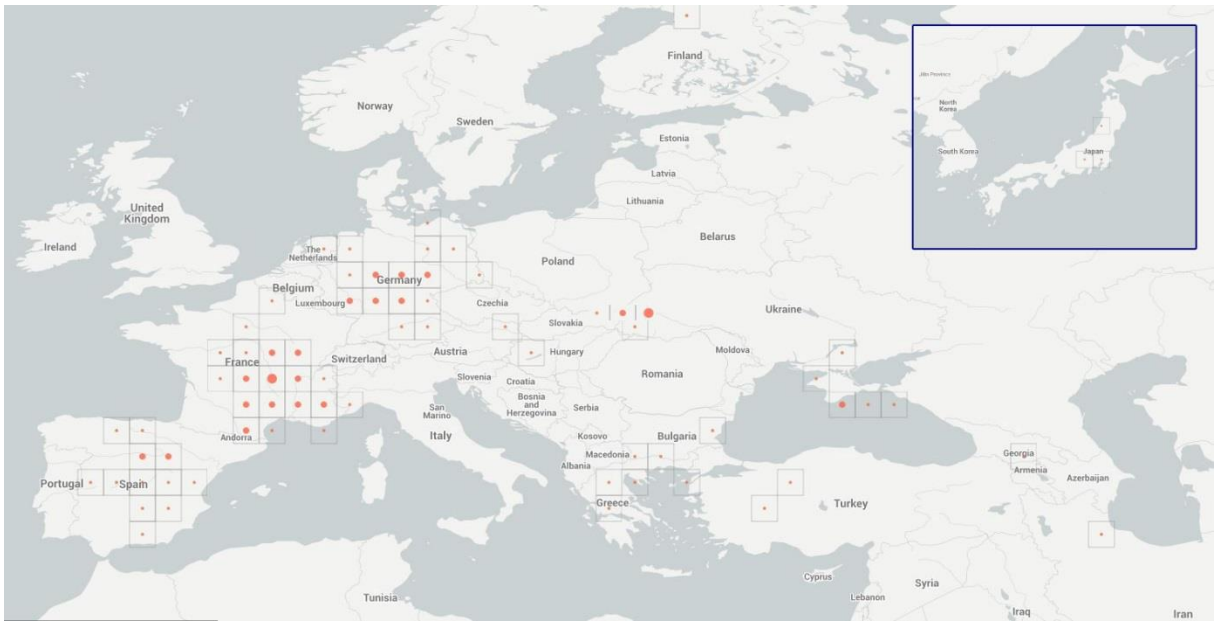

Figure 2.1. The eastern hemisphere (a) locations of V. dubia range from Spain to the Caspian Sea and from Norway to Finland. Two locations are in Japan. The three symbols are circles denoting number of records with small circle less than 10 records, medium circle 10 to 20 records and large circle more than 20 records. The western hemisphere (b) the new range locations span British Columbia, Canada to California, United States and from Washington, United states to Nova Scotia, Canada (GBIF.org).

Table 2.2 locations of *V. dubia*.

Country	locality /province or state	Barcode/ID	Collector	Year	Basis of record	longitude latitude	Institution	References
Algeria	Djebel Herfah and Djebel Tougour Mountains	-	-	1854	Literature	-	-	(Cosson and Maisonneuve 1854)
	Monts de Bellezma (Nord-Est de l'Algérie)	-	-	1962	Literature	5°59'30.99"E 35°32'24.00"N	-	(Quézel and Santa 1962)
	-	-	-	-	Personal communication	-	-	Salima Benhouhou, PC
Austria	Vienna	GZU 110627	Ployel, J.	1859	Specimen	16°22'25.71"E 48°12'29.45"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Vienna	81960	Müllner, M.F.	1878	Specimen	16°22'25.71"E 48°12'29.45"N	Herbarium GZU	(BioCASE)
	Eisenberg	81957	Piers, M.	1889	Specimen	11°53'33.53"E 50°58'6.98"N	GZU	(BioCASE)
	Söchau	80848	Sabransky, H.	1913	Specimen	16° 0'56.39"E 47° 1'55.83"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Söchau	81943	Sabransky, H	1913	Specimen	16° 0'56.39"E 47° 1'55.83"N	Herbarium GZU	(BioCASE)
	Steiermark	GZU	Sabransky, H	1913	Specimen	14°28'11.96"E 47°21'33.65"N	-	(Herbarium WU)
	Vienna	GZU	Rechinger, K	1923	Specimen	16°22'25.71"E 48°12'29.45"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Lainz	81955	Rechinger, K	1923	Specimen	16°16'31.99"E 48°10'24.01"N	Vascular Plant Herbarium Oslo	(BioCASE)
	Lainzer Tiergarten, Vienna	2134177	Rechinger, K	1923	Specimen	16°22'25.71"E 48°12'29.45"N	Vascular Plant Herbarium Oslo	(BioCASE)
	Lackendorf	81944	Melzer, H.	1960	Specimen	16°30'14.74"E 47°35'23.99"N	Herbarium GZU	(BioCASE)

	Between Lackendorf and Unterfrauenhaid	81946	Melzer, H.	1960	Specimen	16°30'14.74"E 47°35'23.99"N	Herbarium GZU	(BioCASE)
	Burgenland	GZU 044461	Melzer, H	1960	Specimen	16°16'7.97"E 47° 9'13.38"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Burgenland	GZU 044440	Melzer, H	1960	Specimen	16°16'7.97"E 47° 9'13.38"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Burgenland	GZU 049613	Melzer, H	1961	Specimen	16°16'7.97"E 47° 9'13.38"N	Herbarium GZU	(Herbarium WU)
	between Lackendorf and Unterfrauenhaid	81947	Melzer, H.	1961	Specimen	16°30'14.74"E 47°35'23.99"N	Herbarium GZU	(BioCASE)
	Burgenland	100285768	Metlesics Hans	1961	Specimen	16.4842 47.5906	Herbarium GZU	(GBIF - Austria)
	Neudorf	81951	Melzer, H.	1963	Specimen	16°26'51.55"E 47°34'57.18"N	Herbarium GZU	(BioCASE)
	Burgenland	GZU 062150	Melzer, H	1963	Specimen	16°16'7.97"E 47° 9'13.38"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Frauenhofen	81953	Melzer, H.	1965	Specimen	15°35'49.15"E 48°41'10.18"N	Herbarium GZU	(BioCASE)
	Niederösterreich	GZU 087898	Melzer, H	1965	Specimen	15°48'17.84"E 48° 6'29.08"N	Herbarium GZU	(Herbarium WU; BioCASE)
	Burgenland	U.1520171	Traxler, G	1965	Specimen	16°16'7.97"E 47° 9'13.38"N	Naturalis Biodiversity Center	(Naturalis)
	Burgenland	U.1520176	Kramer KU; Westra LYT	1966	Specimen	16°16'7.97"E 47° 9'13.38"N	Naturalis Biodiversity Center	(BioCASE)
	Deutschkreuz	2014-0016648	Neumann-Spallart	-	Specimen	16.623442 47.601631	Herbarium W. Natural History Museum, Vienna	(BioCASE)
	-	865675167	Torre Pando	-	Unknown	14°33'0.26"E 47°30'58.43"N	Biologiezentrum Linz	(GBIF - Austria)
	-	862679165	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679154	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)

	-	862679155	Aldasoro, J. J	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679161	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679163	Ricardus (Frère)	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679164	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679157	Dupuy	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679158	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679159	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
	-	862679160	-	-	Specimen	14°33'0.26"E 47°30'58.43"N	Herbarium GZU	(GBIF - Austria)
Azerbajan	Talysh	-	-	-	Literature	48°18'40.00"E 38°41'47.00"N	-	(Tzvelev 1976)
Bosnia and Herzegovina	-	40837	Pohl Henri	1990	Unknown	43.915886 17.679076	-	(BioCASE)
Belgium	-	L.1351359	Crépin, F	1853	Specimen	4°28'23.89"E 50°30'11.45"N	(Naturalis Biodiversity Center)	(Naturalis)
	Ardennes	WAG.1566757	-	1856	Specimen	4.628505 49.762464	Naturalis Biodiversity Center	(Naturalis)
	-	FABR04517	A. Irvine	1861	Specimen	4°28'23.89"E 50°30'11.45"N	Museum national d'Histoire naturelle (MNHN)	(BioCASE)
	-	L.1351370	Crépin, F	1861	Specimen	4°28'23.89"E 50°30'11.45"N	(Naturalis Biodiversity Center)	(Naturalis)
	-	L.1351370	Jansen, P	1936	Unknown	4°28'23.89"E 50°30'11.45"N	Naturalis	(Naturalis)
	-	L.1351359	Wirtgen, PW	-	Unknown	4°28'23.89"E 50°30'11.45"N	(Naturalis Biodiversity Center)	(Naturalis)

Bulgaria	East Stara Planina Mt	B 10 0417270	Th. Raus, F. Pina Gata	1999	Specimen	27.3447 42.4011	Herbarium Berolinense	(BioCASE,Edinburgh, Botanical Museum Berlin,
	Kabelshkovo	281317-1	F.J. Pina & T. Raus	1999	Specimen	27.57 42.66	SEV	And GBIF)
	Fargovo village, Valkosel	108930	Aldasoro, J.J..	2004	Specimen	23°59'11.25"E 41°31'43.86"N	MA	(BioCASE)
	Fargovo village, Valkosel	721300-1	Aldasoro	2004	Specimen	24.0012 41.5707	VAL	(BioCASE)
	Fargovo village, Valkosel	W 2007-0024500	Aldasoro, J.J. Hortus	2004	Specimen	41°34'24"N 24°0'7"E	Herbarium W	(Herbarium WU)
	Rhodopes Occidentales. Carretera entre Koprivlen y Gotse Delchev	728335-1	C. Navarro & al.	2004	Specimen	23.8863 41.5702	SANT	(BioCASE)
	Rhodopes	2134163	C. Aedo, I. Aizpuru, M. L. Alarcón, J. J. Aldasoro, S. Castroviejo, A. G.-Valdecasas, J. Güemes, A. Herrero, C. Navarro, J. Pedrol, A. Prunell, A. Quintanar, E. Rico, V. Rodríguez Gracia, V. Vladimirov	2004	Specimen	25.568108 41.079568	Vascular Plant Herbarium, Oslo (O)	(BioCASE)
	-	80514-1	C.Navarro et all	2004	Specimen	25°29'8.99"E 42°44'1.98"N	VIT	(BioCASE)
	-	-	-	2015	Personal communication	-	Sofia University ‘St. Kliment Ohridski	Prof. Dimitar Dimitrov

	-	862392070	-	-	Specimen	25°29'8.99"E 42°44'1.98"N	Herbarium W	(BioCASE - Austria)
	Fargovo village, Valkosel	137786-1	C. Aedo, A. Aizpuru, M.L. Alarcón, J.J. Aldasoro, S. Castroviejo, A.G. Valdecasas, J. Güemes, A. Her	-	Specimen	41°34'24"N 24°0'7"E	SALA	(BioCASE)
	Zalti Chal village	-	-	-	Literature	25°58′E 41°19′N	-	(Pavlova et al. 2003)
Croatia	Nature park Papuk, Slavonia	-	-	-	Literature	17°39'59.78"E 45°30'2.87"N	-	(Pandža 2010)
	-	-	-	-	Literature	-	-	(Marekovi and Jelaska 2009)
Czech Republic	Znaim	2134165	J. Vetter	1906	Specimen	16° 3'15.36"E 48°51'21.28"N	Vascular Plant Herbarium, Oslo (O)	(BioCASE)
	Bohemia	OHN 67398	Stelzhamer, Joh.	1924	Specimen	14°25'58.35"E 50° 5'6.26"N	OHN	(BioCASE)
	Moravany	2134174	J. Hruby	1935	Specimen	16.576169 49.145560	Vascular Plant Herbarium, Oslo (O)	(BioCASE)
	Jevisovice	11942-1	V. Skalický	1985	Specimen	15.989927 48.987365	VAL	(BioCASE)
	-	-	-	2012	Literature	15°28'22.66"E 49°49'2.97"N	-	(Danihelka et al. 2012)
	-	-	-	2015	Personal communication	-	Institute of Botany, Academy of Sciences of the Czech Republic	Dr. Zdenek Kaplan
	Bohemia	V-172896	Burser, Joachim	-	Specimen	15°28'22.66"E 49°49'2.97"N	UPS	(GBIF; BioCASE)
	Znaim	335009	-	-	Specimen	16° 3'15.36"E 48°51'21.28"N	FML	(BioCASE)
	Znaim.	L.1351360	Oborny, A	-	Specimen	15°28'22.66"E 49°49'2.97"N	(Naturalis Biodiversity Center)	(Naturalis)

	Bohemia	V-172896	Burser, Joachim	-	Unknown	14°25'58.35"E 50° 5'6.26"N	The Museum of Evolution Herbarium (UPS)	(BioCASE)
Egypt	-	-	-	-	Personal communication	-	-	(Ashraf Tawfiek Soliman, Kamal Shaltout, and Ahmed Elkordy)
Finland	Oulu	257201801	Kalleinen, Lassi	2002	Specimen	25.4277 65.0525	FMNH	(BioCASE)
France	Coulanges-lËs-Nevers	1932.1.7.1395	Saul, Casimir	1830	Specimen	3.1875 47.005833	BOUM	(BioCASE)
	Saint-Éloi	19991126019838fel	-	1832	Observation	3.99465 46.2852	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Éloi	20060119160428Esc	Boreau Alexandre (Aucun)	1832	Observation	2°41'32.40"E 50°21'4.90"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Cheverny	19970130151309kel	Monin	1833	Observation	1°27'28.82"E 47°30'0.76"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Autun	20000229145738mot	-	1849	Observation	4.28729 47.0029	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Forgeot	20000229150109mot	-	1849	Observation	4.00986 46.721	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Issy-l'Évêque	20000229151107mot	Carion J.-E.	1849	Observation	4.67665 46.4318	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Cluny	20000229151141mot	-	1849	Observation	3.20441 47.0174	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Coulanges-lès-Nevers	20000229151321mot	-	1849	Observation	3.09272 46.9898	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Marzy	20000229152049mot	-	1849	Observation	1.89962 47.8805	Conservatoire botanique national du Bassin parisien	(BioCASE)

	Orléans	2000022915 4112mot	Dubouché	1849	Observation	2.32348 47.8135	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Benoît-sur-Loire	2000022915 4241mot	Maille	1849	Observation	1.33984 47.2528	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Aignan	2000022915 4325mot	-	1849	Observation	3.62621 46.7645	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Autun	2000022914 5738mot	-	1849	Observation	4.28729 47.0029	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Forgeot	2000022915 0109mot	-	1849	Observation	4.00986 46.721	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Issy-l'Évêque	2000022915 1107mot	Carion J.-E.	1849	Observation	4.67665 46.4318	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Aignan	1997013015 1519kel	Charlot	1849	Observation	1.45038 47.4843	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Loches	1999080310 3256por	-	1852	Observation	1.05074 47.1403	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Autun	1998030614 4036por	-	1859	Observation	4.30719 46.9462	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Issy-l'Évêque	1998030614 4548por	-	1859	Observation	3°58'26.41"E 46°42'28.49"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Cluny	1998030614 4640por	-	1859	Observation	4.30719 46.9462	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Bourgueil	-	-	1874	Unknown	0.168611 47.282487	-	(GBIF)
	Clermont-Ferrand	P00686143	Héribaud	1879	Specimen	1.89962 47.8805	Museum national d'Histoire naturelle (MNHN)	(BioCASE)
	Ferrand	-	-	1879	Specimen	3.087025 45.777222	Museum national d'Histoire naturelle (MNHN)	(GBIF)

	Normier	19981109152702joa	Fautrey F	1883	Observation	4.41261 47.3818	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Normier	19960514110955tru	Fautrey F.	1883	Observation	4.77333 45.7389	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Clamerey	19981109153312joa	Fautrey F.	1884	Observation	4°25'36.16"E 47°23'15.14"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Clamerey	20030507101750Pis	Fautrey	1885	Observation	4°25'36.19"E 47°23'15.26"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Pont-et-Massène	20030507101832Pis	Ricardus (Frère)	1885	Observation	2.42971 44.9227	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Cuffy	19960625150752sab	-	1887	Observation	3.21437 46.99	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Orléans	19970725154843chu	Tristan J.(de)	1890	Observation	4.03709 46.2759	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Cyr-en-Val	19970725155349chu	Tristan J.(de)	1890	Observation	4.30719 46.9462	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Hilaire-Fontaine	19970113151827tru	Gagnepain F., Gillot X.	1895	Observation	4.41261 47.3818	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Hilaire-Fontaine	20000203113331por	Gagnepain F.	1900	Observation	3.62621 46.7645	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Hilaire-Fontaine	20000203113729por	Gagnepain F.	1900	Observation	3°37'34.08"E 46°45'54.47"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Marcigny	20020315110858Per	-	1906	Observation	3.04328 46.9596	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Chambilly	20020325104244Per	-	1906	Observation	4.3152 46.7714	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Bourg-le-Comte	20020314101456Per	-	1906	Observation	3°59'16.72"E 46°18'33.08"N	Conservatoire botanique national du Bassin parisien	(BioCASE)

	Marcigny	-	-	1906	Observation	3.99465 46.2852	Conservatoire botanique national du Bassin parisien	(GBIF)
	Barnay	1998061514 4425mot	Gillot Xavier	1907	Observation	4°20'2.71"E 47° 5'16.67"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Marcigny	2000040517 2206por	-	1907	Observation	3.99465 46.2852	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Chambilly	2000040517 2243por	-	1907	Observation	3.98295 46.3135	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Bourg-le-Comte	2000040517 2313por	-	1907	Observation	3°59'16.72"E 46°18'33.08"N	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Bourg-le-Comte	2000040517 2313por	-	1907	Observation	2.4251 45.3729	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Ferrière-sur-Beaulieu	1999080310 3815por	Dupuy	1908	Observation	2.80836 45.3742	Conservatoire botanique national du Bassin parisien	(BioCASE)
	Saint-Laurent-d'Andenay	1998061515 0609mot	Gandoger	1923	Specimen	4.51182 46.7313	Conservatoire botanique national du Bassin parisien	(BioCASE)
	-	P00686144	C. d' Alleizette	1932	Specimen	2°12'50.46"E 46°13'41.46"N	Museum national d'Histoire naturelle (MNHN)	(BioCASE)
	Saignes (Cantal)	P00686142	H. Bouby	1946	Specimen	3.62621 46.7645	Museum national d'Histoire naturelle (MNHN)	(BioCASE)
	-	L.1351369	Nannenga-Bremekamp, NE	1969	Specimen	2°12'50.46"E 46°13'41.46"N	(Naturalis Biodiversity Center)	(Naturalis)
	Francia	79627-1	C. Bernard	1984	Specimen	2°12'50.46"E 46°13'41.46"N	SALA	(BioCASE)
	Montlaur (dép. Aveyron), nonloin de Verrières	11940-1	C. Bernard & G. Fabre	1984	Specimen	2.557873 43.128738	VAL	(BioCASE)
	Charmoy	2002051510 0352rog	Laroche M., Minois J., SHNC	1990	Observation	25.4277 65.0525	Conservatoire botanique national du Bassin parisien	(BioCASE)

	Le Poët	GAP003907	E. Chas	1991	Specimen	5.897282 44.292116	Herbarium specimens of CBNA (GAP)	(BioCASE)
	Le Poët	-	-	1991	Specimen	5.897282 44.292116	Museum national d'Histoire naturelle (MNHN)	(MNHN)
	Francheville	0E6B72F-AA67-4075-8ADB-EC3D7464E4A9	Jordan Alexis	-	Specimen	4.03709 46.2759	TLMF	(BioCASE)
	-	fr-dep48-nn70721	-	-	Observation	4.4321 47.3659	TELA BOTANICA	(BioCASE)
	-	E00314848	Bentham, G.	-	Specimen	3.21437 46.99	E	(BioCASE)
	-	140502	-	-	Unknown	3.06347 45.0142	MNHN-SPN	(BioCASE)
	-	214223	-	-	Unknown	2°12'50.46"E 46°13'41.46"N	MNHN-SPN	(BioCASE)
	-	fr-dep84-nn70721	Wirtgen P	-	Observation	2°12'50.46"E 46°13'41.46"N	(Naturalis Biodiversity Center)	(BioCASE)
	-	fr-dep03-nn70721	-	-	Observation	0.975755 47.127	TELA BOTANICA	(BioCASE)
	-	60275	-	-	Unknown	3.98295 46.3135	MNHN-SPN	(BioCASE)
	-	fr-dep39-nn70721	-	-	Observation	2°12'50.46"E 46°13'41.46"N	TELA BOTANICA	(BioCASE)
	-	fr-dep07-nn70721	-	-	Observation	2°12'50.46"E 46°13'41.46"N	TELA BOTANICA	(BioCASE)
	-	fr-dep06-nn70721	-	-	Observation	1.33984 47.2528	TELA BOTANICA	(BioCASE)
	-	64984	-	-	Unknown	1.97431 47.8206	MNHN-SPN	(BioCASE)
	-	fr-dep12-nn70721	-	-	Observation	4.67665 46.4318	TELA BOTANICA	(BioCASE)
	-	fr-dep01-nn70721	-	-	Observation	2°12'50.46"E 46°13'41.46"N	TELA BOTANICA	(BioCASE)

	-	fr-dep04-nn70721	-	-	Observation	4.30719 46.9462	TELA BOTANICA	(BioCASE)
	-	fr-dep58-nn70721	-	-	Observation	2°12'50.46"E 46°13'41.46"N	TELA BOTANICA	(BioCASE)
	-	fr-dep71-nn70721	-	-	Observation	2°12'50.46"E 46°13'41.46"N	TELA BOTANICA	(BioCASE)
Germany	Koblenz	WAG.1566752	Wirtgen	1853	Specimen	7.588996 50.356943	WAG	(Naturalis)
	Schwerin	491695	-	1854	Literature	11.4153 53.6485	EMAU	(BioCASE)
	-	BM001134034	Philipp Wilhelm Wirtgen	1859	Specimen	10.393647 51.106563	Natural History Museum (London) Collection Specimens (NHMUK)	(BioCASE)
	Rheinland-Pfalz	E187DF96-D931-43C5-882F-C9AA3862F58E	-	1860	Specimen	7.59948 50.3567	Inatura Dornbirn	(GBIF - Austria)
	Rheinland-Pfalz	W-Hackel 1916-0032038	Schultz, F.W. and Lingenfelder Cent.	1863	Specimen	7°18'32.26"E 50° 7'6.06"N	Herbarium W	Herbarium WU
	Wachenheim	265661	Schultz, F.W.& Lingenfelder Cent	1863	Specimen	8°10'54.47"E 49°26'25.99"N	Herbarium W	(BioCASE)
	Sangerhausen	Z-000069025	C. Lebing	1878	Specimen	11°17'58.22"E 51°28'21.15"N	Herbaria of the University and ETH Zürich	(Herbaria of the University and ETH Zürich)
	Enkheimer Wald-Frankfurt	FR-0090306	M. Dürer	1880	Specimen	8°46'0.00"E 50° 9'0.00"N	Herbarium Senckenbergianum (FR)	(BioCASE)
	Fechenheimer Waldes - Frankfurt	FR-0090307	-	1883	Specimen	8°46'44.12"E 50° 8'29.33"N	Herbarium Senckenbergianum	(BioCASE)
	-	468837	-	1884	Observation	11.4153 53.6485	EMAU	(BioCASE)
	Muschelkalk	2134178	G. Rost, Wislicenus	1899	Specimen	9.850180 49.656992	Vascular Plant Herbarium, Oslo	(GBIF; BioCASE)

							(O)	
	Segnitz	382	Bot. Ver. Würzburg	1899	Specimen	10° 8'27.84"E 49°40'22.91"N	Flora exsiccata Bavarica	(BioCASE)
	Unaterfranken	L.1351365	Rost, G; Wislicenus, WG	1899	Specimen	10°27'5.49"E 51° 9'56.49"N	Vascular Plant Herbarium, Oslo (O)	(Naturalis; BioCASE)
	-	2134178	Rost, G; Wislicenus, WG	1899	Specimen	10°27'5.49"E 51° 9'56.49"N	Vascular Plant Herbarium, Oslo (O)	(Naturalis; BioCASE)
	Wettelroda	OHN 66617	Becker, W.	1899	Specimen	10°27'5.49"E 51° 9'56.49"N	OHN	(BioCASE)
	Wettelroda	2134187	Becker, W.	1899	Specimen	10°27'5.49"E 51° 9'56.49"N	Vascular Plant Herbarium, Oslo (O)	(BioCASE)
	Thuringia	L.1351351	Becker, W	1900	Specimen	10.845346 51.010989	Vascular Plant Herbarium, Oslo (O)	(Naturalis; BioCASE)
	Sangerhausen	2134179	W. Becker	1900	Specimen	11.299505 51.472542	Vascular Plant Herbarium, Oslo (O)	(GBIF; BioCASE)
	-	-	-	1945	Observation	9.9 50.4	BFL	(GBIF)
	Kr. Alsfeld, Gr. Felda, Goldberg	FR-0042993	-	1948	Specimen	9.155 50.6443	Herbarium Senckenbergianum	(BioCASE)
	Kr. Alsfeld, Gr. Felda, Goldberg	FR-0042992	-	1951	Specimen	9.155 50.6443	Herbarium Senckenbergianum (FR)	(BioCASE)
	Kr. Alsfeld, Gr. Felda, am Weidenberg	FR-0042994	T. Romero	1952	Specimen	9.15717 50.6609	Herbarium Senckenbergianum (FR)	(BioCASE)
	Rheinland-Pfalz	MJG-Rheinland Pfalz 005282	Oesau, A	1979	Specimen	7°18'32.26"E 50° 7'6.06"N	Herbarium W	(Herbarium WU)
	Maar	3799-3758	-	1991	Specimen	9.389984 50.675779	Digitization of plant specimens from Rhoen and Vogelsberg (FULD)	(GBIF; BioCASE)
	Stockhausen	25980	J. Müller, K. Lewejohann, A.	1997	Specimen	9.45815 50.5749	(MNHM) Museum of Natural History	(BioCASE)

			Beyer, C. Renker				Mainz	
	FFH-Gebiet 5315-309 Grünland und Höhlen bei Erdbach und Medenbach	2101853346	-	1999	Observation	8.223804 50.688171	Naturgucker	(GBIF; BioCASE)
	Rhein	FR-0090308	-	2000	Specimen	7°26'36.28"E 52°16'53.64"N	Herbarium Senckenbergianum (FR)	(BioCASE)
	Frankfurt am Main	FR-0090308	-	2000	Specimen	8.682127 50.110922	Herbarium Senckenbergianum (FR)	(GBIF)
	Illertal Jedesheim	280958383	-	2008	Specimen	10.115278 48.194443	Naturgucker	(GBIF; BioCASE)
	Schwaben / Bellenberg / Illerberg	116267143	-	2009	Observation	10.123816 48.274055	Naturgucker	(GBIF; BioCASE)
	-	1916-0032038	L. Pignotti	2011	Specimen	10°27'5.49"E 51° 9'56.49"N	Natural History Museum, Vienna - Herbarium W	(BioCASE)
	Landkreis Gießen	FR-0113134	-	2013	Specimen	8.819160 50.595078	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Vogelsbergkreis	FR-0113135	-	2013	Specimen	9.10015 50.763517	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Vogelsbergkreis	FR-0116644	-	2014	Specimen	9.10015 50.763517	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Vogelsbergkreis	FR-0116904	-	2014	Specimen	9.228033 50.739867	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Lahn-Dill-Kreis	FR-0116909	-	2014	Specimen	8.412783 50.6496	Herbarium Senckenbergianum	(GBIF; BioCASE))
	Vogelsbergkreis	FR-0116905	-	2014	Specimen	9.431433 50.5643	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Landkreis Marburg-Biedenkopf	FR-0116908	-	2014	Specimen	8.6636 50.73225	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Vogelsbergkreis	FR-0116906	-	2014	Specimen	9.304983 50.708883	Herbarium Senckenbergianum	(GBIF; BioCASE)

	Vogelsbergkreis	FR-0116914	-	2014	Specimen	9.304983 50.708883	Herbarium Senckenbergianum	(BioCASE)
	Landkreis Gießen	FR-0116911	-	2014	Specimen	8.819783 50.572817	Herbarium Senckenbergianum	(BioCASE)
	Landkreis Gießen	FR-0116912	-	2014	Specimen	8.819783 50.572817	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Landkreis Gießen	FR-0116907	-	2014	Specimen	9.04815 50.571283	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Lahn-Dill-Kreis	B 10 0553631	T. Gregor	2014	Specimen	8.375278 50.5975	Herbarium Berolinense	(GBIF; BioCASE)
	Vogelsbergkreis	FR-0119937	T. Gregor, D.Y. Maguire, S. J. Nowak & R. Sforza 13916	2015	Specimen	9.431433 50.5643	Herbarium Senckenbergianum	(BioCASE)
	Vogelsbergkreis	FR-0119938	-	2015	Specimen	9.431433 50.5643	Herbarium Senckenbergianum	(GBIF; BioCASE)
	Basaltmagerrasen westlich Geiss-Nidda	1184369785	-	2016	Observation	8.958879 50.403511	Naturgucker	(GBIF; BioCASE)
	Göttingen	2134185	Grisebach		Specimen	9.915804 51.541280	Vascular Plant Herbarium, Oslo (O)	(BioCASE)
	-	855687122	Barras	-	Unknown	10°27'5.49"E 51° 9'56.49"N	Inatura Dornbirn	(GBIF - Austria)
	Koblenz	WAG.1566747	Oertel CG	-	Unknown	7.588996 50.356943	WAG.	(BioCASE)
	-	862392624	-	-	Specimen	10°27'5.49"E 51° 9'56.49"N	Herbarium W	(GBIF - Austria)
	-	862679162	-	-	Specimen	10°27'5.49"E 51° 9'56.49"N	Herbarium GZU	(GBIF - Austria)
	-	L.1351351	Láng, AF	-	Unknown	10°27'5.49"E 51° 9'56.49"N	(Naturalis Biodiversity Center)	(Naturalis)
	-	L.1351373	Becker, W	-	Specimen	10°27'5.49"E 51° 9'56.49"N	(Naturalis Biodiversity Center)	(Naturalis)
	-	L.1351351	Tommasini, MGS de	-	Specimen	10°27'5.49"E 51° 9'56.49"N	(Naturalis Biodiversity Center)	(Naturalis)

	-	L.1351365	Herb Bisschop	-	Specimen	10°27'5.49"E 51° 9'56.49"N	(Naturalis Biodiversity Center)	(Naturalis)
	Koblenz	WAG.1566754	Wirtgen	-	Specimen	7.588996 50.356943	WAG	(Naturalis)
	Koblenz	WAG.1566753	Wirtgen	-	Specimen	7.588996 50.356943	Naturalis	(BioCASE)
Georgia	Kartli, Tbilisi	211211	R. I. Gagnidze and Shamil Shetekauri	2003	Unknown	41° 38' 51"N 44° 48' 59"E	The New York Botanical Garden	(Edinburgh)
Greece	Dorkas	1573699	-	1989	Specimen	23.1167 40.9	LD	(BioCASE)
	Sérrai	B 10 0733697	Willing, E. and Willing, B	1992	Specimen	23.5 41.28	Herbarium Berolinense	(Herbarium WU)
	Asvestochorion	-	-	1993	Specimen	23.016667 40.633333	LD	(GBIF)
	-	84737	Gustavsson & Franzén	1979	Specimen	21°49'27.52"E 39° 4'27.15"N	LD	(GBIF-Sweden)
	Metsovo	1778987	-	2005	Specimen	21.116667 39.8	LD	(BioCASE; GBIF-Sweden)
	Metsovo	1799920	-	2005	Specimen	21.15 39.766667	LD	(BioCASE; GBIF-Sweden)
	Rendina, Kardhitsa	B 10 0290546	Willing	2007	Specimen	21.9892 39.0775	Herbarium Berolinense	(BioCASE; Herbarium WU)
	Trikala, SW Koniskos	B 10 0502928	Willing, Rand and Willing, E.	2012	Specimen	21.800102 39.779680	Herbarium Berolinense	(GBIF; Herbarium WU)
	Aitolía kai Akarnanía	B 10 0579988	Willing, Rand and Willing, E.	2013	Specimen	21.87 38.72	Herbarium Berolinense	(Herbarium WU)
	Evrytanía	B 10 0579990	Willing, Rand and Willing, E.	2013	Specimen	21.85 38.76	Herbarium Berolinense	(Herbarium WU)
	Domnista	B 10 0579989	Willing, Rand and Willing, E.	2013	Specimen	21.846380 38.756873	Herbarium Berolinense	(GBIF; BioCASE)

	Etolia-Akarnania	B 10 0579988	Willing, Rand and Willing, E.	2013	Specimen	21.379893 38.708439	Herbarium Berolinense	(GBIF; BioCASE)
	Dráma	B 10 0691629	Willing, Rand and Willing, E.	2015	Specimen	24.43 41.21	Herbarium Berolinense	(Herbarium WU)
	Dráma	B 10 0691631	Willing, Rand and Willing, E.	2015	Specimen	24.44 41.23	Herbarium Berolinense	(Herbarium WU)
Hungary	Vas	GZU	Piers	1889	Specimen	16.681218 47.092911	Herbarium GZU	(Herbarium WU)
	Gyöngyös	2134180	A. de Degen	1899	Specimen	19.929493 47.777265	Vascular Plant Herbarium, Oslo (O)	(GBIF)
	Gyöngyös-Mátra Randgebirge	2134171	Ove Dahl	1902	Specimen	19.950000 47.883333	Vascular Plant Herbarium, Oslo (O)	(GBIF; BioCASE)
	Gyöngyös-Mátra Randgebirge	2134169	Ove Dahl	1902	Specimen	19.950000 47.883333	Vascular Plant Herbarium, Oslo (O)	(GBIF)
	Gyöngyös-Mátra Randgebirge	2134170	Ove Dahl	1902	Specimen	19.950000 47.883333	Vascular Plant Herbarium, Oslo (O)	(GBIF)
	Bács Bodrog	805323-1	J. Prodan	1910	Specimen	21.537611 48.248889	CSIC-Real Jardín Botánico-Colección de Plantas Vasculares (MA)	(GBIF; BioCASE)
	-	WAG.1566758	Wagner, J	1912	Specimen	19.503304 47.162494	(Naturalis Biodiversity Center)	(Naturalis; GBIF)
	-	U.1520177	Wagner J	1912	Specimen	19.503304 47.162494	(Naturalis Biodiversity Center)	(GBIF)
	-	2134176	K. H. Rechinger	1927	Specimen	19.503304 47.162494	Vascular Plant Herbarium, Oslo (O)	(GBIF)
	Srentendre	4382900561	D. Gotthard	1978	Specimen	19° 4'0.70"E 47°40'46.32"N	Museum national d'Histoire naturelle (MNHN)	(Simonkai 1876; GBIF)

	Ungarn	GJO-0074689	Zernig, Kurt Nr	2013	Specimen	18.0795 47.1534	Herbarium GJO	(GBIF - Austria)
	Ungarn	GJO-0075642	Zernig, Kurt Nr	2015	Specimen	18.0795 47.1534	Herbarium GJO	(GBIF - Austria)
	Mount Badacsony	-	-	-	Literature	17°29'38.00"E 46°48'6.00"N	-	(Bauer 2012 et al.)
	Ungarn	AMD.123154	-	-	Specimen	18.0795 47.1534	Naturalis Biodiversity Center (NL) - Botany	(GBIF)
	Ungarn	AMD.123151	-	-	Specimen	18.0795 47.1534	Naturalis Biodiversity Center (NL) - Botany	(GBIF)
Iran	Lissar protected area (N Iran)	-	-	2007	Specimen	5°57'43.60"E 35°31'40.98"N	-	(Hamzeh'ee et al. 2008)
	Gilan	W 2017-0004053	Bidarlord, M	2014	Specimen	48.65 37.96	Naturhistorisches Museum Wien	(Herbarium WU; GBIF - Austria)
Iraq	-	-	-	1968	Literature	-	-	(Bor 1968)
Italy	Monte Amiata, San Salvatore, Siena	-	Fiori	1919	Literature	11°38'0.00"E 42°54'0.00"N	-	(Selvi 2010).
	Vesuv	291424	Tischler, M.B.	1980	Specimen	14°25'35.74"E 40°49'16.67"N	Herbarium W	(BioCASE)
	Sicilia	W 2012-0003904	Tischler, M. B	1980	Specimen	14°39'31.60"E 37°23'53.18"N	Herbarium W	(Herbarium WU)
	Coldiroidi and San Romolo- Itlay	-	-	-	Literature	-	-	(Bicknell 1896)
	Abruzzi	FABR01120	-	-	Specimen	13°43'44.10"E 42°11'31.24"N	Museum national d'Histoire naturelle (MNHN)	(BioCASE)
	abruzzo	-	-	-	Literature	13°43'44.10"E 42°11'31.24"N	-	(Pignatti 1982; Conti and Abbate 2005)
	basilicata	-	-	-	Literature	15°58'11.96"E 40°38'35.08"N	-	(Pignatti 1982; Conti and Abbate

								2005)
	marche	-	-	-	Literature	12°59'23.90"E 43°30'19.94"N	-	(Pignatti 1982; Conti and Abbate 2005)
	piemonte	-	-	-	Literature	7°31'3.32"E 45° 3'5.85"N	-	(Pignatti 1982; Conti and Abbate 2005)
	sardegna	-	-	-	Literature	9° 0'46.41"E 40° 7'15.15"N	-	(Pignatti 1982; Conti and Abbate 2005)
	toscana	-	-	-	Literature	11°14'49.40"E 43°46'6.86"N	-	(Pignatti 1982; Conti and Abbate 2005)
	umbria	-	-	-	Literature	12°37'1.21"E 42°56'26.97"N	-	(Pignatti 1982; Conti and Abbate 2005)
	veneto	-	-	-	Literature	11°41'30.11"E 45°45'36.73"N	-	(Pignatti 1982; Conti and Abbate 2005)
Kuwait	-	-	-	1987-1988	Literature	-	-	(Al Rawi 1987; Boulos 1988)
Libya	-	-	-	1988	Literature	-	-	(Hossain et al. 1988)
Macedonia	-	-	-	2015	Personal communication	-	-	Dr. Renata Arsovska
Morocco	-	-	-	2018	Personal communication	-	-	(Maire 1952; Jean-FrançoisLéger, Mathieu Chambouleyron, and Mohamed Ibn Tattou, PC)

Moldova	Uricani	U.1520170	Dobrescu, C; Eftimie, E	1966	Specimen	28°22'11.59"E 47°24'41.87"N	PACA-AGP - Herbarium Anchieta	(Naturalis)
	-	-	-	-	Literature	28°22'11.59"E 47°24'41.87"N	-	(Tzvelev 1976)
Montenegro	-	TGU	Stešević, Danijela	2002	Specimen	42.44 19.26	-	(Herbarium WU)
Netherlands	Deventer	L.3110823	Jansen, P; Jong de; Gorter, A; Kloos Jr, AW	1936	Specimen	5°17'28.08"E 52° 7'56.54"N	Herbarium Jansen and Wachter	(Naturalis)
Palestine	-	-	-	1932	Literature	-	-	(Post 1932)
Poland	-	-	-	-	Literature	19° 5'51.82"E 51°51'30.36"N	-	(Frey and Paszko 1998)
Romania	Transylvania	L.1351353	Nyárády, EI; Gürtler, C	1913	Specimen	25.222397 46.184056	(Naturalis Biodiversity Center)	(Naturalis)
	Oltenia	AMD.123147	Cirtu, D	1966	Specimen	23.770063 44.342090	(Naturalis Biodiversity Center)	(Naturalis)
	Orsova	654868	Parascan, D. Danciu, M.	1977	Specimen	22.394326 44.728462	BPBM	(BioCASE)
	-	L.1351353	Noë, FW	-	Specimen	24°58'0.33"E 45°56'35.38"N	(Naturalis Biodiversity Center)	(Naturalis)
	Codru-Moma Mountains	-	-	-	Literature	22°10'32.88"E 46°32'11.04"N	-	(Merce et al 2007)
	-	-	-	-	Literature	24°58'0.33"E 45°56'35.38"N	-	(Sârbu et al. 2009)
Russia	-	-	-	-	Personal communication	-	Southern Federal University	Dr. Olga Demina
	Moscow	-	-	-	Personal communication	37°37'2.28"E 55°45'20.97"N	-	Alexey Shipunov
	Makhachkala, Dagestan Republic	-	-	-	Literature	47°29'59.95"E 42°57'59.91"N	-	(Tzvelev 1976)
	-	-	-	-	Literature	44°59'60.00"E 43°35'6.66"N	-	(Komarov 1963; Tzvelev 1976)

Serbia	-	-	-	2015	Personal communication	-	University of Belgrade, Belgrade, Serbia	Dr. Vera Batanjski
	-	-	-	2012	Literature	-	-	(Panjković et al 2012)
Slovakia	-	2010-0000185	-	1906	Specimen	19°41'56.49"E 48°40'8.49"N	Herbarium W	(Naturalis)
	Hajnacka	2134173	F. Švestka	1930	Specimen	19.953033 48.216322	Vascular Plant Herbarium Oslo	(BioCASE)
	-	2304903	Domin, K.	1931	Specimen	19°41'56.49"E 48°40'8.49"N	National Museum of Natural History	(GBIF)
	Levice	U.1520169	Smejkal, M; Vicherek, J	1968	Specimen	19°41'56.49"E 48°40'8.49"N	(Naturalis Biodiversity Center)	(Naturalis)
	Levice	2134168	Smejkal, M; Vicherek, J	1968	Specimen	19°41'56.49"E 48°40'8.49"N	Vascular Plant Herbarium Oslo	(BioCASE)
	-	185100	-	1984	Specimen	19°41'56.49"E 48°40'8.49"N	IBOT SAS	(BioCASE)
	-	-	-	2014	Literature	19°41'56.49"E 48°40'8.49"N	-	(Turis et al. 2014)
	-	-	-	2015	Personal communication	19°41'56.49"E 48°40'8.49"N	Institute of Botany, Slovak Academy of Sciences	Dr. Viera Feráková
	-	-	-	2015	Personal communication	19°41'56.49"E 48°40'8.49"N	Department of Botany, Slovak University of Agriculture	Dr. Pavol Eliáš
	Kusá hora	185102	-	-	Specimen	18°35'56.38"E 48°14'22.37"N	IBOT SAS	(BioCASE)
	-	185101	-	-	Specimen	19°41'56.49"E 48°40'8.49"N	IBOT SAS	(BioCASE)
Spain	Guadarrama, Madrid	2365638	Lange, v. s	1861-1862	Literature	4° 5'15.16"W 40°40'23.48"N	(Willkomm, M. & Lange, J. (1861-1862)	(Anthos)
	El Escorial (Madrid)	3285-1	Torre Pando	1873	Specimen	4° 8'51.82"W 40°35'20.55"N	HGM	(BioCASE)
	Granada	2511484	-	1905	Literature	3°35'54.81"W 37°10'38.41"N	(Hervier, J. 1905)	(Anthos)

	Granada	2511485	-	1905	Literature	3°35'54.81"W 37°10'38.41"N	(Hervier, J. (1905)	(Anthos)
	sierra del Brezo, Palencia	1540463	-	1917	Literature	4°45'42.00"W 42°49'34.00"N	(Gandoger, M. 1917)	(Anthos)
	Cervera, Palencia	1540464	-	1917	Literature	4°29'57.45"W 42°52'0.81"N	(Gandoger, M. 1917)	(Anthos)
	Espagne	G-G-314938/2	Sennen, E.-M	1918	Specimen	3.64957 40.22683	Geneva Herbarium – General Collection (G)	(BioCASE)
	Cardeñajimeno, Burgos	117744	-	1924	Literature	3°37'11.57"W 42°19'48.38"N	Font Quer, P.	(Anthos)
	Espagne	G-G-314940/2	Sennen, E.-M	1926	Specimen	3.64957 40.22683	Geneva Herbarium – General Collection (G)	(GBIF)
	Sierra de Castril	G-G-314926/1	E. Reverchon	1930	Specimen	-1.64 40.46	Geneva Herbarium – General Collection (G)	(BioCASE)
	Nocedo, León	2075191	J. Borja Carbonell, Cámara Niño & Roja	1953	Literature	5°23'58.26"W 42°53'38.15"N	(Borja Carbonell, J. 1953)	(Anthos)
	Reinosa, Cantabria	1534646	Borja	1953	Literature	4° 8'21.34"W 42°59'58.30"N	(Guinea, E. 1953)	(Anthos)
	Morcuera	1370113	Segura, A.	1962	Observation	-2.1 41.1	Fund. Biodiversidad	(BioCASE)
	Barriomartín	1370117	Segura, A.	1966	Observation	-2.1 41.1	Fund. Biodiversidad	(BioCASE)
	Oncala	-	-	1966	Specimen	-2.280615 41.969895	Fund. Biodiversidad	(GBIF; BioCASE)
	Segovia	85331-1	E. Valdés-Bermejo	1974	Specimen	4° 6'32.07"W 40°56'34.33"N	SALA	(BioCASE)
	Riofrío	114035-1	E. Valdés-Bermejo & G. López	1974	Specimen	4° 9'2.65"W 40°52'24.55"N	Colección de plantas vasculares del herbario de la Universitat de València(VAL)	(BioCASE)
	Valdemeca, Cuenca, Spain	35583	G. López	1977	Specimen	1°44'36.74"W 40°13'29.65"N	SANT	(BioCASE)

	Sierra de Valdemeca	234417-1	G. López	1977	Specimen	1.750000 40.166667	SANT	(GBIF)
	Campillo de Ranas, Guadalajara	993098	Rivas-Martínez, S.	1980	Literature	3°18'51.63"W 41° 5'8.52"N	(Rivas-Martínez, S. 1980)	(Anthos)
	Cerezo de Abajo, Segovia	1114352	-	1980	Literature	3°35'30.16"W 41°13'4.08"N	(Rivas-Martínez, S. 1980)	(Anthos)
	Urraca-Miguel, Ávila	1110619	-	1980	Observation	4°31'21.49"W 40°40'19.04"N	Fund. Biodiversidad	(Anthos)
	Navares de las Cuevas	40559-1	T. Romero	1983	Specimen	3°45'1.77"W 41°24'48.43"N	SALA	(BioCASE)
	Navares de las Cuevas, Segovia	43678-1	E. Rico & T. Romero	1983	Specimen	3°45'1.77"W 41°24'48.43"N	SALA	(BioCASE)
	Arguijo, Soria	86767	-	1983	Literature	2°30'45.08"W 41°59'18.39"N	(Mendiola, M.A. 1983).	(Anthos)
	La Póveda de Soria, Soria	86774	-	1983	Literature	2°30'12.22"W 42° 0'42.10"N	Fund. Biodiversidad	(Anthos)
	Castillejo de Mesleón, Segovia	40561-1	T. Romero	1984	Specimen	3°36'4.34"W 41°16'50.99"N	SALA	(BioCASE)
	Soto de Sepúlveda, Segovia	569362-1	T. Romero	1984	Specimen	3°34'7.59"W 41°16'10.06"N	MA	(BioCASE)
	Arcones	40560-1	T. Romero	1985	Specimen	3°43'26.95"W 41° 7'5.41"N	SALA	(BioCASE)
	Sierra Pradales, Segovia	49275-1	X. Giráldez, E. Rico & T. Romero	1985	Specimen	3°45'38.00"W 41°27'2.00"N	SALA	(BioCASE)
	Cordovilla de Aguila	620379-1	C. Aedo	1985	Specimen	-4 42	MA	(BioCASE)
	Sierra del Almuerzo, Soria	85033	-	1985	Observation	2°15'4.00"W 41°50'25.00"N	Fund. Biodiversidad	(Anthos)
	Campo Azalvaro, Ávila	2655244	-	1985	Literature	4°40'32.59"W 40°39'54.16"N	(Rivas-Martínez, S. & Belmonte, D. 1985).	(Anthos)
	Trescasas	807647-1	R. García	1985	Specimen	-4.03 40.95	MA	(BioCASE)
	Cordovilla	620379-1	C. Aedo	1985	Specimen	-4.21 42.81	MA	(GBIF)

	Sotosalbos, Segovia	997626403	R. García	1988	Specimen	-3.94 41.03	MA	(GBIF)
	Guijuelo	47198-1	X. Giráldez, E. Rico & J. Serradilla	1988	Specimen	5°40'29.00"W 40°33'32.42"N	SALA	(BioCASE)
	Oteruelo, Madrid	2778221	-	1988	Literature	3°51'11.25"W 40°54'51.77"N	(Fernández González, F. 1988)	(Anthos)
	Madrid	2778222	-	1988	Observation	3°42'13.64"W 40°25'0.39"N	Fund. Biodiversidad	(Anthos)
	Madrid	2778223	-	1988	Literature	3°42'13.64"W 40°25'0.39"N	(Fernández González, F. 1988)	(Anthos)
	Pinilla del Valle, Madrid	2778224	-	1988	Observation	3°49'13.35"W 40°55'35.41"N	(Fernández González, F. 1988)	(Anthos)
	Madrid	2778226	-	1988	Literature	3°42'13.64"W 40°25'0.39"N	(Fernández González, F. (1988)	(Anthos)
	Sotosalbos	807646-1	R. GarcÃa	1988	Specimen	-3.94 41.02	MA	(BioCASE; Anthos)
	Guijuelo, Salamanca	65378	-	1989	Literature	5°40'29.00"W 40°33'32.42"N	(Serradilla Rodríguez, J. (1989)	(Anthos)
	Navares de las Cuevas, Segovia	92474	-	1989	Observation	3°45'1.77"W 41°24'48.43"N	Fund. Biodiversidad	(Anthos)
	Arcones, Segovia	92475	-	1989	Observation	3°43'26.95"W 41° 7'5.39"N	Fund. Biodiversidad	(Anthos)
	Segovia	G-G-314921/1	Charpin, A. & P.-A. Loizeau	1989	Specimen	-3.96528 40.82444	Geneva Herbarium – General Collection (G)	(GBIF; Anthos)
	Cármenes	50962-1	F. Llamas and C. Acedo	1991	Specimen	-5.52 43.01	LEB	(GBIF; BioCASE; Anthos)
	Sierra de Castril, Granada	1654546	Morales, C. and Passera, C.	1992	Literature	-2.1 37.1	(Vizoso, M.T. et al 2002)	(BioCASE; Anthos)
	Trescasas, Segovia	70482	-	1995	Literature	4° 2'13.12"W 40°57'43.18"N	(García Adá, R. 1995).	(Anthos)
	Sotosalbos, Segovia	70483	-	1995	Literature	3°56'27.55"W 41° 2'5.58"N	(García Adá, R. 1995).	(Anthos)
	Morcuera, Soria	1370113	Segura, A	1998	Literature	3°12'58.23"W 41°27'50.99"N	(Segura Zubizarreta, A et al 1998)	(Anthos)

	Villaverde del Monte, Soria	1370115	-	1998	Observation	2°40'18.04"W 41°48'48.91"N	Fund. Biodiversidad	(Anthos)
	Barriomartín, Soria	1370117	Segura, A	1998	Literature	2°29'36.59"W 41°59'51.63"N	(Segura Zubizarreta, A et al 1998)	(Anthos)
	Póveda de Soria, Soria	1370119	-	1998	Observation	2°30'12.22"W 42° 0'42.10"N	Fund. Biodiversidad	(Anthos)
	Oncala, Soria	1370121	-	1998	Literature	2°18'43.74"W 41°58'15.49"N	(Segura Zubizarreta, A et al 1998)	(Anthos)
	Sierra del Almuerzo, Soria	1370123	-	1998	Literature	2°15'4.00"W 41°50'25.00"N	(Segura Zubizarreta, A et al 1998)	(Anthos)
	Noguera	100036-1	E. Rico & F. Conti	1999	Specimen	1°35'44.83"W 40°27'20.46"N	SALA	(BioCASE)
	Granada	1654546	Morales, C. and Passera, C	2002	Observation	3°35'54.81"W 37°10'38.41"N	Fund. Biodiversidad	(Anthos)
	Cardeñajimeno, Burgos	2601502	-	2006	Literature	3°37'11.57"W 42°19'48.38"N	(Alejandre Sáenz, J.A. et al 2006)	(Anthos)
	Cardeñajimeno, Burgos	2601499	-	2006	Literature	3°37'11.57"W 42°19'48.38"N	(Alejandre Sáenz, J.A. et al 2006)	(Anthos)
	Cardeñajimeno, Burgos	2601501	-	2006	Literature	3°37'11.57"W 42°19'48.38"N	(Alejandre Sáenz, J.A. et al 2006)	(Anthos)
	Pantano de Arlanzón, Burgos	2601503	-	2006	Literature	3°20'15.48"W 42°15'20.17"N	(Alejandre Sáenz, J.A. et al 2006)	(Anthos)
	Noguera de Albarracín, Teruel	2736259	G. Mateo, J. Fabado & C. Torres	2006	Literature	1°35'48.77"W 40°27'31.62"N	(Mateo Sanz, G et al 2006)	(Anthos)
	Bronchales, Teruel	2736260	-	2006	Literature	1°35'17.14"W 40°30'31.75"N	(Mateo Sanz, G et al 2006)	(Anthos)
	Purujosa	79291-1	P.M. URIBE-ECHEBARRIA	2007	Specimen	-1.793 41.667	VIT	(GBIF)
	Noguera	206499-1	G. Mateo, C. Torres & J. Fabado	2007	Specimen	-1.602 40.476	VAL	(GBIF; BioCASE)
	Cercedilla	10395-1	Barras	-	Unknown	4° 3'24.66"W 40°44'26.34"N	SEV-Historico	(BioCASE)
	Campo Azalvar	P22723	Rivas Martinez, Salvador & Belmonte, Dolores	-	Observation	-4.53 40.64	Fund. Biodiversidad	(BioCASE)

	Madrid	U-P04232	-	-	Observation	-3.71 40.91	SIVIM	(BioCASE)
	Madrid	-	-	-	Literature	3°42'13.64"W 40°25'0.39"N	(Morales 2003)	(Morales 2003)
	Andalusia	-	-	-	Literature	4°43'37.57"W 37°32'46.00"N	-	(Blanca et al. 2011)
	Madrid	U-P07492	-	-	Observation	-3.71 40.91	SIVIM	(BioCASE; Anthos)
	Urraca-Miguel	1110619	-	1980	Literature	-4.1 40.1	(Rivas-Martínez, S. 1980)	(BioCASE; Anthos)
	Cerezo de Abajo	1114352	-	-	Observation	-3.1 41.1	Fund. Biodiversidad	(BioCASE; Anthos)
	Trescasas	70482	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(BioCASE; Anthos)
	Sierra del Almuerzo, Soria	85033	-	1985	Literature	2°15'4.01"W 41°50'25.00"N	(Bachiller Cacho, D. 1985).	(BioCASE; Anthos)
	Arguijo	86767	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(BioCASE; Anthos)
	Soria	86774	-	1983	Observation	-2.1 41.1	(Mendiola, M.A. 1983).	(BioCASE; Anthos)
	Villaverde del Monte	1370115	-	1998	Literature	-2.1 41.1	(Segura Zubizarreta, A et al 1998)	(BioCASE; Anthos)
	Soria	1370119	-	1998	Literature	-2.1 41.1	(Segura Zubizarreta, A et al 1998	(BioCASE; Anthos)
	Oncala	1370121	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(BioCASE; Anthos)
	Soria	1370123	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(BioCASE; Anthos)
	Segovia	92474		1989	Literature	-3.1 41.1	(Romero Martín, T. & Rico Hernández, E. 1989)	(BioCASE; Anthos)
	Segovia	92475	-	1989	Literature	-3.1 40.1	(Romero Martín, T. & Rico Hernández, E. 1989)	(BioCASE; Anthos)
	Cármenes	1478631	-	1997	Literature	5.52129 43.00885	(Llamas, F. & Acedo, C. (1997).	(BioCASE; Anthos)

	Sierra de Castril	L.1351385	Reverchon, E	1903	Specimen	-3.36222 38.02389	(Naturalis Biodiversity Center)	(Naturalis)
	Sierra de Castril	L.1351475	Reverchon, E	1903	Specimen	-3.36222 38.02389	(Naturalis Biodiversity Center)	(Naturalis)
	Urraca-Miguel, Avila	218710051	-	-	Observation	40.1 -4.1	Fund. Biodiversidad	(GBIF; Anthos)
	Cardeñajimeno, Burgos	218710108	-	-	Observation	42.1 -3.1	Fund. Biodiversidad	(GBIF; Anthos)
	Pineda de la Sierra, Burgos	2601500	-	2006	Literature	3°17'49.34"W 42°12'56.94"N	(Alejandre Sáenz, J.A. et al 2006)	(Anthos)
	Gata, Montehermoso, Cáceres	218710042	-	-	Observation	-6.1 40.1	Fund. Biodiversidad	(GBIF; Anthos)
	Sierra de Gredos, Madrigal	218710041	-	-	Observation	-5.1 40.1	Fund. Biodiversidad	(Anthos)
	Sierra de Castril, Suerte Somera, Granada	218710368	-	-	Observation	-2.1 37.1	Fund. Biodiversidad	(Anthos)
	Cármenes, León	218710223	Llamas, F. and Acedo, C.	1991	Observation	-5.1 42.1	Fund. Biodiversidad	(GBIF; BioCASE; Anthos)
	Cármenes, León	894161781	Llamas, F. and Acedo, C.	1991	Specimen	-5.1 42.1	Fund. Biodiversidad	(BioCASE; Anthos)
	sierra del Brezo, Palencia	218710295	-	-	Observation	-4.1 42.1	Fund. Biodiversidad	(GBIF; BioCASE; Anthos)
	Cervera, Palencia	218710296	-	-	Observation	-4.1 42.1	Fund. Biodiversidad	(Anthos)
	Cordovilla de Aguilar, Palencia	30617293	C. Aedo	1985	Specimen	-4.22 42.81	MA	(GBIF; BioCASE)
	Cervera de Pisuerga, Palencia	217822170	-	-	Observation	-4.1 42.1	Fund. Biodiversidad	(GBIF; Anthos)
	Reinosa, Soria	218710291	-	-	Observation	-3.1 42.1	Fund. Biodiversidad	(GBIF; Anthos)
	Morcuera, Soria	218710141	Segura, A.	1962	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Villaverde del Monte, Soria	218710142	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)

	Sierra del Almuerzo, Soria	218710146	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Barriomartín, Soria	218710143	-	1966	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Póveda de Soria, Soria	218710144	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Oncala, Soria	218710145	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Sierra del Almuerzo, Soria	218710618	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Arguijo, Soria	218710632	-	-	Observation	-2.1 41.1	-	(GBIF)
	Oncala, Soria	767258330	-	1966	Specimen	2°18'43.74"W 41°58'15.49"N	PAMP	(GBIF; Anthos)
	La Póveda de Soria, Soria	218710633	-	-	Observation	-2.1 41.1	Fund. Biodiversidad	(GBIF; Anthos)
	Noguera, Teruel	142940017	E. Rico & F. Conti	-	Specimen	-1.64 40.46	SALA	(GBIF; Anthos)
	Noguera, Teruel	997642738	E. Rico & F. Conti	1999	Specimen	-1.64 40.46	MA	(GBIF)
	Teruel	895220891	G. Mateo, C. Torres & J. Fabado	2007	Specimen	-1,631 40,476	VAL	(GBIF; Anthos)
	Teruel	895198344	J. Fabado, G. Mateo & C. Torres	2006	Specimen	-1,626 40,476	VAL	(GBIF; Anthos)
	Guijuelo, Salamanca	218710595	-	-	Observation	-5.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE; Anthos)
	Arcones, Segovia	218710682	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE; Anthos)
	Trescasas, Segovia	218710603	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE)
	Navares de las Cuevas, Segovia	218710681	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE)
	Sotosalbos, Segovia	218710604	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE)

	Cerezo de Abajo, Segovia	218710062	-	-	Observation	-3.1 40.1	Fund. Biodiversidad	(GBIF; BioCASE; Anthos)
	Trescasas, Segovia	997626341	R. García	1985	Specimen	-4.03 40.95	MA	(GBIF)
	Madrid	617458638	-	-	Literature	-3.71 40.91	(Sardinero, S.2004)	(GBIF; Anthos)
	El Cuadrón, Madrid	-	-	-	Observation	3°39'35.65"W 40°56'36.70"N	Fund. Biodiversidad	(Anthos)
	RascafrÃa - Oteruelo,	2778222	-	1988	Observation	3.87122051 40.908825	(Fernández González, F. (1988).	(GBIF; BioCASE)
	Jaén - Le Pozo. /Comunidad Autónoma de Andalucía	G-G-314932/1	Reverchon, E.	-	Specimen	-3.36222 38.02389	Geneva Herbarium - General Collection (G)	(GBIF; BioCASE)
	Jaén - Sierra de Castril. /Comunidad Autónoma de Andalucía	G-G-314928/2	Reverchon, E.	-	Specimen	-3.36222 38.02389	Geneva Herbarium - General Collection (G)	(GBIF; BioCASE)
	Guijuelo, Salamanca	218710595	-	-	Observation	-5.1 40.1	Fund. Biodiversidad	(Anthos)
	Madrid	-	-		Specimen	-3.703790 40.416775	Fund. Biodiversidad	(GBIF)
Syria	-	-	-	1932	Literature	-	-	(Post 1932)
Tunisia	-	-	-	1952	Literature	-	-	(Maire 1952)
	-	-	-	2018	Personal communication	-	-	Mounir Mekki
Turkey	-	L.1351375	Balansa (Orient series), B	1856	Specimen	35°14'35.96"E 38°57'49.48"N	(Naturalis Biodiversity Center)	(Naturalis)
	-	L.1351380	Balansa (Orient series), B	1857	Specimen	37°58′43″N 48°42′21″E	(Naturalis Biodiversity Center)	(Naturalis)
	-	L.1351374	Balansa (Orient series), B	1857	Specimen	35°14'35.96"E 38°57'49.48"N	(Naturalis Biodiversity Center) (NL)	(Naturalis)
	-	L.1351378	Balansa (Orient series), B	1857	Specimen	35°14'35.96"E 38°57'49.48"N	(Naturalis Biodiversity Center)	(Naturalis)

	-	L.1351379	Balansa (Orient series), B	1857	Specimen	35°14'35.96"E 38°57'49.48"N	(Naturalis Biodiversity Center)	(Naturalis)
	Akscheher	5662	Bornmüller, Joseph Friedrich Nicolaus	1899	Specimen	32°28'60.00"E 37°52'0.00"N	Royal Botanic Garden Edinburgh Herbarium (E)	(Royal Botanic Garden; GBIF)
	Izmir	5072	Alava, Reino Olavi	1966	Specimen	27° 7'43.39"E 38°25'7.86"N	Royal Botanic Garden Edinburgh Herbarium (E)	(Royal Botanic Garden)
	Yamanlar Dag	E00356759	-	1966	Specimen	27.233333 38.533333	Royal Botanic Garden Edinburgh Herbarium (E)	(GBIF)
	Yamanlar Dag	E00356755	-	1966	Specimen	27.233333 38.533333	Royal Botanic Garden Edinburgh Herbarium (E)	(GBIF)
	Izmir	5105	Alava, Reino Olavi & Bocquet, Gilbert Francois	1966	Specimen	27° 7'44.11"E 38°25'8.88"N	Royal Botanic Garden Edinburgh Herbarium	(Royal Botanic Garden)
	Eskisehir	498	Ekim, T.	1970	Specimen	31°24'55.47"E 38°21'21.28"N	Royal Botanic Garden Edinburgh Herbarium	(Royal Botanic Garden; GBIF)
	Ala Dag	4246	R. J. Soreng & Y. Serengil	1993	Specimen	31.6833 40.6069	National Museum of Natural History (NMNH)	(GBIF)
	Afyon	-	-	2011	Specimen	30.8714 38.9808	Museum national d'Histoire naturelle (MNHN)	(GBIF)
	Afyon	10636	L. J. Gillespie, E. Cabi, R. J. Soreng & K. Boudko	2011	Specimen	30.8714 38.9808	Museum national d'Histoire naturelle (MNHN)	(GBIF)
	Edirne	9259	R. J. Soreng, M. Kaya & E. Kurt	2015	Specimen	26°33'38.99"E 41°40'28.00"N	Museum national d'Histoire naturelle (MNHN)	(GBIF)
	-	-	-	2015	Personal communication	-	Universty of Sutcu Imam, Kahramanmaras-Turkey	Dr. Yusuf Ziya KOCABAS
	-	MW073326 4	-	-	Specimen	35.243322 38.963745	Moscow Digital Herbarium	(GBIF)

							(Moscow State University)	
	Edirne	-	-	-	Literature	26°33'38.99"E 41°40'28.00"N	-	(Davis 1965)
	Istanbul	-	-	-	Literature	28°58'36.98"E 41° 0'18.99"N	-	(Davis 1965)
	Izmir	-	-	-	Literature	27° 7'44.11"E 38°25'8.88"N	-	(Davis 1965)
	Usak	-	-	-	Literature	29°24'29.48"E 38°40'56.28"N	-	(Davis 1965)
	Konya	-	-	-	Literature	32°28'59.96"E 37°52'0.01"N	-	(Davis 1965)
	Eskisehir	-	-	-	Literature	30°31'14.17"E 39°46'35.84"N	-	(Davis 1965)
	Ankara	-	-	-	Literature	32°51'14.80"E 39°55'14.77"N	-	(Davis 1965)
	Adana	-	-	-	Literature	35°19'16.63"E 37° 0'0.21"N	-	(Davis 1965)
	Malatya Province	-	-	-	Literature	37°57'13.07"E 38°24'5.42"N	-	(Arabaci and Yildiz 2004)
	-	-	-	-	Literature	36°57'14.79"E 37°45'1.09"N	-	(İlçim et al. 2008)
	-	-	-	-	Literature	33°36'48.32"E 40°36'4.83"N	-	(Duran and Dural 2003)
	cankiri	-	-	-	Literature	29°58'59.01"E 40° 9'0.47"N	-	(Ocak et al. 2009)
	Bilecik	-	-	-	Literature	30°31'50.35"E 36°40'58.37"N	-	(Arabaci and Yildiz 2004)
Ukraine	-	MW0601849	Egorova O.	1901	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	Bereg	OHN 45697	Margittai, A.	1930	Specimen	22.637095 48.197187	Oskarshamn herbarium (OHN)	(GBIF)
	-	OHN 45697	Margittai, A.	1930	Specimen	31.165580 48.379433	OHN	(BioCASE)
	-	MW0601845	Smirnov P. and Derviz T.	1952	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)

-	MW0601847	Smirnov P. and Derviz T.	1952	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601848	Smirnov P. and Derviz T.	1952	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601846	Smirnov P. and Derviz T.	1952	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601866	Smirnov P.	1958	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601865	Smirnov P.	1960	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601851	Kurchenko E.	1962	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601864	Smirnov P.	1963	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601863	Smirnov P.	1963	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601850	Grosset G.	1964	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601852	Burmistrova L.	1964	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601853	Burmistrova L.	1964	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601855	Shvedchikova N.	1978	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601862	Shvedchikova N.	1978	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601859	Shvedchikova N.	1978	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601861	Shvedchikova N.	1978	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601858	Shvedchikova N.	1978	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
-	MW0601857	Shvedchikova N.	1979	Specimen	31.165580 48.379433	Moscow University Herbarium (MW)	(GBIF)
-	MW0601860	Shvedchikova N.	1979	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)

	-	MW0601856	Shvedchikova N.	1987	Specimen	31.165580 48.379433	Moscow University Herbarium (MW)	(GBIF)
	Ukrainian Carpathian Mountains	BRNU 649773	Prokešová, H. andKalníková, V	2016	Specimen	23.06 48.14	Herbarium WU	(Herbarium WU)
	-	MW0234940	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	--	MW0234940	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234936	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234942	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234939	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234938	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234937	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234941	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0234943	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	-	MW0601854	-	-	Specimen	31.165580 48.379433	Moscow University Herbarium	(GBIF)
	Ukrainian Carpathian Mountains	-	-	-	Literature	25°30'0.00"E 46°59'60.00"N	-	(Tzvelev 1976)
United Kingdom	Grays Chalk Quarry, Essex	-	A. Copping	1986	Literature	0°18'57.47"E 51°29'13.17"N	-	(Copping 1987)

Chapter 3: A bottleneck for microbes in seeds of *Ventenata dubia, Bromus tectorum,* and *Boechera stricta.*

Abstract

Plants host diverse, endophytic microbiomes in their roots, leaves, and stems, but seeds represent a bottleneck. Seeds are strongly defended against most microbes and those that can infect seeds are highly competitive and commonly exclude one another. In a survey of isolation frequencies of culturable microbes of seeds (i.e., rates of vertical transmission) in 98 plant species from 39 families, this hypothesis of a seed bottleneck for microbes was confirmed since almost all of the surface-sterilized seeds hosted either zero or one culturable endophyte. Recent literature indicates an extremely low average isolation frequency of two or more microbes per seed. In this exploratory, follow-on study, we surveyed two invasive plants *Ventenata dubia, Bromus tectorum,* and one plant native to North America, *Boechera stricta*. We varied age of seed, surface-sterilization protocol, and isolation medium to see if we could find any indication of a departure from the bottleneck. Seeds of *V. dubia* from years ranging from 1984 to 2017, totaled 3,400 seeds, and they were plated to four media (MGA 2.5, PDA, Kings, and filter paper). Seeds of *B. tectorum* from dated from 1987 to 2017 and totaled 500 seeds and they were plated on PDA alone. Besides, 1,080 seeds of North American native plant, *B. stricta,* were tested for the bottleneck and for exclusionary interactions that might be responsible by varying the method of surface-sterilization, genotypes, and comparing inoculated versus uninoculated plants in a flowing stage. The overall result in this study out of 4980 seeds, 82 % of the seeds were ‘zeros’ no isolates found; 17% were ‘one’ with a single endophyte and 1% with two culturable endophytes. Comparing with Newcombe et al. (2018), there was 70% ‘zeros’, 27% were one, and 3% two or more culturable endophytes suggesting confirmation of the relationships of plant to microbe vertical transmission.

Introduction

Ventenata dubia is a new invader in the Pacific Northwest. It commonly infests areas in which range management is ineffective or poor. It was first documented in the United States in 1956 (Northam and Callihan 1994), and its recent expansion has seriously degraded the quality of pastures, Conservation Reserve Program (CPR) fields, and hay fields. *B. tectorum* is an invasive grass in North America with a serious economic impact. The genus *Boechera* (family: Brassicaceae) is primarily North American; *B. stricta* is widespread in North America and has been the focus of studies in ecology and evolution (Song et al. 2006). It is most diverse in the western U. S. A. (Song et al. 2006) where it inhabits subalpine meadows, river edges, and forest understories. *Boechera stricta* is a perennial species, and it is well known, morphologically and genetically, as a perennial crucifer

species closely related to the model plant *Arabidopsis thaliana* (Song et al. 2006).

Vertical or maternal transmission occurs when offspring receive their microbiota from their maternal parents. Vertical transmission has been defined as "the direct transfer of infection from a parent organism to its progeny" (Raghavendra et al. 2013), and it is thought to "favor evolution toward mutualism and benign parasitism" (Raghavendra et al. 2013). Also, vertical transmission is considered a classic example of mutualism or parasitism, and it is transmitted from parent to offspring by seeds (Hodgson et al. 2014).

Fungi as an example for endophytes, may be transmitted either horizontally or vertically. Horizontally transmitted fungal endophytes are often transmitted by sexual spores that can be spread by the wind (Rodriguez 2009). The spread via horizontal transmission is similar to the transmission of pathogens. Vertically transmitted fungal endophytes are generally asexual and transmit via the fungal hyphae that penetrate the host's seeds (Selosse et al. 2004). The importance of symbiosis to the plants is providing essential nutrient for the plants, protecting the plant from the pathogens and abiotic stresses.

A bottleneck for microbes in seeds had been confirmed by Raghavendra et al., 2013. Seeds always have a low isolation frequency of microbes compared to the vegetative parts (leaves and stem) because developing seeds are better defended against microbes, it called ‘optimal defense’, even their own seeds’ microbes inoculated in the flowers it would fail to infect the seeds as in *Centaurea stoebe* seeds (Raghavendra et al., 2013). In addition to seeds' optimal defense in *C. stoebe* seeds, there was microbe’s interaction, resulting in a limit of one microbe per seeds. Out of 8763 seeds of *C. stoebe*, only 26% of the seeds had one culturable endophyte and 74% of the seeds had no culturable endophyte. Trying to increase the number of endophytes per seeds, *C. stoebe* flowers were inoculated with mixes of two seeds isolates. The result was the same, only one isolate per seed. An example of the mix of seed isolates was *Cladosporium* with *Botrytis*; the result of this in the seeds was *Cladosporium* because *Cladosporium* excluded *Botrytis*. Another mix was *Botrytis* and *Fusarium*, and the results were *Fusarium* because *Fusarium* excluded *Botrytis*. Last mixed were three endophytes *Botrytis*, *Cladosporium*, *Fusarium*, inoculated to flowers, and the result was *Fusarium* excluded *Botrytis* and *Cladosporium*. These are exclusionary interactions, and this means it is difficult to increase the number of isolates per seed because there are inhibitory interactions between microbes (Raghavendra et al., 2013).

The objective of this research effort was to identify any vertically-transmitted endophytes of *V. dubia, B. tectorum* and *B. stricta*, and test for deviation from the ‘zero/one' pattern. Zero means the absence of culturable endophytes on a particular isolation medium; one means the presence of one culturable endophyte per seed. With each of the three plant species, we also varied other factors (i.e.,

age of the seeds, type of medium, inoculated versus uninoculated, method of surface-sterilization, and genotypes). Much of this experimentation was exploratory. Factors varied for *V. dubia* included seed age and medium. For *B. tectorum* seed age alone was varied. Finally, for *B. stricta* we inoculated flowers with leaf and seed fungi; the surface-sterilization method varied as did genotypes of *B. stricta*.

Materials and Methods

Planting and Incubation

For *V. dubia* the first samples of 1150 seeds were collected from 2006 to 2017 from different sites in the USA. One site was in Serbia (sampled by Dr. Vera Batanjski -Faculty of Biology, University of Belgrade, Belgrade, Serbia). Table 3.1 represents the locations of *V. dubia* seeds sampling and the years and month if available. All seeds of this first sample were surface-sterilized by dipping in 6% sodium hypochlorite for two minutes and then rinsed by sterile distilled water three times. Seeds were then placed on *Fusarium*-selective medium (MGA 2.5) prepared as in Castellá et al. (1997). Ten seeds per Petri dish were then kept in the dark at room temperature for about 2 to 3 months for observation (Figure 3.1 and Figure 3.2).

In the summer of 2016, a second sample was collected by Dr. Prather from sites in several states (Table 3.1). The protocol for sampling was ten plants per site with each plant separately bagged. After getting the plants, five plants were selected having at least ten seeds each, and each batch of 10 seeds was separated from the other batches in envelopes even though in the surface-sterilization they were separated to reduce contamination between the seeds. The number of seeds per site was 50 seeds. The total number of seeds was 950 seeds in this year (2016). All seeds were surface-sterilized and washed as above, and groups of 10 seeds plated to *Fusarium*-selective medium and incubated as above.

The third sample of nine hundred seeds was collected in the same year as above (Table 3.1). They were surface-sterilized by dipping in 6% sodium hypochlorite for two minutes and then washed in distilled sterile water three times. The 900 seeds were divided by 3 for three types of medium (PDA, Kings medium, and sterile filter paper), 300 seeds for each medium. Ten seeds were placed in each plate of PDA, Kings medium, and sterile filter paper, a total number of plates for each medium was 30, and each of them had ten seeds. Three media were used to see if that would make any difference in the results. Plates were kept at room temperature for about 2 to 3 months of observation.

A fourth sample comprised 400 seeds total of *V. dubia* collected in 1984, 2015, and 2017 from different sites (Table 3.1). *Ventenata dubia* seeds from various locations were surface-sterilized by dipping in 6% sodium hypochlorite for two minutes, and then they were washed in distilled sterile water three times. Seeds plated on 40 PDA plates, and each PDA plate had ten seeds total of *V. dubia.*

Bromus tectorum seeds were from different years (1987, 1990, 2001, 2011, and 2017) (Table

3.2). These were surface-sterilized by dipping in 6% sodium hypochlorite for two minutes and then rinsing in distilled sterile water three times. Each plate of PDA received ten seeds. Table 3.2 is for the location if it available and the year of sampling *B. tectorum* seeds (Figure 3.1) and (Figure 3.2).
For *Boechera*, the first part used four surface-sterilizations methods (seed disinfestation). These methods have been used to isolate vertically transmitted endophytes (VTEs) from *Boechera* plants grown in the greenhouse. One hundred seeds were used for each method: 1) surface-sterilization (1 min in 96% ethanol, 5 min in 6% sodium hypochlorite (NaOCl), 30 sec 96% ethanol), and rinsing (5 min in distilled sterile water (SDW) (Ganley and Newcombe 2006), 2) surface-sterilization (15 min in 3% NaOCl) and rinsing two times (5 min rinse in SDW) (Bacon and White 1994). 3) surface-sterilization (5 min in 3% NaOCl) and rinsing two times for 5 min in SDW. 4) Gentle method: seeds disinfestation in 5 min in distilled water (DW), rinsing for 5 min in SDW. After each method, seeds were air dried in the hood on a sterile paper towel, then placed on potato dextrose agar (PDA), five seeds per plate. Seeds were imprinted on PDA for each method to test the efficacy of disinfestation (Figure 3.1) and (Figure 3.2). All plated seeds were observed for the number of endophytes in each seed, and type of endophytic bacteria or fungi. For fungi identification of genus was done and no farther identification for bacteria.

Fungal inoculation

The second part was the inoculation of *B. stricta* in the greenhouse. Table 3.3 lists inoculants used for *B. stricta*'s flowers. Flowers of *B. stricta* plants were inoculated by Dr. Newcombe with bulked inoculum from all of the following leaves and seed fungi plates: Boe5, 35, 20, 17, FZ102, Boe3, Boe51, and Boe 40 (Table 3.3). Two hundred ml of distilled water were used to wash spores and scraped mycelium from each of eights leaves and seed fungi plates (Boe5, 35, 20, 17, FZ102, Boe3, Boe51, and Boe 40) provided by Posy Busby (Assistant Professor at Oregon State University). Two hundred ml were applied as a spray on April 2016 to each of eight over-wintered outdoor plants in Moscow, ID that had been flowering. Moist incubation lasted for 12 hours (plants were bagged subsequent to spraying). There were seven inoculated plants, and they were from two genotypes which were LTM and SAD12. Three of the seven were the 'SAD12' genotype, and four were 'LTM'. Also, there were seven control plants. All leaves and inflorescences were sprayed with either water for control plants or bulk inoculum for treatment plants. "The maternal SAD12 locality in Colorado is a sagebrush grassland in a river valley occurring at an elevation of 2,530 m. This population produces predominately Met-derived 6-methylsulfinyl hexyl glucosinolate (6MSOH)" (Schranz et al. 2009) and "the paternal LTM population from Idaho grows in a subalpine meadow occurring at an elevation of 2,390 m. Val-derived 1-methyl ethyl glucosinolate (1ME) is the predominant glucosinolate produced by LTM" (Schranz et al. 2009).

After 3 to 4 weeks, seeds from both control and inoculated plants were collected by Dr. Newcombe. Four groups of seeds were brought to the lab in separate envelopes: SAD12 – Control, SAD12 - Inoculated, LTM –Control, LTM – Inoculated. One hundred seventy seeds from each group have been used in this part, and the total seeds were 680. The disinfestation method used in this part was gentle rinse five min in distilled water (DW), then five min in SDW, with Petri plates used for each solution and keeping genotypes separate by using one set of plates for each of them. After that, the seeds were air dried in the hood on sterile paper towels. Then seeds were then placed on Petri plates (16-17 seeds per plate) containing PDA; dishes were sealed with Parafilm to prevent drying and any contamination. Seeds were observed, and any endophytes recorded. There was not any specific reason to choose LTM and SAD12 genotypes in this part, but they were the only available.

Calculation of Isolation Frequency and Relative Abundances

Isolation frequency and relative abundances calculated for each microbe.

$$\text{Isolation frequency} = \frac{\text{Total number of seeds yielding isolates}}{\text{Total number of seeds sampled}}$$

$$\text{Relative abundances} = \frac{\text{Total number of isolates for each microbe}}{\text{Total number of isolates of microbes}}$$

Results

The results reflected either zero, one or two endophytes observed. There were 399 fungi out of 3,400 seeds of *V. dubia*, and 153 bacteria out of 3,400 seeds. 15.4 % of seeds had one endophyte, and only 0.4% had two endophytes and the rest, 84.2% of the seeds, were ‘zero’ pattern (Table 3.4). There were not any seeds that had more than two endophytes in the results.

For *B. tectorum* there were 118 fungi out of 500 seeds, and 161 bacteria out of 500 seeds. 48.2 % had one endophyte, and only 3.8 % had two endophytes. The rest (48 %) of the seeds had no endophyte (Table 3.4).

Table 3.5 presentes the results and details of each group that had been tested. For example, in the first row, there are 200 *V. dubia* seeds, and they are 33 years old, the surface sterilizations method was dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times. The result, there were not any seeds having more than two endophytes, and the relative abundances for two or more is zero. The column of microbes in ≥ Two indicates if there were two or

more microbes, what they were, (fungi, bacteria, or both) and this case it was none because the result of this group was ‘zero’ or ‘one’. For the next column (3% Expected) here is comparing the result of this group of 200 seeds with Newcombe et al. 2018 results which they expected to find 3% of the seeds have two or more endophytes. Results presented here are similar, with low rates of vertical transmission.

There were no differences in the results for *V. dubia* and *B. tectorum* for two endophytes. In most cases, the percentages of two endophytes were lower than 3 % comparing with Newcombe et al. study in 2018. There were only two cases in *B. tectorum* seeds for two endophytes more than 3 %. The first case, the percentage was 10% and the second one 6% out of 100 seeds (Table 3.5).

Similarly, in *B. stricta* there were 62 fungi and 27 bacteria out of 1080 seeds. 8.24 % of the seeds had one endophyte (‘one’ pattern), and 91.76% had zero endophytes while two did not occur in *B. stricta.* Even with the different surface-sterilizations methods, inoculated flowers with a group of fungi never resulted in > 2 fungi found in the seeds (Table 3.3 and 3.4).

The overall result, out of 4980 seeds, the majority of seeds were ‘zero’ pattern, with about 82%, 17% of the seeds had one endophyte and only 1% with two endophytes. As in Table 3.5, there were no seeds that had more than two endophytes in all of the three plant species. The organisms that were found in each plant species were fungi or bacteria, are listed: *V. dubia* seeds had *Alternaria*, *Cladosporium*, *Chaetomium*, *Cochliobolus*, *Penicillium*, *Fusarium*, *Aspergillus*, *Rizpous*, *Epicoccum*, and Bacteria. *Alternaria* was predominant in *V. dubia*. *B. tectorum* had *Penicillium*, *Cladosporium*, *Aspergillus*, and bacteria. Bacteria were predominant in *B. tectorum*. *B. stricta* had *Aureobasidium*, *Penicillium*, *Mucor*, and bacteria. *Aureobasidium* was predominant in *B. stricta*.

Discussion

With the total number of seeds, 4980, for all three species, there were only 1% of seeds having two culturable endophytes per seed. Even for *B. stricta* with inoculated flowers (Table 3.3), the result was zero or one endophyte, and there was no more than one endophyte. Also, none of the fungi derived from leaves and seed, and inoculated to *B. stricta* flowers were found in the *B. stricta* seeds. Fungi isolated from leaves and seed of *B. stricta* did not infect the flowers (developing seeds). With this result, seeds comprised a bottleneck for microbial colonization, because seeds are different than other plant parts in being more protected (with stronger defenses), so this results in a low number of internal microbes, lower than numbers in leaves or roots. Seeds have a strong defense against any microbe, a situation potentially explained by an optimal defense. Exclusionary interactions, as we confirmed in this study and previously in Raghavendra et al., 2013. A previous result was less than 4% of the seeds having two or more endophytes (Newcombe et al. 2018), and here the result is 1% for two

or more with using different factors (age of the seeds, type of the medium, inoculating the flower with fungi, surface-sterilizations methods, and genotypes).

Developing seeds are expected to strongly defend against microbial attack (Raghavendra et al. 2013), a situation known as the optimal defense theory (Rhoades 1979) which offers an analytical outline for the distribution of various defenses used by plants. One of its expectations is that the plant will assign defenses according to the role of tissue value, i.e., the cost of having that tissue removed. For example, younger leaves have higher concentrations of defense chemicals than older leaves (McCall and Fordyce 2010). Optimal defense theory is based on two important suppositions: 1) defenses are beneficial; compared to organisms that are not defended, defenses increase capability to withstand parasites, predators, or pathogens; 2) defenses are costly, when there are no parasites, predators, or pathogens, defenses decrease fitness as compared to organisms that do not use defenses. Optimal defense theory applies to protection from parasites, predators, or pathogens.

Seeds are not the same as the other plant parts. In regard to microbial infection, seeds tend to be lower in the diversity of microbes compared to vegetative counterparts (Arnold 2007). Seeds seem to have better defensive power than roots, leaves, and stems (Arnold 2007). Optimal defense theory does predict more powerful defense of seeds than vegetative plant parts, even though this theory was created with herbivory in mind instead of microbial attack (Stamp 2003). Consistent with this, seeds are predicted to retain the greatest amounts of defense compounds inside the plant (Zangerl and Berenbaum, 1997).

Few studies have focused on seed endophytes, compared with leaf and stem endophytes (Qi et al. 2012). As an example, for the bottleneck in *Centaurea stoebe* seeds, out of 8763 seeds, only 26% of the seeds had one culturable fungal isolates, and 74% of the seeds had none ‘zero' (Raghavendra et al. 2013). From a *Lupine* seeds study, there were surface sterilized symptomatic and surface sterilized asymptomatic seeds. Out of 3,200 seeds, the results were zero, one, or two endophytes per seed. However, instances of two endophytes per seed were rare (Alomran et al. 2013). Another study involved 800 surface-sterilized seeds of *Pinus monticola*. Endophytes found in only 16 seeds and only one endophyte per seed, so that means zero endophytes per seed was the most common (Ganley and Newcombe 2006). Only in one time was the two-isolates substances high: in *B. tectorum* collected in 1987, it was 10% out of 100 seeds. The seeds were 33 years old, so they were kept in the storage for a long time and the storage condition would have increased the number of endophytes in these seeds. However, 10% is still a low number comparing with plant's parts, leaves, stems.

In this experiment we did not have an equal number of seeds in each plant species, we did not follow one protocol of surface-sterilization or use the same type of isolation medium or have the same

age of the seeds, because it is an exploratory study testing three plant species for bottleneck and exclusionary interactions for *B. stricta*. As the result, above indicate, we confirm the bottleneck for all plant species assessed, and the exclusionary interactions for *B. stricta*.

Literature Cited

Alomran M M (2012) Mycoflora of Lupine Seed, with Emphasis on Lupinus Albus (Master dissertation, Washington State University)

Arnold A E (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. Fungal biology reviews 21:51-66

Bacon C W (2018) Stains, media, and procedures for analyzing endophytes. In Biotechnology of endophytic fungi of grasses. CRC Press. Pp. 59-68

Castellá G, Bragulat M R., Rubiales M V, Cabañes, F J (1997) Malachite green agar, a new selective medium for Fusarium spp. Mycopathologia 137:173-178

Ganley R J, Newcombe G (2006) Fungal endophytes in seeds and needles of *Pinus monticola*. Mycological research 110: 318-327

Hodgson S, de Cates C, Hodgson J, Morley N J, Sutton B C, Gange A C (2014) Vertical transmission of fungal endophytes is widespread in forbs. Ecology and evolution 4:1199-1208

McCall A C, Fordyce J A (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences?. Journal of Ecology 98:985-992

Newcombe G, Harding A, Ridout M., Busby P (2018) A hypothetical bottleneck in the plant microbiome. Frontiers in microbiology 9:1645

Northam, F. E., & Callihan, R. H. (1994). New weedy grasses associated with downy brome. US Department of Agriculture, Forest Service, Intermountain Research Station, General Technical Report, INT-313. 211–212

Qi F, Jing T, Zhan Y (2012) Characterization of endophytic fungi from Acer ginnala Maxim. in an artificial plantation: media effect and tissue-dependent variation. PLoS One 7: e46785

Raghavendra K H, Newcombe G, Shipunov A, Baynes M, Tank D (2013) Exclusionary interactions among diverse fungi infecting developing seeds of *Centaurea stoebe*. FEMS microbiology ecology. 84:143-153

Rhodes D F (1979) Evolution of plant chemical defense against herbivores In GA Rosenthal & DJ Janzen (Eds.) Herbivores: Their interaction with secondary plant metabolites. Pp 3–54

Rodriguez, R. J., White Jr, J. F., Arnold, A. E., & Redman, A. R. A (2009) Fungal endophytes: diversity and functional roles. *New phytologist*, *182*(2), 314-330.

Schranz M E, Manzaneda A J, Windsor A J, Clauss M J, Mitchell-Olds T (2009) Ecological genomics of *Boechera stricta*: identification of a QTL controlling the allocation of methionine-vs branched-chain amino acid-derived glucosinolates and levels of insect herbivory. Heredity, 102: 465

Selosse, M. A., Baudoin, E., & Vandenkoornhuyse, P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. *Comptes rendus biologies*, *327*(7), 639-648.

Song B H, Clauss M J, Pepper A, Mitchell-Olds T (2006) Geographic patterns of microsatellite variation in *Boechera stricta*, a close relative of Arabidopsis. Molecular Ecology 15;357-369

Stamp N (2003) Out of the quagmire of plant defense hypotheses. The Quarterly review of biology 78:23-55

Zangerl A R, Berenbaum M R (1997) Cost of chemically defending seeds: furanocoumarins and Pastinaca sativa. The American Naturalist 150:491-504

Table 3.1. locations of *V. dubia* seeds samples.

location	Sample date or year
Pullman, WA	1984
Rosalia, WA	1984
Phillips Farm, Latah County Idaho. Paradise Ridge, Latah County Idaho	April,14,2014
Phillips Farm, Latah County Idaho	February, 2014
Belgrade, Serbia	2015
Mountain Home, ID	June, 2011
Murphy, ID	June, 2012
Moscow, ID	2015
Moscow, ID	2015
Murphy, ID	June, 2012
Castle Rocks, ID	June, 2011
Payette, ID	June, 2013
Indian Valley, ID	April, 2006
Fishtrap, WA	July 15, 2016
French Corner, ID	June 28, 2016
Moscow, ID	June 23, 2016
Cambridge, ID	June 28, 2016
French Corner, ID	June 28, 2016
Council, ID	June 28, 2016
Alpine, ID	June 28, 2016
Keating, OR	June 27, 2016
Lewiston, ID	July 11, 2016
Athol, ID	July 12, 2016
Rathdrum, ID	July 12, 2016
Castle Rocks, ID	June 28, 2016
Laclede, ID	July 15, 2016
Usk, WA	July 12, 2016
Usk, WA	July 12, 2016
Lenore, ID	July 11, 2016
Mountain Home, ID	June 29, 2016
Mountain Home, ID	June 29, 2016
Alpine, ID	June 28, 2016
Palouse, WA	2017

Table 3.2. locations and year of *B. tectorum* seeds samples.

Name	Year	location
B. tectorum	1987	-
B. tectorum	1990	-
B. tectorum	2001	-
B. tectorum	2011	-
B. tectorum	2017	Cheney, WA

Table 3.3. Source and identity of inoculants for *B. stricta* flowers.

Isolate Number	Host	Identities based on ITS sequences
Boe3	B. stricta leaf endophyte	*Alternaria mali* or A. longipes (equal matches)
Boe5	B. stricta leaf endophyte	Ascochyta arabiei, Didymella phacae
17	B. stricta leaf endophyte	No good match,

20	B. stricta leaf endophyte	Hyalodendriella betulae
35	B. stricta leaf endophyte	Dothidea sambuci
Boe 40	B. stricta leaf endophyte	Penicillium raistrickii
Boe 51	B. stricta leaf endophyte	Davidiella macrospora/Clad. macrocarpum
FZ102	B. stricta seed endophytes	Botrytis

Table 3.4 'zero/one' pattern of number endophyte for *V. dubia*, *B. tectorum*, and *B. stricta seeds.*

			Relative Abundance		**% seed with # of isolates per seed**		
Plant Species	**Seeds sampled (N)**	**Total isolates**	**Bacteria**	**Fungi**	**Zero**	**One**	**Two**
Ventenata dubia	3400	552	0.28	0.72	0.842	0.154	0.004
Bromus tectorum (cheatgrass)	500	279	0.58	0.42	0.478	0.48	0.038
Boechera stricta	1080	89	0.3	0.7	0.9	0.1	0

Table 3.5 Results

Plant species	Age of the seeds	Medium	Surface Sterilizations method	Seeds sampled (N)	≥ Two endophytes	Relative abundances for ≥ Two	Microbes in ≥ Two	3% Expected	Observed	Chi-square	P-value
V. dubia	33 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	200	0	0	None	6	0	6.091	0.0135
V. dubia	2 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	3	0.03	Association of bacteria with fungi, or two fungi	3	3	3.046	0.080
V. dubia	Less than a year	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	300	4	0.013	Association of bacteria with Fungi	9	4	1.966	0.160
V. dubia	Less than a year	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	2	0.02	Association of bacteria with Fungi	3	2	0.2051	0.650
V. dubia	9 years	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three	50	0	0	None	1.5	0	1.523	0.217

			times								
V. dubia	4 years	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	200	1	0.005	Two fungi	6	1	3.635	0.056
V. dubia	3 years	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	0	0	None	3	0	2.956	0.085
V. dubia	2 years	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	0	0	None	3	0	2.956	0.085
V. dubia	One year	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	400	2	0.005	Two fungi	12	2	7.2701	0.007
V. dubia	Less than a year	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three	300	0	0	None	9	0	8.869	0.002

			times								
V. dubia	Less than a year	MGA 2.5	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	950	2	0.0021	Association of bacteria with Fungi	29	2	23.4	0.000
V. dubia	Less than a year	Kings	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	300	0	0	None	9	0	8.869	0.002
V. dubia	Less than a year	Filter paper	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	300	0	0	None	9	0	8.869	0.002
B. tectorum	31 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	10	0.1	Fungi and bacteria together	3	10	4.0313	0.044
B. tectorum	28 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three	100	3	0.03	Fungi and bacteria together	3	3	0	1.

			times								
B. tectorum	17 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	0	0	None	3	0	3.046	0.080
B. tectorum	7 years	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	0	0	None	3	0	3.046	0.080
B. tectorum	Less than a year	PDA	Dipping in 6% sodium hypochlorite for two minutes, and then rinsed by sterile distilled water three times	100	6	0.06	Fungi and bacteria together	3	6	1.0471	0.306
B. stricta	Less than a year	PDA	1 min in 96% ethanol, 5 min in 6% Sodium hypochlorite (NaOCl), 30 sec 96% ethanol), and rinsing (5 min in distilled sterile water (SDW	100	0	0	None	3	0	3.046	0.080
B. stricta	Less than a year	PDA	(15 min in 3% NaOCl) and rinsing for two times (5 min rinse in SDW)	100	0	0	None	3	0	3.046	0.080

B. stricta	Less than a year	PDA	(5 min in 3% NaOCl) and rinsing two times for 5 min in SDW. 4)	100	0	0	None	3	0	3.046	0.080
B. stricta	Less than a year	PDA	surface-sterilization in 5 min in distilled water (DW), rinsing for 5 min in SDW	100	0	0	None	3	0	3.046	0.080
B. stricta, LTM genotype (inoculated flower)	Less than a year	PDA	surface-sterilization in 5 min in distilled water (DW), rinsing for 5 min in SDW	170	0	0	None	5.1	0	5.178	0.022
B. stricta, SAD12, genotype	Less than a year	PDA	surface-sterilization in 5 min in distilled water (DW), rinsing for 5 min in SDW	170	0	0	None	5.1	0	5.178	0.022
B. stricta, LTM genotype (inoculated flower)	Less than a year	PDA	surface-sterilization in 5 min in distilled water (DW), rinsing for 5 min in SDW	170	0	0	None	5.1	0	10.355	0.001
B. stricta, SAD12 genotype	Less than a year	PDA	surface-sterilization in 5 min in distilled water (DW), rinsing for 5 min in SDW	170	0	0	None	5.1	0	5.178	0.022

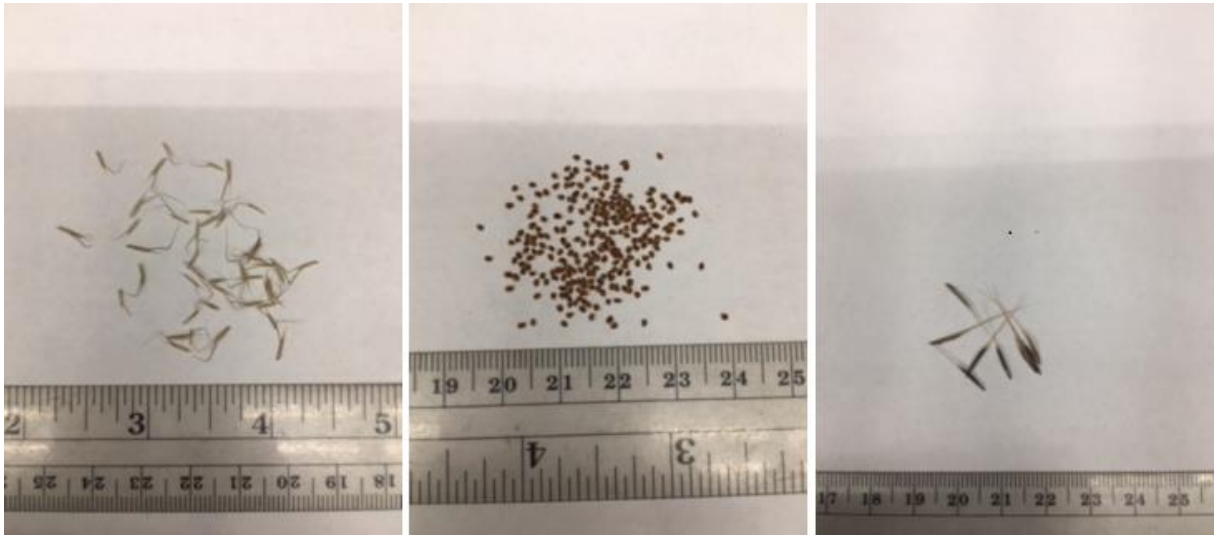

Figure 3.1 from (left) Seeds of *V. dubia*, *B. stricta*, and *B. tectorum.*

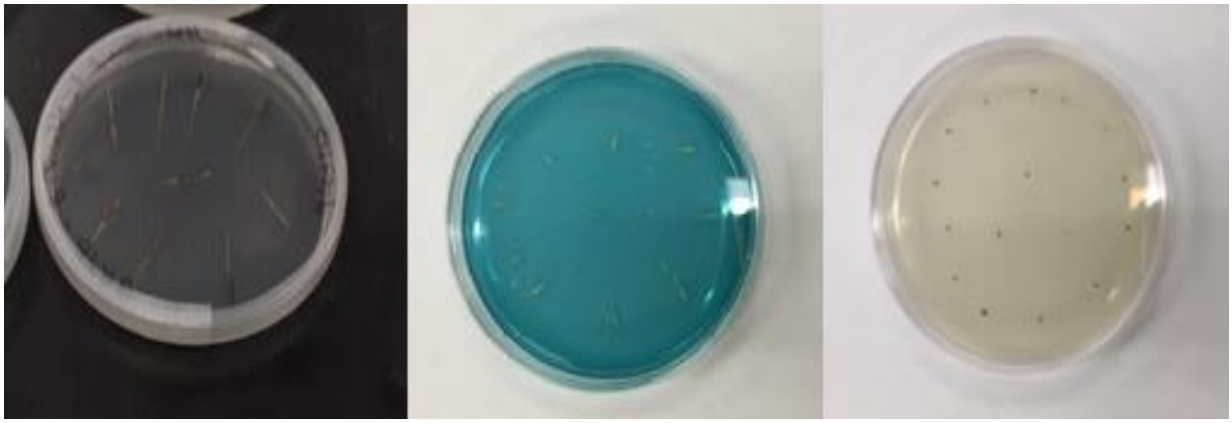

Figure 3.2 from (left) Seeds of *B. tectorum* on PDA, *V. dubia* on MGA 2.5, *B. stricta* on PDA.

Chapter 4: *Aspergillus tubingensis* is a seedlings pathogen of date palm

Abstract

Many diseases of the date palm are known. But pathogens that might affect seed germination and seedling emergence from soil are poorly studied, perhaps because date palm cultivars are propagated vegetatively. Here we determined the effects of date seed fungi on germination and emergence of 600 seeds overall (200 of each of three cultivars: Thoory, Halawi, and Barhi). In each cultivar, 100 seeds were from Saudi Arabia (part of the native range), and 100 were from the southwestern USA (where the date palm was introduced around 1765). Just four genera (i.e., *Alternaria, Aspergillus, Chaetomium, Penicillium*) were isolated overall from the surface-sterilized date seeds, and *Alternaria* was found in only one cultivar in the American Southwest. *Aspergillus* was in highest relative abundance among the remaining three at 39%, and it was significantly more common overall in Saudi Arabian seeds than in American seeds (116 versus 39, respectively; χ^2=51.58, p<0.0001). *Aspergillus* reduced germination of seeds (χ^2=22.13, p<0.0001) and then also reduced emergence when germinated and non-germinated seeds were planted in potting mix in a greenhouse (χ^2=77.42, p<0.0001). In contrast, *Penicillium* species were more common in American seeds than Saudi seeds (χ^2=14.57, p=0.0001); *Penicillium* did not affect germination (χ^2=3.43, p=0.06) although it did have a marginally positive effect on seedling emergence (χ^2=5.52, p=0.019). In a second experiment with 17 Halawi seeds, fungus-free germinants were inoculated with *A. tubingensis* or not and then planted. Controls emerged whereas *Aspergillus*-inoculated seeds did not. Our findings show that *Aspergillus tubingensis* is a seedborne pathogen that has not previously been reported as a pathogen of date palm. Our findings also suggest that *Aspergillus* may be more common in seeds in the native range of its host than in its introduced range.

Introduction

The date palm, *Phoenix dactylifera*, belongs to the family Arecaceae. The date palm has been cultivated for at least five millennia in desert oases from North Africa to Southwest Asia, including the Persian Gulf (Abdelmonem and Rasmy 2007). The date palm was introduced to Spain, and the Spanish, in turn, introduced date culture to North America, almost three hundred years ago in the desert oasis of Mission San Ignacio of Baja California Sur in Mexico. The date palm has flourished there ever since, and it was subsequently introduced to other parts of the North American Southwest (the states of California and Arizona) as well as to Australia, India, Pakistan, South Africa, and South America (Chao and Krueger 2007). Over the years, the United States has imported much propagative material of date palm, including seed for selection of cultivars (Fairchild 1908; McCarthy 2012). Cultivars, once selected, are then propagated vegetatively. Many popular date cultivars are now grown

in both the native and introduced ranges.

Plants often host fewer diseases where they are introduced versus where they are native; this is part of the enemy release. Mitchell and Power (2003) showed that this was true for certain obligate parasites that might be left behind in the native range when the plant is introduced elsewhere: rust fungi (Uredinales), powdery mildew fungi (Erysiphales), smut fungi (Ustilaginales) and viruses. Date palms are not known to host any rust or powdery mildew fungi anywhere in the world, but they do host a widespread, 'falsesmut' fungus (in Exobasidiales rather than Ustilaginales) (Farr and Rossman, 2018). However, other pathogens might also be more common in date palm's native range than in its introduced range. Given vegetative propagation, the same cultivars (genotypes) can be contrasted in the two ranges, and here we consider that comparison for seed-borne pathogens of three cultivars.

We obtained seeds from the same cultivars sampled in both the native range and the American Southwest. Seeds of the date palm are relatively large (from 0.5 grams to 4 grams) (Zaid and de Wet 2002), and they can remain viable for an extraordinary period of up to 2000 years under certain climatic conditions of high temperatures and low precipitation (Sallon et al. 2008). Overall, thirteen genera of fungi have been reported from seeds (*Alternaria, Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Penicillium, Phialophora, Rhizopus, Scytalidium, Thielavia, Trichoderma,* and *Ulocladium*) but none have been investigated for effects on seed germination and seedling emergence (Al-Sheikh 2009; Bokhary 2010; Al Hazzani et al. 2014).

Objective

The objectives of this study were to determine the seedborne fungi in date palm seed of the same cultivars in Saudi Arabia and the American Southwest and then determine the effects of these fungi on germination and emergence of seedlings.

Materials and Methods

Seed germination and seedling emergence

Seeds of three date palm cultivars (cultivars 'Thoory', 'Halawi', and 'Barhi') were obtained from local markets in Riyadh, Madinah, Albir, and Thadq in Saudi Arabia (Figure 4. 1) and ordered online in the United States (cv. 'Thoory' from Oasis Date Gardens in Thermal, California; 'Halawi' from Sun Organic Farm in San Marcos, California; 'Barhi' from Fresh Date by Anderson in Thermal, California). All seeds were from fruits harvested in 2015. One hundred seeds of each of the three cultivars were from Saudi Arabia, and 100 were from the American source. Thus, the total number of seeds was 600. All seeds were surface-disinfested by dipping in 1% sodium hypochlorite for two minutes, and then washed in sterile distilled water (SDW) three times. From ten to 13 seeds were placed in each 25 cm Petri dish depending on the size of the seeds. In each dish, seeds were placed on a sterilized paper towel that had been moistened with 20 ml of SDW. SDW was also added as needed

over time to prevent seeds from drying out and to allow seeds to germinate (Al-Sheikh 2009). The plates were incubated at room temperature (22° C) with continuous white fluorescent lights. After ten days, seed germination and fungal identity were recorded every two days for 24 days. Representative isolates of fungal genera were recovered from each cultivar and maintained on PDA. Ungerminated seeds and germinants were then moved to the greenhouse where they were planted in ‘conetainers' filled with Sunshine #1 potting mix. Seeds and germinants were kept in the greenhouse for 115 more days to observe seedling emergence. The greenhouse temperatures were maintained between 20°C and 24°C, with a 16:8 hour, day-night cycle.

Identification of Seedborne Fungi

Fungi emerging from, or present on, surface-disinfested seeds were recorded and examined under first a dissecting and then a compound microscope to identify each to genus. Appropriate mycological text was consulted Malone et al (1997). Two isolates (one *Aspergillus*, ex date USA; one *Penicillium*, ex date Saudi Arabia) were identified to species level using a sequence-based approach. In short, the total genomic DNA was extracted and a part of the calmodulin (*CaM*) and β-tubulin (*BenA*) gene amplified and sequenced according to the method described in Samson et al. (2010). The generated sequences were compared against an in-house sequence database containing all *Aspergillus* and *Penicillium* reference sequences. The isolates were deposited in the CBS culture collection housed at the Westerdijk Fungal Biodiversity Institute (*Aspergillus tubingensis* CBS 144784and *Penicillium* sp. CBS 144783).

Koch’s Postulates / Aspergillus experiment

After first observing the effects of fungi on seed germination and emergence in the initial, 600-seed experiment described above and identifying *Aspergillus*, the latter was the focus of a second experiment to test Koch’s Postulates. In this part, seventeen seeds of Halawi cultivar and 14 different *Aspergillus* isolates were used. Three seeds were used as controls and fourteen seeds inoculated for testing Koch's Postulates. All seeds were surface-sterilized by dipping in 1% sodium hypochlorite for two minutes and then washed in SDW three times. Each seed was placed in a ten cm diameter Petri dish over a sterilized paper towel with 10 ml of SDW; more SDW was added as needed to prevent seeds from drying out and to allow seeds to germinate. Fourteen different *Aspergillus* isolates were added to these fourteen seeds as spore suspension, so that means each seed was inoculated by an *Aspergillus* isolate which was different than used for the other seeds. The spore suspension was done by adding SDW by transfer pipette to the *Aspergillus* PDA plate and then the transfer pipette was used to add a small amount of spore suspension directly applied to the surface of each seed. Then after 30 days, the seeds that only had *Aspergillus* without any other seed-borne fungi were transferred to the greenhouse to test the effect of *Aspergillus* only. For the control seeds, one of them has been removed

from this experiment because of the appearance of another fungus (*Penicillium*) on the surface of the seed, as well from fourteen inoculated seeds one of which had also been removed from this experiment because the appearance of another fungus (*Penicillium*) on the surface of the seed. The total number of seeds transferred to the greenhouse was fifteen seeds, two controls and thirteen inoculated with *Aspergillus*. Seeds were planted in Cone tainers pot with potting mix, and each seed was labeled. Seeds were kept in the greenhouse for 121 days with watering four days a week and the greenhouse conditions (20°-24°C, 16:8 day: night cycle).

Repeated Koch's Postulates for Aspergillus tubingensis

Koch's Postulates were repeated using an *Aspergillus tubingensis* which was identified previously as described. Two hundred seeds of Halawi cultivar were used to test Koch's Postulates, with only *A. tubingensis*. One hundred seeds were used as controls and 100 seeds inoculated with *A. tubingensis*. All seeds were surface-sterilized by dipping in 1% sodium hypochlorite for two minutes and then washed in SDW three times. Ten seeds were placed in each 25 cm Petri dish over a sterilized paper towel with 10 ml of SDW; more SDW was added as needed to prevent seeds from drying out and to allow seeds to germinate. *A. tubingensis* isolate WAS was added to these 100 seeds as spore suspension. The spore suspension was done by adding SDW by transfer pipette to the *A. tubingensis* PDA plate and then used the transfer pipette to add a small amount of spore suspension directly applied to the surface of each seed. Then after 30 days, all seeds were transferred to the greenhouse to test the effect of *A. tubingensis*. Seeds were planted in Cone tainers pot with potting mix, and each seed was labeled. Seeds were kept in the greenhouse for 121 days with watering four days a week and the greenhouse conditions (20°-24°C, 16:8 day: night cycle). After 121 days and after taking all the data, dry weight of all aboveground parts for both control and inoculum were recorded.

Statistical Analyses

Survival analysis was carried out to determine how fungi affected seedling emergence over time using RStudio version 3.6.0. Chi-square analyses were performed for 2x2 contingencies. T-Test was done for a dry weight of aboveground's parts in Koch's Postulates for *A. tubingensis.*

Results

Only one fungal species per seed was isolated in 54.5% or 327 of the 600 seeds. A further 238 seeds, or 39.7%, yielded no fungi. Just 35 (5.8%) of the seeds yielded two species per seed. Table 4.1 shows the number of isolates of fungi per cultivar, Thoory, Halawi, and Barhi, from each country, Saudi Arabia, and the United States. For example, in Thoory from Saudi Arabia (Th-SA), there were four isolates of *Aspergillus*, 14 isolates of *Chaetomium*, 13 isolates of *Penicillium*, and one unknown fungus, so the total isolated fungi were 31 from Th-SA. A total of 397 fungal isolates were thus obtained, and these belonged to four genera: *Alternaria, Aspergillus, Chaetomium, Penicillium* (Table

4.1).

The relative abundance of each genus was as follows: 39.0% *Aspergillus,* 33.8% *Penicillium,*18.4% *Chaetomium,* and 8.6% *Alternaria* (Table 4.1). The two most abundant genera, *Aspergillus* and *Penicillium,* were recorded to a varying extent in all cultivars from both Saudi Arabia and the American Southwest. *Alternaria* was relatively rare and was only found in one cultivar, 'Barhi', in American seeds. Phenotypic diversity was observed in the detected *Alternaria* isolates, and 79 % would have been identified as *Ulocladium* using the classical, phenotype-based classification system of those genera (Simmons 2007).

The *Aspergillus* sect. *Nigri* isolate (CBS 144784) was identified as *Aspergillus tubingensis* using partial calmodulin and tubulin gene sequencing (*BenA* 99.8 %; *CaM* 99.5 %) (similarity percentages with the type strain of the species). Sequence analysis revealed that the *Penicillium* isolate belongs to section *Canescentia*. The isolate could not be identified to species level and probably represents a new taxon. Most closely related species are *P. yarmokense* (*BenA* 97.8 %; *CaM* 97.9 %), *P. murcianum* (*BenA* 97.4 %; *CaM* 97.7 %), *P. canescens* (*BenA* 96.2 %; *CaM* 98.6 %) and *P. arizonense* (*BenA* 96.5 %; *CaM* 98.2 %).

Aspergillus sp. were more common in Saudi (116 seeds) than American (39 seeds) sources (χ^2=51.58, p<0.0001). *Penicillium* sp. was the reverse: more common in American seed (i.e., 97 seeds) than Saudi (37 seeds) (χ^2=14.57, p=0.0001). *Chaetomium* sp., the genus with the third highest relative abundance (73 seeds from which isolates were obtained) was equally common in American (45) and Saudi (28) seed (χ^2=2.02, p=0.15).

Seed germination was recorded in the lab prior to planting all seeds, both germinated and non-germinated, in potting mix in the greenhouse to determine emergence. Germination in Petri dishes in the lab was affected by fungi associated with the seeds. The growth of *Aspergillus* sect. *Nigri* reduced germination of seeds (χ^2=22.13, p<0.0001) and then also strongly reduced emergence when germinated, and non-germinated seeds were planted in potting mix in a greenhouse (χ^2=77.42, p<0.0001). In contrast, the *Penicillium* species present did not affect germination (χ^2=3.43, p=0.06) although it did have a marginally positive effect on seedling emergence (χ^2=5.52, p=0.019). The third most abundant fungus in date seeds was *Chaetomium* sp. represented a third pattern of effects on germination and emergence. It slowed germination of seeds (χ^2=20.19, p<0.0001) but did not reduce emergence (χ^2=3.80, p=0.051) (Table 4.2).

Survival analysis for all fungi

Presence of *Aspergillus* decreased seedling emergence. On the other hand, *Penicillium* and *Chaetomium* did not decrease it and even increased it as the values were significantly higher than those of no fungi seeds (Figure 4.8).

Koch's Postulates / Aspergillus experiment

Seeds inoculated with *Aspergillus* have resulted in slower or no emergence seedlings. Difference between the control and the seeds with *Aspergillus* is clear in Figure 4.2. Delayed emergence caused by *Aspergillus* (left) versus control, emerged seedling (right) is apparent. The two controls seeds had become seedlings (Figure 4.3), and one infected seed with *Aspergillus* had been delayed in the emergence, and just the root had been showing (Figure 4.3), and other infected seeds did not emerge. All of that was after four months of observation.

Repeated Koch's Postulates for Aspergillus tubingensis

Emergences of seedling with *A. tubingensis* have resulted in slower or no emergence when the seed was inoculated with *A. tubingensis*. Difference between the control and the seeds with *A. tubingensis* is clear in Figure 4.4. Delayed emergence caused by *A. tubingensis* (right) versus control, emerged seedling (left) (Figure 4.5, 4.6, and 4.7).

Survival analysis for Aspergillus tubingensis

Seedling emergence was affected by *A. tubingensis* and not in controls where fungi were absent in the seeds. Presence of *A. tubingensis* decreased seedling emergence as in (Figure 4.9). However, when *A. tubingensis* absent, there was no effect in the seedling emergence. From the t-test, the means of the dry weight of aboveground for control is 0.545311475 and for inoculated is 0.286470588. That means *A. tubingensis* affected the seedling emergence and the weight aboveground by decreasing the weight of inoculated plants.

Discussion

Aspergillus tubingensis has never been reported from date palm before in any disease context, and this is thus the first report of this species as a pathogen of *Phoenix dactylifera*. *Aspergillus niger*, on the other hand, another member of *Aspergillus* section *Nigri* (Samson et al. 2014; Varga et al. 2011), has been reported as a postharvest pathogen causing fruit rot in Spain (Abdullah et al. 2010; Palou et al. 2016). In cases where *A. tubingensis* is known to be a plant pathogen, it has also been associated with fruit rot (Anderson and Thrane 2006). This is thus the second report of *A. tubingensis* as a pathogen beyond the association with fruit rot; the first report was that *A. tubingensis* as a leaf spot pathogen of *Jatropha curcas* in China (Guo et al. 2017). If more emergence assays were performed with seedborne microbes, it seems probable that more 'cryptic pathogens' would be discovered, even in genera such as *Aspergillus*. An example, albeit from a different genus, is the recent finding that *Sydowia polyspora* can act as a pre-emergent pathogen of *Pinus ponderosa* (Ridout and Newcombe 2018).

Many fungi have been implicated in fruit rot of date palm (Al Sheikh 2009; Palou et al. 2013), including both *Aspergillus* sp. belonging to the *A. niger* clade (Palou et al. 2016), and *Penicillium* sp.

(Palou et al. 2013). Yet, with respect to seed germination and seedling emergence, *A. tubingensis* and *Penicillium* sp. had opposing effects; the former with decidedly negative effects versus those of the latter that trended positively. This suggests that members of the fruit rot community switch to more specific and varied roles when it comes to seed germination and seedling performance.

Differences in the communities of seedborne fungi in native and introduced ranges have been reported before (Shipunov et al. 2008). That the pathogenic *A. tubingensis* was more common in Saudi seeds than American counterparts of three date cultivars confirms the expectation of enemy release for a cryptic, pre-emergent pathogen. It is interesting that *Penicillium* sp. was more common in American seeds and exerted a positive effect on seedling emergence. Novel mutualisms such as that are sometimes seen in plants in their introduced ranges (e.g., Baynes et al. 2012).

Most of the 600 seeds (94.2%) yielded either one fungus per seed (54.5% or 327 of the 600 seeds) or were fungus-free (238 seeds, or 39.7%). Only 5.8% yielded two fungi per seed. These findings confirm the hypothetical bottleneck in the plant microbiome that was recently proposed (Newcombe et al. 2018). Two reasons have been invoked to explain such a bottleneck: optimal defense theory and exclusionary interactions among seed-infecting fungi (Raghavendra et al. 2013). According to the Primary Symbiont Hypothesis that is based on the bottleneck, the identity of each microbe in a seed then matters, as effects on seedlings can change with primary symbiont identity. That is, in effect, what we saw in this study.

Literature Cited

Abdelmonem A M, Rasmy M R (2007) Major diseases of date palm and their control. Commun. Inst. For. Bohem 23: 9-23

Abdullah S K, Monfort E, Asensio L, Salinas J, Llorca L L, Jansson H B (2010) Soil mycobiota of date palm plantations in Elche, SE Spain. Czech Mycol *61*:149-162

Al Hazzani A A, Shehata A I, Rizwana H, Moubayed N M, Alshatwi A A, Munshi A, Elgaaly G (2014) Postharvest fruit spoilage bacteria and fungi associated with date palm (*Phoenix dactylifera L*) from Saudi Arabia. African Journal of Microbiology Research 8:1228-1236

Al-Sheikh H (2009) Date-palm fruit spoilage and seed-borne fungi of Saudi Arabia. Res J Microbiol *4*:208-13

Andersen B, Thrane U (2006) Food-borne fungi in fruit and cereals and their production of mycotoxins. Springer, Boston, MA: In Advances in Food Mycology. Pp 137-152

Baynes M, Newcombe G, Dixon L, Castlebury L, O'Donnell K (2012) A novel plant–fungal mutualism associated with fire. Fungal biology 116:133-144

Bokhary H A (2010) Seed-borne fungi of date-palm, *Phoenix dactylifera L*. from Saudi Arabia. Saudi Journal of Biological Sciences 17:327-329

Chao C T, Krueger R R (2007) The date palm (*Phoenix dactylifera L*): overview of biology, uses, and cultivation. HortScience 42:1077-1082

Guo J W, Gao Y, Li C Y, Yang L F, Tian X J, Hong L, Kong Q, Zhang YG, Li W J (2017) First Report of Leaf Spot Disease Caused by *Aspergillus tubingensis* on Jatropha curcas in Yunnan, China. Plant Disease 101:505

Fairchild D (1908) Seeds and plants imported during the period from July 1, 1906 to December 1, 1907. Inventory No. 13: Nos. 19058 to 21730. *USDA Bur. PI. Indust. Bull. 132*

Farr D F, Rossman AY. Fungal databases, systematic mycology and microbiology laboratory, ARS, USDA. http://nt.ars-grin.gov/fungaldatabases/ Retrieved 2018

Malone J P, Muskett A E (1997) Seed-borne fungi. Description of 77 fungus species. The International Seed Testing Association. Pp 179-384

McCarthy M A (2012) Date Palms in the Desert: Reimagining and Cooperating with Nature in Arid Arizona. Arizona Journal of Interdisciplinary Studies 1: 39

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens. Nature 421:625 -627

Newcombe G, Harding A, Ridout M, Busby P (2018) A hypothetical bottleneck in the plant microbiome. Frontiers in microbiology 9:1645

Palou L, Montesinos-Herrero C, Taberner V, Vilella-Esplá J (2013) First report of *Alternaria alternata* causing postharvest black spot of fresh date palm fruit in Spain. Plant disease 97: 286-286

Palou L, Rosales R, Taberner V, Vilella-Espla J (2017) Incidence and etiology of postharvest diseases of fresh fruit of date palm (*Phoenix dactylifera L.*) in the grove of Elx (Spain). Phytopathologia Mediterranea 55:391-400

Raghavendra A K H, Newcombe G, Shipunov A, Baynes M, Tank D (2013) Exclusionary interactions among diverse fungi infecting developing seeds of *Centaurea stoebe*. FEMS microbiology ecology 84:143-153

Ridout M, Newcombe G (2018) *Sydowia polyspora* is both a Foliar Endophyte and a Preemergent Seed Pathogen in *Pinus ponderosa*. Plant disease 102:640-644

Sallon S, E Solowey, Y Cohen, R Korchinsky, M Egli, I Woodhatch, O. Simchoni, M. Kislev (2008) Germination, genetics, and growth of an ancient date seed. Science 320:1464-1464

Samson R A, Visagie C M, Houbraken J, Hong S B, Hubka V, Klaassen C H, Perrone G, Seifert K A, Susca A, Tanney J B, Varga J (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. Studies in mycology 78: 141-173

Shipunov A, Newcombe G, Raghavendra A K, Anderson C L (2008) Hidden diversity of endophytic fungi in an invasive plant. American Journal of Botany 95:1096-1108

Simmons E G (2007) Alternaria: An Identification manual. CBS Fungal Biodiversity Centre, Series No.6, Utrecht, The Netherlands. 775p

Varga J, Frisvad J C, Kocsubé S, Brankovics B, Tóth B, Szigeti G, Samson R A (2011) New and revisited species in Aspergillus section Nigri. Studies in Mycology 69:1-17

Zaid A, P F de Wet (2002) Date palm cultivation. FAO Plant Production and Protection Paper 156. http://www.fao.org/docrep/006/Y4360E/y4360e0g. htm#bm16. Accessed: 2017

Table 4.1 Number of isolates of fungi per cultivar (Th, Thoory; HA, Halawi; Ba, Barhi) and range (SA, Saudi Arabia; USA, American Southwest) for a total of 397 isolates per 600 seeds. Relative abundances are shown on the bottom line as percentages of the total number of isolates.

	Alternaria	***Aspergillus***	***Chaetomium***	***Penicillium***	**Unknown**	**Total**
Th-SA	0	4	14	13	1	32
Th-USA	0	8	27	73	0	108
Ha-SA	0	45	14	19	0	78
Ha-USA	0	6	1	2	0	9
Ba-SA	0	67	0	5	0	72
Ba-USA	34	25	17	22	0	98
TOTAL	34	155	73	134	1	397
Percentages	8.56%	39%	18.38%	33.75%	0.25%	

Table 4.2 Germination in the lab followed by seedling emergence in potting mix in the greenhouse.

Seed fungus	**(# of emerged seedlings)**	**(# planted that did not emerge)**	**Average Number of Days for emergence**	**Number of seeds germinated in the lab**	**Number of seeds that did not germinate in the lab**
None	166	72	62.55	160	78
Penicillium	86	19	63.31	81	24
Penicillium with *Aspergillus*	5	7	64.2	9	3
Alternaria	21	10	62.3	25	6
Alternaria with *Penicillium*	1	1	63	1	1
Alternaria with *Aspergillus*	0	1	-	0	1
Penicillium with *Chaetomium*	12	3	66	10	5
Chaetomium	44	9	67	18	35
Aspergillus with *Chaetomium*	4	1	72.75	2	3
Aspergillus	31	106	71.7	58	79
Unknown fungi	1	0	70	1	0
Total	371	229		365	235

Table 4.3 T-Test of two-sample assuming unequal variances for aboveground dry weight

	Inoculum	**Control**
Mean	0.286470588	0.545311475
Variance	0.01344664	0.066346851
Observations	17	61
Hypothesized Mean Difference	0	
df	60	
t Stat	-5.971892886	
P(T<=t) one-tail	6.84443E-08	
t Critical one-tail	1.670648865	
P(T<=t) two-tail	1.36889E-07	
t Critical two-tail	2.000297822	

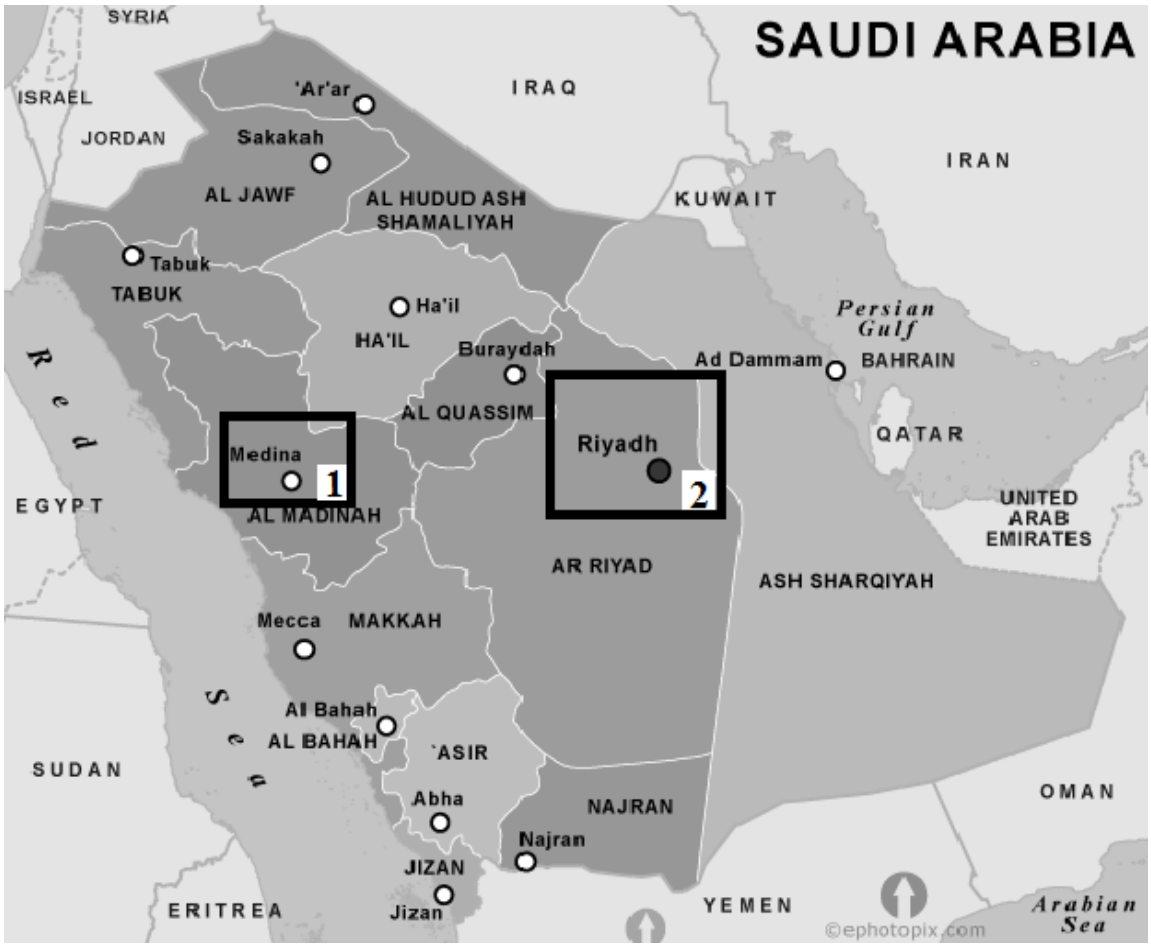

Figure 4. 1 Sampling area in Saudi Arabia: 1. Madinah, and 2: Riyadh, Albir, and Thadq.

Figure 4. 2 Delayed emergence caused by *Aspergillus* (left) versus fungus-free, emerged seedling (right).

Figure 4. 3 Delayed emergence caused by *Aspergillus* (left) versus fungus-free, emerged seedling (right).

Figure 4. 4 Plates of date palm seeds from the (left) first day in the lab, control plete after one-month, infected seeds with *A. tubingensis* after one-month.

Figure 4. 5 Seedlign in the greenhouse after 121 days, from the (left) control and (right) infected with *A. tubingensis*.

Figure 4. 6 Seedlign after 121 days, from the (left) control and (right) infected with *A. tubingensis*.

Figure 4. 7 Seedlign after 121 days, from the (left) control and (right) inoculated with *A. tubingensis*.

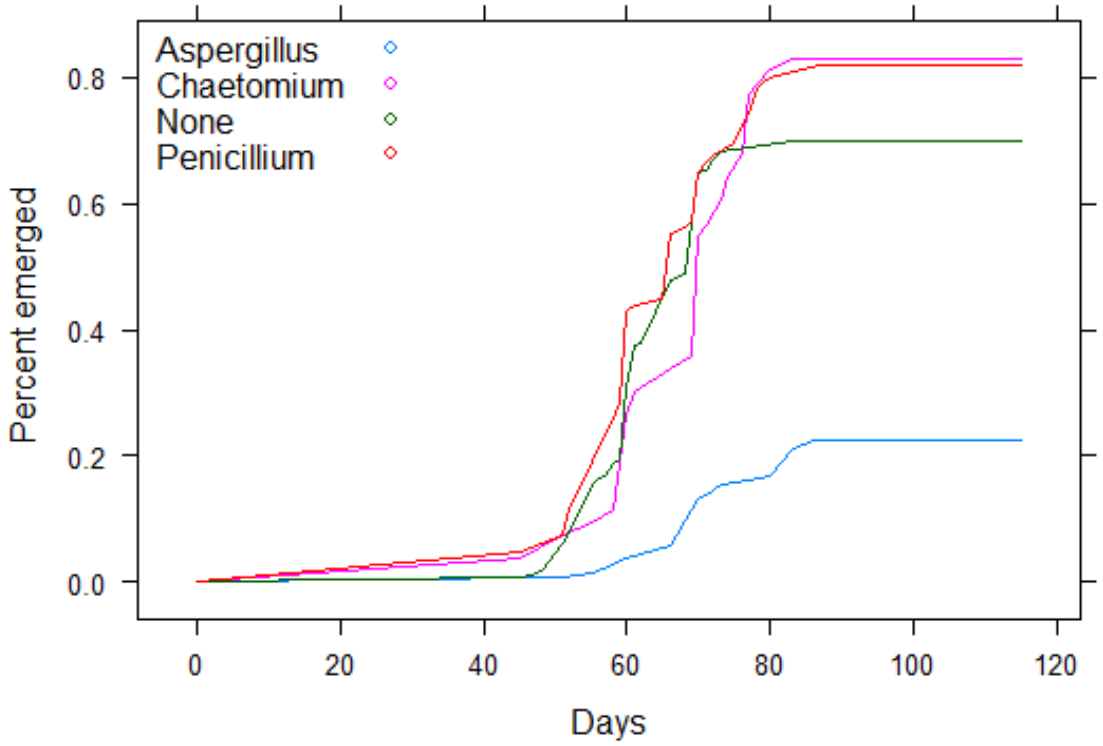

Figure 4.8 Survival analysis of emergence of date palm seedlings with or without fungi in the seeds.

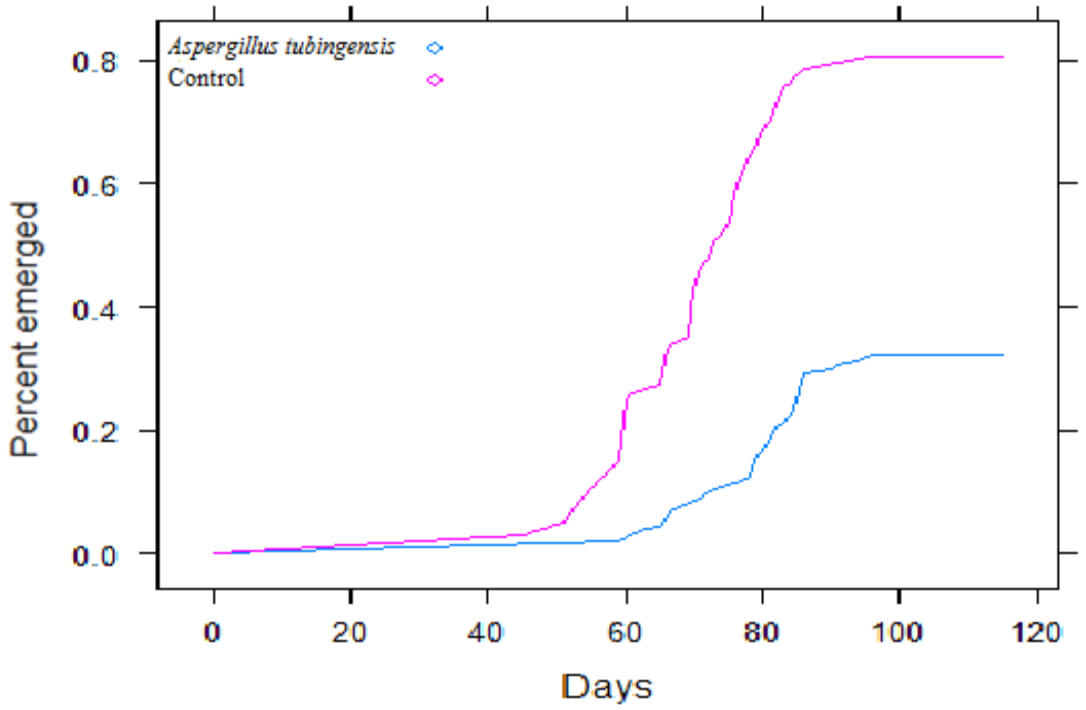

Figure 4.9 Survival analysis of emergence of date palm seedlings with or without *A. tubingensis*.

Epilogue & Future Directions

Investigating plant pathologies in a globally interconnected world. This dissertatiuon outlines work conducted to characterize plant microbe relationships. The first chapter is Review about *Ventenata dubia* and the second is *Ventenata dubia*'s Native Range Provides Insight into its Potential Distribution in North America and Directs Search Efforts to Areas that may Contain Pathogens Useful for Biological Control. These two chapters about are the grass plant *V. dubia* that has become a dangerous invader of the Palouse region (eastern Washington and northern Idaho). *V. dubia* is without any pathogens in its invaded area in eastern Washington and northern Idaho. The long-term goal is to introduce classical biocontrol pathogens from the native range to the invaded range. Biological control is the best option for this invasive plant and to find a pathogen for controlling this issue, we should search in its native range. It has been thought it's a native of North Africa, as one of the common names is North Africa grass here in the United States. After collecting data about *V. dubia*, it is almost exclusively reported in southern Europe and western Asia.

Chapter three, A bottleneck for microbes in seeds of *Ventenata dubia*, *Bromus tectorum*, and *Boechera stricta* presents a survey of isolation frequencies of culturable microbes of three plant species, done to compare with Newcombe et al. (2018). The result confirmed the hypothesis of a seed bottleneck for vertical transmission microbes.

Chapter four, *Aspergillus tubingensis* is a pre-emergent pathogen of date palm seedlings, is a survey of seed-borne fungi for both native range, Saudi Arabia, and introduced range of date palm, United States. Then an experiment of seed-borne fungi effects in seedlings was done as well. The result of it. *A. tubingensis* affected germination and emergence.

The core of this dissertation is to identify plant pathologens related to native and invasive species and identify natural controls to prevent ecosystem collapse and negative impacts. Plants are essential for life on earth because they are providing food, medicines, and oxygen for breathing. There are more than 390,000 plant species, and about 2,000 new species discovered annually. However, about 8,800 of plant species are threatened. Critical threats to plants are climate change, invasive species, and pathogens. Climate Change includes higher temperatures from global warming, and the result may decrease global crop production in the coming years. Invasive species, non-native species, additionally recognized as alien or invasive species, can be brought to an ecosystem intentionally or unintentionally. The impacts of exotic species can be through competition with native plants for soil resources and plant's physical spaces, and the result could be, invasive species can speed the decline of native plant species. Plant pathogens are causing several billions of dollars annually in losses in crops, pastures, and forests. So why we need to protect plants? Because plants have a significant role by, providing us with clean air, food, medicines. Plants are extraordinarily valuable economically.

Invasive plants have been getting more attention lately. A primary take away from this research is the importance for scientists and nonscientists to work together to protect native plant species and habitat. Here are some important steps that are necessary for engagement across the globe to limit plant species travel and negative impact. As I am from another country, my interest also lies in translation of findings from the USA where this research is conducted, to my home country. In this essence, below are several potential areas for future directions to build on this research.

- First and foremost, the critical need to monitor, prepare, and gage the risks of a future invasion of plants that will change the local area ecosystem. There are many proactive steps that management and science's communities can take to reduce the threats of invasive plants.
- Follow up with standardized information networks on invasive plants, standardizing the language.
- Use invasion risk assessments to become aware of areas where invasion is most likely to occur due to global change, and plants movement.
- Share data throughout global data networks to facilitate control efforts. For example, sharing a record of invasive plant distribution and control or the management information to reduce searching time and efforts.
- Knowing endangered species is important to prevent the extinction of any species.
- Start education programs, for example, invasive plant species stewardship programs as below for details.

Invasive Plant Species Identification and Stewardship Program

This is a potential future direction program; the vision for this program is to educate any community about their region's native plants and plant communities. The program could be for training the public and volunteers about the native plants and habitats. Training can be in the classroom or the field. Trainers need to be invasive species experts and well versed about the subject, for example, academics, organizations, or government agencies.

Goals of this program:

- Decrease in invasive cover by removing plants and follow up after the invasive species removal.
- Increase tree planting.
- Find the best management practices that appear to have been followed and report it.

Roles of this Program Should be

- Understanding the benefit of this program will help inform the trainers about the impact of invasive plants and how this could affect their community's natural resources and quality of life.
- Offering the highest quality training.
- Making the program available and open to a wide range of individuals.
- Encouraging and supporting native plant recognition in their community and try not to lose the native plants.
- Using a variety of methods for beginner volunteers to increase the awareness of the program.
- To educate about the threatened species and encourage volunteers to adopt one or more rare plants and provide them with the tools and experience required to locate and record observations for endangered species.
- Organized technical training workshops to make sure that volunteers will have the appropriate professional and safety training and equipment they need and are adequately prepared to search for their adopted plants.
- Workshops from professional botanists are necessary to educate how to identify the rare plants, survey methods, and data collection methods. Also, how to collect specimens and photographs.

Important Points to Learn and to do as a Member of Program:

- To identify local native plants.
- To learn about the ecology of local plant communities and wildlife habitats.
- To learn how to identify and control invasive plants.
- Help to educate the public about native plants and habitats.
- Help to restore our habitat with native plants and restoring habitat.
- To share working experience with others in the same community and provide guidance and inspire others.
- To raise public awareness of the program to other people to get as much as volunteers and participants.
- Use a variety of methods for beginner volunteers and increase awareness of the program.

This program can be a partnership between universities and local agencies, thus providing access for research, outreach, and learning environment.

The part of personal communications and how to get the right contact information in this dissertation, took me a long time because sometimes I had to contact 2 to 3 people to get someone's

email address. I think the next step is to have a global scientists' network, that has contact information that can be helpful for scientific projects. Also, the same concept could be used for recording plant species and have all the information about the abundance or rarity. Professional people can add their findings in it with proof of what they want to add. Most importantly, to have 4 or more languages in this global network, because one of the challenges for me was how to get all this information for locations of *V. dubia* when some of the information was not in English or my native language (Arabic).

Another future vision is the establishment of a Professional herbarium program in each country, with face to face and online access for ease of use across long distances. Contact information for employees of the herbarium is provided in case of need for more services or collaboration. This facility will make the process much easier and timely, everything easier and faster in case of needing herbarium services for projects instead of spending months or years collecting data; we can get the data in a simple way in a short amount of time. This program will be costly, may be granted funding, or fees generated from services; the expense with specific outcomes is preferred to lost data.

Lastly, noted during the work on this dissertation is the "Folk taxonomies," or use of the common name in scientific papers as an important issue for consideration. General information about the common names for plant species has been used for a long time. Regrettably, there is no standardization. Each country and regions within countries have their particular common names for plants. People in everyday life, and researchers, often use common names because they are simple to pronounce and, therefore, easier to keep in mind and remember. For many practical purposes within any region, common names may be used quickly and easily. However, the potential for miscommunication, confusion, and error is increased as the common names are not shared across regions as was encountered while researching and writing *V. dubia* chapter number 2. The potential for error is a critical reason why common names should not be used in scientific papers, only scientific names, and characterization.